Review Article
Realm of Thermoalkaline Lipases in Bioprocess Commodities

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For decades, microbial lipases are notably used as biocatalysts and efficiently catalyze various processes in many important industries. Biocatalysts are less corrosive to industrial equipment and due to their substrate specificity and regioselectivity they produced less harmful waste which promotes environmental sustainability. At present, thermostable and alkaline tolerant lipases have gained enormous interest as biocatalyst due to their stability and robustness under high temperature and alkaline environment operation. Several characteristics of the thermostable and alkaline tolerant lipases are discussed. Their molecular weight and resistance towards a range of temperature, pH, metal, and surfactants are compared. Their industrial applications in biodiesel, biodetergents, biodegreasing, and other types of bioconversions are also described. This review also discusses the advance of fermentation process for thermostable and alkaline tolerant lipases production focusing on the process development in microorganism selection and strain improvement, culture medium optimization via several optimization techniques (i.e., one-factor-at-a-time, surface response methodology, and artificial neural network), and other fermentation parameters (i.e., inoculums size, temperature, pH, agitation rate, dissolved oxygen tension (DOT), and aeration rate). Two common fermentation techniques for thermostable and alkaline tolerant lipases production which are solid-state and submerged fermentation methods are compared and discussed. Recent optimization approaches using evolutionary algorithms (i.e., Genetic Algorithm, Differential Evolution, and Particle Swarm Optimization) are also highlighted in this article.

1. Introduction

Lipases are lipolytic enzymes, triacylglycerol acylhydrolases (EC 3.1.1.3) that catalyze the hydrolysis of triacylglycerols to glycerol and free fatty acids [1]. Lipases are ubiquitous and versatile in which they can act as hydrolases or synthases and, like any other biocatalysts, they have the ability to catalyze reactions under various conditions with a very high degree of substrate specificity [1–3]. Hence, lipases reduce the formation of by-products from “reaction” process which is one of few factors for their green and environmental friendly properties [1, 2]. For decades, microbial lipases were remarkably used as biocatalyst and their market values in various fields and industries such as food (i.e., aroma), agriculture, cosmetics (i.e., esters), medicine, pharmaceutical (i.e., drugs), detergent (fat/oil removal), fat-processing (i.e., dairy), oleochemical (biodiesel), leather, paper (pitch/sap removal), and textile are increasingly growing [1–8]. Previous study indicates that demands for lipases as biocatalysts are significant, and report launched by the Freedonia Group in 2014 showed that the world demand for lipases is projected to increase 6.2% annually to US$ 345 million in 2017 [1]. To date, lipases are placed at the top third rank behind proteases and amylases and lipases annual market is expected to reach about 590.5 million dollars by 2020 [4].

Lipases synthesized by microbes play important roles as natural functional proteins in which their type and function in bacteria, actinomycetes, and fungi may differ mainly depending on its constitutive genetic makeup and biological evolution in order to optimize their behavioural condition in complex environment under normal-stress condition as well as for their living and survival [8–12]. Lipases show interfacial
activation, which is due to the existence of a hydrophobic lid in most lipases. It covers the active center in the inactive closed conformation of the lipases [13]. In its open conformation form, the active center is accessible for the substrates [14, 15]. The change between closed to open forms occurs in the occurrence of a substrate emulsion or a lipid interphase that opens the lid, as a result of which the lipases are activated [13]. Several instrumental techniques for structural analysis such as X-ray crystallography, circular dichroism, Fourier transform infrared (FTIR), electrospray ionization (ESI), and matrix-assisted laser desorption/ionization (MALDI) have been performed to elucidate the molecular structure of lipases. Three-dimensional (3D) structures of many lipases share similar \( \alpha/\beta \) hydrolase fold and have the same catalytic mechanism in which active site is formed by a catalytic triad of serine (Ser), aspartic acid (Asp)/glutamic acid (Glu), and histidine (His) [14, 16]. Lipases also share a consensus pentapeptide sequence of Gly-X-Ser-X-Gly motif, whereby X may be any amino acid residue [14]. For instance, X-ray crystallography analysis of protein structure-function relationships of Bacillus sp. L2 lipase revealed that topological organization of \( \alpha/\beta \)-hydrolase fold consisting of 11 \( \beta \)-strands, 13 \( \alpha \)-helices, serine-113, histidine-358, and aspartate-317 with single Ca\(^{2+}\) and Zn\(^{2+}\) was found in this lipase molecule [17]. Recently, amino acid sequence of lipase from S. arlettae JPBW-1 was characterized using MALDI-TOF-MS analysis and comparative modelling approach was performed via ROBETTA server to postulate a structure-activity relationship of the lipase [16]. In one study, it was found that TA lipase from Cohnella sp. A01 was structurally determined to be 37.5% \( \alpha \)-helix, 12.8% \( \beta \)-sheet, 22.7% \( \beta \)-turn, and 27% random coil [18]. Although several lipases have been industrially produced, the discovered lipases are not tolerant to high temperature and alkaline environment, thus easily losing their 3D conformational structure at certain period of time [1–3]. Moreover, improvement of lipases stabilities via lipase modification and immobilization also leads to some drawbacks such as high cost of production and time-consumption and some strategy is subjected to "trial and error" [13]. In some applications, immobilized lipase (heterogeneous form) is not preferred; instead free lipases are more suitable for some functions such as in laundry industry [19, 20].

To date, microbial lipases which are stable at high temperature and resistance to alkaline pH known as thermoalkaline (TA) lipases have been reported as new potential biocatalyst. TA lipases may differ from non-TA lipases such as in their primary sequences, molecular weights (MW), optimal pH and temperature, substrate and positional specificities, cofactors, and cellular locations [1, 2]. TA lipases have been given more attention due to their nature which is thermostable and alkaline tolerant in immobilized or in free lipases form. Although several TA lipases have been discovered, they are still under extensive research particularly in the development of fermentation process. Moreover, details of industrial fermentation techniques of TA lipases production are rarely reviewed and some knowledge owned by producing company or entity has been already patented [1]. This review describes and discusses the characteristics and potential applications of TA lipases, as well as the development of TA lipases production via process fermentation and its optimization approaches.

2. Characteristics of TA Lipases

2.1. Molecular Weight. The MW of the mesophilic enzymes is about the same to that of thermophilic enzymes with nearly similar 3D structures [11, 68]. Most of the thermophilic enzymes appears as small-size monomer and compacted proteins which may confer a higher thermostability as compared to that of the bulky proteins of mesophilic enzymes [31]. In fact, thermophilic enzymes have about double the number of subunits than mesophilic enzymes where enzymes with small subunits are more thermostable as compared to large enzyme with no subunits [68]. Several studies have reported that the MW of polypeptide chain of TA lipases can range from 21 to 67 kDa. This refers to molecular weight of the TA lipases from B. stearothermophilus, A. cinnamonoea BCRC 35396, Geotrichum candidum, T. atroviride LIPB, T. atroviride LipA, Acinetobacter sp. AU07, Microbacterium sp., T. thermophilus, Bacillus sp. RSJ-1, B. thermoleovorans ID-1, Bacillus LBN2, Cohnella sp. A01, B. licheniformis H7, and B. sonorensis 4R which were 67.0 kDa, 60.0 kDa, 59.0 kDa, 57.0 kDa, 50.0 kDa, 45.0 kDa, 40.0 kDa, 39.0 kDa, 37 kDa, 34.0 kDa, 33.0 kDa, 29.5 kDa, 22.0 kDa, and 21.87 kDa, respectively, as determined using sodium dodecyl sulfate- (SDS-) polyacylamide gel electrophoresis (PAGE) [4, 9, 11, 18, 23, 26, 27, 29–33, 54, 69–71].

2.2. Temperature and pH. Thermophilic lipases are usually optimally active between 60 and 80°C and some lipases are relatively thermostable with optimal active temperature of 40 to 60°C. For instance, TA lipase from Bacillus thermoleovorans ID-1, isolated from hot springs in Indonesia, had an optimal activity at 70–75°C and pH 7.5 [31]. TA lipase ID-1 exhibited 50% of its original activity after incubation at 60°C (for 60 min) and at 70°C (for 30 min) and its catalytic function was activated in the occurrence of Ca\(^{2+}\) or Zn\(^{2+}\) [31]. On the other hand, TA lipase from Bacillus sp. LBN2 had optimal activity of 60°C and pH 10 which was slightly different as compared to TA lipase ID-1 [9]. It is well-accepted that enzyme resistant to thermodenaturation is also resistant to extreme pH [68]. Thermostable lipase from Geotrichum candidum showed stability at varying range of pH (5–12) and thermostability (15–65°C) [71]. The lipase LBN2 was found to be stable in the pH range of 8–11 and retained 90% activity at 60°C for 1h [9]. TA lipase 3646 from the Cohnella sp. A01 expressed in E. coli BL21 (DE3) exhibited maximum activity at 70°C and pH 8.5 [18]. The TA lipase 3646 was also highly stable at the pH range of 8.5 to 10.0 for 180 min [18]. On the other hand, T. atroviride 676 lipase retained 78.9% of its original catalytic activity at 65°C and approximately 98% of its original activity at abrad range of pH values from 3.0 to 8.0 [33]. TA lipase from both Microbacterium sp. and Bacillus sp. RSJ-1 exhibited maximal hydrolytic activity at a temperature of 50°C and a pH of about 8.5 [23, 30]. Moreover, TA lipase RSJ-1 was very stable at 50°C for 60 min and retained 90% of its initial activity for 120 min [30]. However, its half-life (t\(_{1/2}\)) at temperature ranging from 55°C
to 75°C was decreased from 240 min to 30 min, respectively [30]. The TA lipase RSJ-1 was also highly stable in a pH range of 8.0 to 9.0 for 120 min [30]. Similarly, TA lipase from Acinetobacter sp. with sequence similar to GDGL family of lipases had an optimum temperature and pH of 50°C and 8.0, respectively [27]. The TA lipase from T. coremiiforme V3 which was cloned into plasmid pPICZ α A and overexpressed in P. pastoris X33 showed temperature tolerance at 60°C and retained about 45% of lipase activity at 70°C after 60 min [72]. In contrast, lipase from T. harzianum IDM41D had a relatively low thermotolerance (40°C) as compared to previously stated lipases [73]. The lipase IDM41D was stable at a pH range of 8.0–10.0 with an optimum enzymatic activity at pH 8.5 for 60 min [73]. The lipase from A. cinnamomea BCRC 35396 was found to be stable in pH 7–10 with optimum catalytic activity at pH 8.0 but had a very low activity at pH more than 10 [11]. The lipase BCRC35396 activity was also significantly stable at temperature range of 25–60°C, with maximal activity noted at 45°C [11]. Thermotolerance of TA lipase from B. sonorensis 4R was significant at 80°C due to its hyperthermophilic nature. This TA lipase 4R had a decreasing half-life (t1/2) pattern at temperature ranging from 80°C to 120°C for 150 min to 50 min, respectively [4, 45]. However, TA lipase 4R was highly activated and stabilized by the presence of Mg2+ which prolonged its t1/2 values at 80°C from 150 min to 180 min [4]. TA Lipase 4R was also highly active at pH 9.0 for 160 min [4]. On the other hand, TA lipase from T. thermophilus expressed in P. pastoris worked efficiently at pH range from 8.9 to 10.5 and showed its maximum activity at 8.0, respectively [27]. The TA lipase from T. coremiiforme V3 which was cloned into plasmid pPICZ α A and overexpressed in P. pastoris X33 showed temperature tolerance at 60°C and retained about 45% of lipase activity at 70°C after 60 min [72].

2.3. Salts, Metals and Inhibitors. Most TA lipases were also activated by the presence of several ion salts but greatly inhibited by some other heavy metals and chemicals. It has been reported that salts and metals ions have various effects on TA lipases catalytic activity. For instance, preincubation of TA lipase 4R with inorganic salts (MgSO4 and CaSO4) stimulated lipase activity by 249.94% and 30.2%, respectively [4, 19]. In contrast, the catalytic activity of TA lipase 4R was greatly reduced in the presence of other inorganic and metal salts [CoCl2, CdCl2, HgCl2, CuCl2, and Pb(NO3)2], enzyme inhibitors [phenylmethylsulfonyl fluoride (PMSF), orlistat], oleic acid, iodine, chelating agent [ethylene-diaminetetraacetic acid (EDTA)], and dissociating agent (urea) [4]. Unlike TA lipase 4R, TA lipase from Cohnella sp. A01 is not a metalloenzyme as there is no reduction in catalytic activity after treatment with EDTA [18]. On the other hand, the catalytic activities of both LipA and LipB lipases were completely inhibited by 10 mM PMSF and 10 mM EDTA [15]. Further metal ions analysis demonstrated that both LipA and LipB are metalloenzymes containing one Ca2+ and one Mn2+ ion per monomeric lipase unit [15]. LipA and LipB probably have a structural motif of Ca and Mn-binding site [17]. Moreover, thermal stability of both LipA and LipB is dependent on the addition of 1 mM Mn2+ which enhanced their catalytic activities at 96°C by 3-fold and increased the durations of their thermostability up to 4 h at 60°C and 75°C, respectively [15]. Ca2+ and Mn2+ also enhanced T. harzianum IDM41D lipase activity while other metallic ions did not give any effect [73]. TA lipase from T. thermophilus was also promoted by Ca2+ and inhibited by Zn2+ and Cu2+ [29]. The TA lipase from thermophilic Bacillus sp. RSJ-1 exhibited enhanced lipase activity in the presence of Ba2+, Na+, Mg2+, and Ca2+ and was greatly inhibited by Zn2+, K+, Co2+, and Cs+ [30]. The EDTA did not affect TA lipase activity from a thermophilic Bacillus sp. RSJ-1 [30]. The Microbacterium sp. lipase was completely inhibited by PMSF but minimal inhibition was observed when incubated with EDTA and dithiothreitol (DTT) which indicated the presence of metal ions and disulfide bridge (S-S) [23]. The reason that lipase being activated by some metal ions such as Ca2+ and Zn2+ was due to the presence of structural motif of Ca-binding EF-hand (i.e., consists of aspartate, glutamine, and glutamate amino acids residues) and Zn2+ -binding motif (i.e., consists of two histidine and two aspartic acid amino acids residues) which involves ionic and polar dipole interactions [17]. Some metal ions located a bit further from the catalytic amino acid residue and thus are not likely to contribute in the catalytic activity but rather possibly play a role in an improved stability [17].

2.4. Surfactants and Organic Solvents. It is well-known that enzyme resistant to thermodenaturation is also resistant to surfactants and organic solvents [68]. Microbacterium sp. lipase activity was stimulated by Triton X-100 and SDS and inhibited by polysorbate-20 (Tween-20) and polysorbate-80 (Tween-80) [23]. TA lipase from Acinetobacter sp. AU07 was inhibited by detergents like SDS and exhibited minimal loss of lipase activity when incubated with hydrogen peroxide (H2O2), Tween-80, and Triton X-100 [27]. The hydrolytic activity of the TA lipase from Acinetobacter sp. AU07 was specific to moderate chain fatty acid esters 4-nitrophenyl palmitate which was used as a substrate [27]. On the other hand, the presence of various oxidants, reductants, and some surfactants reduced TA lipase RSJ-1 activity [30]. Nevertheless, TA lipase from G. stearothermophilus was stable towards a variety of surfactants (i.e., Tween-20, Tween-80, Triton–X100, and SDS), anionic detergent (i.e., sodium cholate, sodium taurocholate), and oxidizing agents (i.e., H2O2, sodium perborate, and sodium hypochlorite) in which more than 82% of its relative activity was retained [26]. Lipases are normally less active in water-free solvents as compared to aqueous solution due to the restricted conformational flexibility and rigidification of enzyme conformations. However, TA lipase from
Lipases in their immobilized form usually enhance their cost-effective enzymatic process [23, 34]. Heterogeneous lipases that were found stable at high temperature were important for their catalytic activity [2, 32, 56]. Table 1 summarized the characteristic of TA lipases.

3. The Applications of TA Lipases

3.1. Biodiesel. The transesterification of vegetable oils to biodiesel using chemocatalyst (i.e., CuO/Zeolite) has been industrially implemented due to its very high conversion rates and short production time [38, 78–81]. However, this process is stable at relatively low temperature around 40°C [74]. Unlike TA lipases, commonly used lipases faced loss of catalytic activity at high temperature [77]. Moreover, immobilized lipases which were found stable at high temperature were employed for bioconversion of products which eventually add cost to production [74]. However, immobilized lipases were not suitable for single use application such as detergent. Moreover, the cost for immobilization is far expensive as compared to free lipases. In general, the suitability and major applications of TA lipases can be divided, but not limited, into three main industries (Table 2).

3.2. Biodetergent. In detergent, chemocleansing agents may be composed of a mixture of cationic [i.e., dodecyl dimethyl benzyl ammonium chloride (DDBAC)], anionic surfactants [i.e., linear alkylbenzene sulfonates (LAS), sodium dodecylbenzenesulfonate (SDBS), and SDS], nonionic surfactants [i.e., Tween 80, Triton X-100, and fatty alcohol polyoxyethylene ether (FAPOE)], and builders (i.e., phosphates, carbonates) [21, 35, 83]. Some of them are nonbiodegradable and toxic to aquatic organisms in which recent study showed that chemocleansing agents such as FAPOE and DDBAC at 1 μg/mL were toxic to aquatic zebrafish larval with defect in their locomotor activity and significant physical deformation [84]. Moreover, auxiliary sewage treatment removes only a small proportion of wastes (i.e., phosphorus) from the influent, while the bigger proportion remaining are released to stream, river, lake, and estuary via wastewater effluent [21, 84]. Therefore, TA lipases serve as an alternative to replace conventional chemocatalysts for laundry cleaning and dishwashing [35, 52, 53]. Studies have proven that incorporation of TA lipases in detergent does not just improve cleaning performance but also promotes environmental sustainability [35]. TA lipases are very stable at high temperature and alkaline environment which is the optimum condition required for maximal cleaning process. Most of them are also compatible with detergents components and resistant to inhibition [22, 24, 25, 50]. TA lipases are also available in their natural free form, soluble, and readily incorporated in liquid-based detergent [35, 83]. TA lipase from Bacillus LBN2 is found stable in some surfactants (i.e., ionic and nonionic) and commercial detergents at range of 0.1% to 10% (w/v) [9]. For instance, Triton x-100 and I44 stimulated the TA lipase activity up to 60% due to an increased number of turnovers of lipase by surface active agents [9]. This can be further thermal stability, mechanical strength, chemical-physical stability, lipophilic-lipophilic nature, amount of active lipase, renewability, and remaining functionality [82]. Examples of TA lipases that have shown their potential application for biodiesel production are TA lipases from Geobacillus thermodenitrificans AV-5, Microbacterium sp., and lipZ01 expressed in Pichia pastoris GS11 [23, 34, 44]. The purified lipase lipZ01 favourably hydrolsed triacylglycerols contain acyl chain lengths more than 8 carbon atoms, and the bioconversion amount of biodiesel production was almost 92% in a methanolysis process using MeOH and olive oil [44]. Biodiesel was produced via immobilized lipase Microbacterium sp. (immobilized on Celite and charcoal support) catalyzed methanolysis process, which enabled a yield of up to 95.1% biodiesel [23]. On the other hand, maximum yield of 94% of biodiesel can be achieved from alcoholysis of Jatropha oil in t-butanol using immobilized Enterobacter aerogenes lipase at an oil:MeOH molar ratio of 1:4, 50 U of biocatalyst/g of oil, and a t-butanol:oil volume ratio of 0.8:1 at 55°C after 48h of reaction time [47]. Immobilized lipase mediated transesterification of Simarouba glauca oil has been successfully carried out under n-hexane solvent system for maximum biodiesel production (91.5% fatty acid methyl esters) which is considered as an economical process and facilitates lipase reusability via sustainable approach [48].
### Table 1: Characteristics of TA lipases.

| TA lipases                | MW   | T (°C) | pH  | Stimulants        | Inhibitors       | Chemicals with tolerance                  | References |
|---------------------------|------|--------|-----|-------------------|------------------|-------------------------------------------|------------|
| *Pseudomonas* sp. lipase  | -    | 90     | 11  | -                 | -                | -                                         | [21]       |
| BSK-L lipase              | -    | 30–60  | 8   | Mn^{2+}, K^{+}, Zn^{2+}, Fe^{3+}, Ca^{2+} | -                | SURF, oxidants, detergents                | [22]       |
| *Microbacterium* sp. lipase | 40.0 | 50     | 8   | Tri-X-100, SDS    | PMSF, EDTA, DTT, Tw-20, Tw-80 | -                                         | [23]       |
| PS3 lipase                | 31.4 | 55     | 7   | Tri-X-100, Mg^{2+} Ca^{2+} | M+ (A), SDS, EDTA, CTAB, Tw-80, glycerol | Solvents (A)                                    | [24]       |
| S. *aureus* lipase        | -    | 60     | 12  | -                 | -                | -                                         | [25]       |
| S. *pasteuri* lipase      | 56.0 | 50     | 9.0 | -                 | -                | -                                         | [8]        |
| *B. steaothermophilus* lipase | 67.0 | 55     | 9–13| Mg^{2+}           | -                | SURF, oxidants, nonpolar solvent          | [26]       |
| BCRC 35396 lipase         | 60.0 | 25–60  | 7–10| -                 | -                | -                                         | [11]       |
| *Acinetobacter* AU07 lipase | 45.0 | 50     | 8   | -                 | -                | -                                         | [27]       |
| LipB lipase               | 57.0 | 96     | 8   | Mn^{2+}, Ca^{2+}  | PMSF, EDTA       | -                                         | [15]       |
| LipA lipase               | 50.0 | 96     | 8   | Mn^{2+}, Ca^{2+}  | PMSF, EDTA       | -                                         | [15]       |
| Lip 256 lipase            | 33.0 | 80     | 9   | Na^{+}, Fe^{3+}, K^{+}, Fe^{2+}, Sr^{2+} | Ca^{2+}, Mg^{2+}, Cu^{2+}, Solvent (D) | Glycerol, MaCN, pyridine, urea             | [28]       |
| *T. thermophilus* lipase  | 39.0 | 40–70  | 9.5 | Ca^{2+}           | Zn^{2+}, Cu^{2+} | -                                         | [29]       |
| RSJ-1 lipase              | 37.0 | 50     | 8–9 | Ca^{2+}, Na^{+}, Mg^{2+}, Ba^{2+} | Ca^{2+}, K^{+}, Co^{3+}, Zn^{2+} | EDTA                                        | [30]       |
| ID-1 lipase               | 34.0 | 70–75  | 75  | Ca^{2+}, Zn^{2+}  | -                | -                                         | [31]       |
| LBN2 lipase               | 33.0 | 60     | 10  | -                 | -                | -                                         | [9]        |
| Cohnella sp. A01 lipase   | 29.5 | 70     | 8.5 | -                 | -                | EDTA                                      | [18]       |
| 4R lipase                 | 21.9 | 80     | 9.0 | Mg^{2+}           | M+ (B), PMSF, orlistat, OA, I^{−}, EDTA, urea | -                                         | [4]        |
| H7 lipase                 | 22.0 | 90     | 9   | Ca^{2+}, Co^{3+} and Zn^{2+} | -                | -                                         | [32]       |
| *T. atroviride* 676 lipase | -    | 65     | 3–8 | -                 | -                | Solvents (B)                              | [33]       |
| AV-3 lipase               | 50.0 | 65     | 9   | -                 | -                | -                                         | [34]       |
Table 1: Continued.

| TA lipases                  | MW  | T (°C) | pH   | Stimulants                  | Inhibitors       | Chemicals with tolerance | References |
|-----------------------------|-----|--------|------|-----------------------------|------------------|--------------------------|------------|
| BTS-3 lipase                | 31.0| 55–70  | 8–10.5| K$$^+$$, Fe$$^{3+}$$, Hg$$^+$$, Mg$$^{2+}$$ | Al$$^{3+}$$, Co$$^{3+}$$, Mn$$^{2+}$$-Zn$$^{2+}$$ | -           | [35]       |
| CCR11 lipase                | 11.0| 60     | 9-10 | Ca$$^{2+}$$, Tri-X-100      | PMSE, SDS, Tw-80, Tw-20, butanol | -           | [36]       |
| T. lanuginosus lipase In1    | 33.0| 60–70  | 8–12 | Ca$$^{2+}$$                  | Fe$$^{2+}$$, Zn$$^{2+}$$, K$$^+$$, Ag$$^+$$ | -           | [37]       |
| B. thermocatenulatus lipase | 16.0| 60–70  | 7.5–8| -                           | -                | -                        | [38]       |
| HF544325 lipase             | 27.0| 45     | 8    | -                           | -                | -                        | [39]       |
| S. arlettiae JPBW-1 lipase  | 45.0| 30–90  | 7–12 | Mn$$^{3+}$$, Ca$$^{3+}$$ and Hg$$^{2+}$$ | K$$^+$$, Co$$^{3+}$$, Fe$$^{3+}$$ | Solvents (C), Tw-80, Tw-40, EDTA | [16, 40]   |
| Bacillus sp. A30-1 lipase   | 65.0| -      | -    | -                           | -                | -                        | [41]       |
| Staphylococcus aureus       | 25.0| 52     | 11   | Ca$$^{2+}$$ and Tween-80    | SDS              | -                        | [42]       |
| Lipase L2                   | 43.0| 70     | 9    | Ca$$^{2+}$$, K$$^+$$, Na$$^+$$, Mn$$^{2+}$$ | EDTA, PMSE, pepstatin A, BME, DTT | -           | [17, 43]   |

Note. MW, molecular weight; T, optimal or favourable temperature of enzyme activity; OA, oleic acid; Tw-20, Tween-20; Tw-80, Tween-80; SURE, surfactants; BME, 2-mercaptopropanol; solvent (A): methanol, ethanol, acetone, benzene, chloroform, xylene; solvent (B): benzene, xylene, n-hexane, methanol, ethanol, and toluene up to 30%; solvent (C): kerosene, n-dodecane, and hexane; solvent (D): acetone, MeOH, trichloromethane, petroleum ether, hexane, isopropanol, DTT, EDTA, polyhexamethylene biguanide, DMSO, benzene, Tri-X-100, Tw-20, Tw-80, SDS; M+ (A): Cu$$^{2+}$$, Fe$$^{3+}$$, Zn$$^{2+}$$, and Co$$^{3+}$$; M+ (B): Co$$^{3+}$$, Cd$$^{2+}$$, Hg$$^{2+}$$, Cu$$^{2+}$$, and Pb$$^{2+}$$; I$$^-$, iodine.
Table 2: Industrial application of TA lipases.

| Fields         | Process                                           | Products            | Microbial origin of lipases                                                                 | References          |
|----------------|---------------------------------------------------|---------------------|-------------------------------------------------------------------------------------------|---------------------|
| Renewable energy | Transesterification of oils/alcoholysis/methanolysis/interesterification | Biodiesel           | Geobacillus thermodemirificans AV-5, Microbacterium sp., lipZ01 expressed in Pichia pastoris GS11, Brevibacterium halotolerans PS4, Talaromyces thermophilus, Bacillus stearothermophilus, Thermosyntropha lipolytica, Staphylococcus aureus Al.A1, Bacillus methylotrophicus PS3, Staphylococcus aureus SAL3, Bacillus sp. BSK-L, Geobacillus zalihae | [23, 34, 44–48] |
| Laundry/dishwashing | Hydrolysis of lipid/ester bonds                  | Biodetergent        | Staphylococcus aureus AL.A1, Bacillus methylotrophicus PS3, Staphylococcus aureus SAL3, Bacillus sp. BSK-L, Geobacillus zalihae | [15, 19, 20, 22, 24–26, 39, 49–53] |
| Leather        | Hydrolyse grease or fat from leather             | Biodegreasing agent | Geobacillus thermoleovorans DA2, Staphylococcus aureus, Staphylococcus arlettae JPBW-1         | [42, 54, 55]       |
| Cosmetic       | Esterification of fatty acids and other compounds | AG, plasticizer, fatty acids, and esters | Thermomyces lanuginosus, Rhizomucor miehei, Pseudomonas cepacia, Candida antarctica               | [5, 6, 56]         |
| Food           | Transesterification/interesterification/acidolysis and esterification | AG, plasticizer, fatty acids, and esters | Rhizomucor miehei, Rhizopus oryzae NRRL 3562, Candida antarctica                         | [6, 16, 47, 57]    |
| Bioremediation | Hydrolysis of oils                               | Biodegrading agent   | Staphylococcus pasteurii, Ochrobactrum intermedium strainMZV101, Bacillus sonorenensis 4R, Aspergillus ibericus, Aspergillus awam, Aspergillus niger | [4, 41, 44, 58] |
| Pharmaceutical | Transesterification/aminolysis                   | Plasticizer and drugs | Candida antarctica, Candida rugosa                                                        | [2, 6]              |

Note: AG, acylglycerols.
explained by a decrease in the surface tension of the aqueous phase which maintains the open conformation of lipase and facilitates substrate binding to catalytic site of the lipase that improves its activity [9]. However, TA lipase from Bacillus LBN2 was inhibited by Tween-40 and Tween-80 after 1 h of incubation at 50 °C [9]. On the other hand, lipase from B. thermoleovorans was strongly inhibited by SDS which may be due to the alteration of the active site conformation of the lipase molecular structure that resulted in inhibition and partial inactivation [31, 54]. On the contrary, TA lipase from Bacillus steatotherephillus showed its stability and compatibility with commercial detergents [26]. BSK-L lipase was very stable when formulated in detergent and its components such as oxidising agents and surfactants, hence, help to improve detergent effectiveness and washing performance in removing oil stains [22]. In dishwashing experiment, T1 lipase was proven to be stable in most nonionic surfactants and a mixture of sodium carbonate (Na₂CO₃) and glycine (Gly) [52, 53].

3.3. Biodegreasing. In leather industry, enzymes are necessary to facilitate process and improve leather quality during different stages in leather manufacturing, such as dehairing, curing, soaking, dining, picking, tanning, and degreasing [3]. Enzyme reduces discharges and waste disposed from different stages of leather manufacturing which has been reported to give health hazards [3]. Being thermostable and alkali-tolerant, TA lipases are also suitable as biodegreasing agents in leather industry where they remove pitch and excessive oil from leather, improving the quality of leather [12]. Examples of TA lipases that have manifested their potential application as biodegreasing agent are Geobacillus thermoleovorans DA2 and Cohnella sp. A01 lipases [54]. Hide and skin contain proteins and fat in the collagen fibers which must be removed prior to tanning process. Lipases specifically degrade fats, unlike damaging the leather structure [41]. Removing fats via lipases-degreasing process will minimise hazard and environmental pollutions. For leather made of bovine hide and sheepskins, lipases allow improvement of its tensile strength [41].

3.4. Natural Flavour and Pharmaceutical. TA lipases also act as biocatalyst for the synthesis of several flavour esters such as isoamyl acetate via transesterification process of short chain carboxylic acids (i.e., vinyl acetate) and alcohols (i.e., isoamyl alcohol) [85, 86]. Flavour esters give a “Natural” taste, odour, and smell for prospect application in food commodity. For example, immobilized lipase-mediated transesterification of isoamyl acetate and other flavour esters under solvent and solvent-free environment is considered as economical biotechnological approach for flavour ester production via continuous mode of operation [85, 86]. A substantially high isoamyl acetate production (95%) was obtained via enzymatic synthesis of isoamyl alcohol with vinyl acetate using immobilized Rhizopus oryzae NRRL 3562 lipase at 8 h reaction time [86]. In the application of TA lipases for pharmaceutical commodity, immobilized lipase from Candida rugosa has also been used to synthesize lovastatin, a drug which reduces serum cholesterol levels [41]. Lipases may be used as digestive aids and as the activators of Tumor Necrosis Factor (TNF) to treat malignant tumors [41]. The production of fatty alkanolamides in a solvent-free enzymatic process via amino-lysis of linoleyl ethyl ester with several amino-alcohols leads to the development of chemoselective synthesis of new active molecules for cutaneous application [87]. Candida antarctica lipase B has been used for by the synthesis of the serotonin reuptake inhibitor (S)-(+) -cericlamine [88].

4. Source of TA Lipases

4.1. Wild Strains. Although lipases are ubiquitous, the organisms producing TA lipases are rarely isolated. The nature’s genetic reservoir which is the main sources for TA lipases could be identified via functional screening or from the DNA extracted from previously unknown organisms using bioinformatic [17]. Mostly, thermophilic microorganisms are very rich and important sources for TA lipases and they are normally isolated from the soil of areas with special conditions. For example, several extracellular TA lipases have been identified from Gram positive bacterial strains such as Geobacillus thermoleovorans (i.e., strain DA2, ID-1), Geobacillus thermodenitrificans (i.e., IBRL-nra, AV-5), B. steatotherephillus, Cohnella thermotolerans, and Thermosyntropha lipolytica DSM 11003 [12, 26, 54]. These thermophilic organisms grow optimally at temperature between 50 and 80 °C.

Some other extracellular TA lipases have been also identified from mesophiles. For instance Aeribacillus sp. SSL096201, Bacillus sp. LBN 2, B. sonorensis 4R, B.licheniformis H7, B. pseudofirmus, B. odyssey, B. pumilus HF544325, and Microbacterium sp. [4, 9, 23, 32, 59, 60]. Gram negative bacterial strains also produce TA lipases such as Acinetobacter sp. (i.e., strain AU07, BK44), Pseudomonas sp., and Enterobacter sp. Bni2 [21, 27, 61]. On the other hand, fungus strains producing TA lipases such as Talaromyces thermophilus, Thioderma atroviride 676, Curvularia sp. DHE 5, and Antrodia cinnamomea BCRC 35596 have been reported [11, 20, 29, 33, 57]. Most of TA producing strains were isolated from several places like desert ecosystem, hot spring, oil-contaminated soil, soil samples of olive oil mill, mushroom spring, alkaline hot spring, pulp and paper mill effluent sludge, alkaline lake, and soil of slaughter house [6, 39, 80]. Table 3 summarized the wild-type (WT) strain of TA lipases.

On the other hand, extremophiles such as hyperthermophiles are also sources for TA lipases. They grow optimally at high temperatures between 80 and 110 °C and some even reached up to 113 °C [46, 89]. Only represented by few bacterial and archaean species, these microorganisms have been isolated from marine and terrestrial hot ecosystems. TA lipases from hyperthermophiles developed a special structure-function relationship of high thermal stability and optimum activity at temperatures greater than 70 °C [89]. Some of these enzymes are active and thermostable at temperatures up to 110 °C and above [89]. Active at very high temperatures, hyperthermophilic enzymes normally do not work well at temperatures below 40 °C [46]. For instance, both B. sonorensis 4R and Bacillus sp. HT19 produced hyperthermostable alkaline lipases [4, 28].
| Type               | Microorganisms                        | Origin                                      | References |
|-------------------|---------------------------------------|---------------------------------------------|------------|
| Hyperthermophile  | *B. sonorensis* 4R                    | Thar desert ecosystem                       | [4]        |
|                   | *Bacillus* sp. HT19                   | Hot spring                                  | [28]       |
|                   | *Geobacillus thermoleovorans* DA2     | desert/hot springs of Southern Sinai        | [54]       |
|                   | *Geobacillus thermoleovorans* ID-1    | Water/soil of the thermal springs           | [31]       |
|                   | *Cohnella thermotolerans*             | Alkaline Lunar Lake                         | [18]       |
| Thermophile       | *Thermosyntrophus lipolytica* DSM 11003| Alkaline hot springs of Lake Bogoria        | [15, 39]   |
|                   | *B. stearothermophilus*               | Olive oil mill                              | [26]       |
|                   | *Staphylococcus aureus*               | Barbeque shop soil                          | [42]       |
|                   | *S. aureus* ALA1                     | Dromedary milk                              | [49, 51]   |
|                   | *Staphylococcus pasteuri*             | Oil mill effluent                           | [8]        |
|                   | *B. sibtilis* BSK-L                   | Soil                                        | [22]       |
|                   | *Pseudomonas* sp.                     | Sludge/sediment of Pulp and Paper Mill      | [23]       |
|                   | *Acinetobacter* sp.                   | Distillery waste                            | [27]       |
| Mesophile         | *Aerobic* sp. SSSL096201              | Lonar lake water                            | [59]       |
|                   | *Bacillus* sp. LBN 2                  | Soil sample of hot spring                   | [9]        |
|                   | *B. methylotrophicus* PS3             | Soil of service station                     | [50]       |
|                   | *B. licheniformis* H7                 | Soil of the Hayran thermal springs          | [32]       |
|                   | *B. pumilus* HFS44325                 | Tannery waters in the old medina of Fez     | [60]       |
|                   | *Staphylococcus arlettae* JPBW-1      | Rock salt mine                              | [55]       |
| Gram negative     | *Ochrobactrum intermedium* MZV101      | Gheynarje Nir hot spring                    | [58]       |
| Mesophile         | *Enterobacter* sp. Bn12               | Soils/wastewaters from leather/edible oil industries | [61] |
|                   | *Acinetobacter* sp.                   | Distillery waste                            | [27]       |
|                   | *Pseudomonas* sp.                     | Local compost                               | [21]       |
| Fungus            | *Talaromyces thermophilus*            | Soil from thermal station                   | [20, 29]   |
|                   | *Trichoderma asperelliforme* 676      | Amazon forest soil                          | [33]       |
|                   | *Curvularia* sp. DHE 5                | Soil samples from Mit Ghamr                 | [57]       |
| Mesophile         | *Antrodia cinnamomea* BCRC 35396      | Taiwan Bioresources Collection              | [11]       |
|                   | *Aspergillus* sp. strain O8           | Diesel contaminated soil                    | [62]       |
4.2. Mutant Strains. Several TA lipases-producing strains have been improved via mutagenesis to obtain strains capable of producing a substantially high amount of TA lipases and even more stable at higher temperature and alkaline pH as compared to WT strains. For instance, mutant strain *P. cyclopium* has been developed using methods of error-prone PCR-direct evolution and site-directed mutagenesis with an improved thermal stability of alkaline lipases (i.e., L41P, G47I, and PCL) [90]. The mutant lipases exhibited an optimal temperature of 5°C higher than the WT strain and half-life of 7 to 13-fold higher than that of WT strain at 45°C [90]. The mechanism responsible for the thermal stability improvement was elucidated from the lipase structure related to leucine-41-proline and glycine-47-isoleucine that could stabilize the PCL structure via hydrophobic-hydrophilic interactions, helix propensity, and proline substitution. The thermal stability for D31I lipase (70.6°C) was higher than WT T1 lipase (68.5°C) [56]. The stability of T1 lipase from *G. zahihae* strain was improved via insertion of an extra ion pair at the intraloop and the interloop mutant D311E via site-directed mutagenesis [52, 56]. On the other hand, mutant S385E lipase which was developed via amino acid substitution strategy has also been reported. It had an increased duration of thermostability and even higher optimal temperature (80°C in Tris–HCl pH 8) as compared to other mutants and WT lipase (70°C in Gly–NaOH pH 9) [43].

4.3. Recombinant Strains. TA lipases production was also improved through recombinant techniques. For example, TA lipase TTL gene from *T. thermophilus* was successfully expressed in *Pichia pastoris* with an enhanced extracellular production [29]. The recombinant plasmid pPIC9K-TTL was constructed by inserting TTL gene into the downstream of AOX1 promoter and α-factor, which was then transformed into *P. pastoris* via electroporation, producing more than 200 positive transformants [29]. The TA lipase gene from *T. coremiiforme* V3 was also cloned into plasmid pPICZαA and overexpressed in *P. pastoris* X33 [91]. Another lipase gene like Gene lipZ01 was expressed in *P. pastoris* G515 to produce 42 U/mL recombinant lipase LipZ01 (50 kDa) with maximum activity at optimal temperature and pH value of 45°C and 8.0, respectively [44].

On the other hand, lipase 3646 gene from *Cohnella* sp. A01 was constructed using pET26b(+) vector and expressed in *E. coli* BL21 (DE3) in which the purified lipase exhibited maximal activity at 70°C and pH 8.5 [18, 76]. Lip 42 gene from *Bacillus* sp. 42 was also cloned in *E. coli* BL21 (DE3) [92]. Some recombinant proteins produced by *E. coli* have few drawbacks such as intracellularly produced lipases and therefore the production is normally proportional and limited by the number of cells [92, 93]. When it comes to downstream processing, extracellular lipases may be preferred as compared to intracellular lipases due to the tedious cell disruption process in order to release the enzymes. Compared with *E. coli* and *S. cerevisiae*, the methylotrophic yeast *P. pastoris* has several benefits as a host for the biomanufacture of recombinant heterologous enzymes, such as easiness of genetic manipulation, high cell density, elevated levels of productivity, the capability to carry out multifaceted posttranslational modifications, and very small secretion levels of endogenous proteins [91].

5. Biomanufacturing

5.1. Inoculums Size. In general, inoculums size can range from 1 to 5% (v/v) for optimum TA lipase production depending on strains and fermentation medium (Table 4). For bacterial strain, inoculums were normally prepared using 6 to 16 h old culture before inoculated into basal medium. For instance, inoculums size of 5% (v/v) of a 12 h old culture of *T. lipolytica* was used to produce extracellular TA lipase. Inoculums size of 5% (v/v) of a 12 to 16 h old culture of recombinant *E. coli* BL21 harbouring lip42 gene has been also reported. In response of surface methodology (RSM) optimization study, a very low inoculums size was suggested for the production of lipase from *Acinetobacter* sp. AU07 (0.44%, v/v) [27]. Meanwhile, the production of TA lipase from *B. sonorensis* 4R and *G. thermoleovorans* DA2 was conducted at inoculums size of 5% (v/v) and 4% (v/v), respectively [4, 12]. It has been reported that *Bacillus* sp. LBN2 at inoculums size of 10^6^ cells per mL was inoculated into medium for maximal for lipase activity [9]. For fungus strain, inoculums were normally prepared using 2 to 10 d old culture before being inoculated into basal medium. For example, *P. chrysogenum* and *A. flavus* strains were prepared using 10 d old culture with 10^6^ spores per gram dry substrate (gds) in solid state fermentation (SSF) [41, 94]. In another study, 1 × 10^7^ spores per mL of *A. niger* J-1 and *A. terreus* NCIFT 4269.10 from a 7 d old culture were used as inoculums [95]. A relatively low inoculums size of 1% (v/v) of filamentous fungi *T. atroviride* 676 has been suggested [6, 33]. It is essential to determine the optimum inoculums size for optimal number of active microbial cells needed for TA lipases production. Large inoculums size not only can cause overproduction of microbial mass and inefficient mass transfer but also is unsuitable for scale up process and production in large bioreactor.

5.2. Carbon and Nitrogen Sources. A variety of carbon and nitrogen containing substrates could be used as carbon and nitrogen sources for TA lipases fermentation. The type, amount, and ratio of carbon and nitrogen sources are important for microbial growth and optimal production of TA lipases. The most favourable carbon sources for TA lipase production by various thermophilic microbes are varied. Olive oil containing large percentage of monounsaturated fats was mostly used as carbon source and in some cases as an inducer for lipase production. For instance, TA lipase was optimally produced by *B. licheniformis* Ht7, *A. cinnamomea* BCRC 35396, *B. thermoleovorans* ID-1, *Bacillus* sp. IH1-91, and *T. atroviride* 676 using 1–5% olive oil as carbon source in 24 h to 168 h fermentation [11, 33]. This is potentially due to TA lipase substrate specificity to unsaturated fatty acid. In addition to this, isolate *B. thermoleovorans* ID-1 could also cultivate on several lipid substrates such as oils (i.e., olive, mineral, and soybean oil), emulsifiers (i.e., Tween-20, Tween-40, and Tween-66), and triglycerides (i.e., triolein, tributyrin). Another lipase production by *Bacillus* species like
| Strain/origin | IS (%) | Medium composition (%), w/v or v/v | Temp (°C), agitation (rpm) | pH | Other minerals (%), w/v or v/v | Duration (h) | Activity (U/mL or other units) | References |
|--------------|--------|-----------------------------------|---------------------------|----|--------------------------------|-------------|-------------------------------|------------|
| B. stearothermophilus | 1 | Xylose, 1; peptone, 1; olive oil, 1 | 55, 200 | 11 | MgSO₄, Tw-80 | 48 | 1800–2500 | [26] |
| Geobacillus sp.DA2 | 4 | Galactose, 1; (NH₄)₂PO₄, 0.5 | 60, 120 | 10 | - | 48 | 1000 | [54] |
| Bacillus sp.ID-1 | - | Olive oil, 1.5 | 65, - | - | - | 12 | 520 (IU) | [31] |
| C. thermotolerans | 5 | Maltose, 1; starch, 1; NB; tributyrin, 1 | 60, 120 | - | - | 72 | - | [18] |
| B. methylophilus | 10 | Xylose, 1; YE, 1; olive oil, 1 | 30, - | 7 | Ca³⁺; Tw-80, 1 | 72 | 360 (IU/mL) | [50] |
| S. aureus ALA1 | - | Coconut oil mill waste, 2 | 35, 120 | 7 | NaCl, 0.2; olive oil, 1 | 24 | 12 | [52] |
| S. pasteurii | 2 | Olive oil, 1 | 70, - | 8 | - | 7 | 1339 (μM/min) | [59] |
| B. subtilis BSK-L | 1 | Yeast extract, 0.5; BE, 0.5; peptone, 1 | 37, 200 | 9 | NaCl, 7 | 48 | 20 | [8] |
| Microbacterium sp. | - | Tributyrin, 1; NaNO₃, 0.0085; Fe(CH₃COO)₃, NH₄, 0.005 | 37, - | 8.5 | MgSO₄, 0.02; KH₂PO₄, 0.68; Na₂HPO₄, 2H₂O, 0.78 | 72 | 355 (U) or 3.2 (U/mg) | [23] |
| Aericibacillus sp. SSL | - | Yeast extract, 1; olive oil, 1 | 70, - | 8 | - | 44 | 6000 | [64] |
| Bacillus sp. LBN 2 a | 1 | Soybean oil, 0.5 | 35, 100 | 8 | - | 3 | - | [55] |
| B. sonorensis 4R | 5 | Tween-80, 1; glucose, 1; (NH₄)₂SO₄, 0.2 | 80, - | 9 | K₂HPO₄, MgSO₄, NaCl, CaCO₃, CaSO₄, FeSO₄, MnCl₂, ZnCl₂ (2 to 0.001) | 96 | 177 (U/mg) | [4] |
| B. licheniformis H7 | - | Olive oil, 1 | 30, - | - | - | 24 | 15.9 (U/g) | [32] |
| B. coagulans BTS-3 | - | Mustard oil, 1; yeasts: YE, 1; BE, 1.5 | 55, - | 8.5 | - | 48 | 116 | [63] |
| B. thermoleovorans | 1 | NB, 0.325; olive oil, 2.5 | 55, 150 | 6.5 | CaCl₂, 0.1%; gum Arabic, 1 | 44 | 6000 | [64] |
| B. pumilus | HF544325 | Casein peptone, 1.7; YE, 0.5 | 37, 200 | 7.4 | Glucose, 0.25 | 72 | 14 | [60] |
| S. arlettae JP-BW-1 | 10 | Soybean oil, 5 | 35, 100 | 8 | - | 3 | - | [55] |
| Pseudomonas sp. b | 2 | Glucose, 1; NH₄Cl, 0.5; ECO, 2; (NH₄)₂HPO₄, 0.3 | 37, 250 | 7 | K₂HPO₄, 0.3; KH₂PO₄, 0.1; MgSO₄, 7H₂O, 0.01 | 24 | 24 (U/mg) | [21] |
| O. intermedium | - | Yeast extract, 0.5; olive oil, 1; NH₄Cl, 0.1 | 60, 180 | 10 | MgSO₄, 7H₂O, FeSO₄, 7H₂O, CaCl₂, 7H₂O, K₂HPO₄ (1 to 0.1) | 72 | - | [65] |
| T. thermophilus | - | Tributyrin - olive oil emulsion | 50, - | 9.5 | - | 73–10 (kU/mg) | [20, 29] |
| T. atroviride 676 | 1 | Tween-80, 0.5; olive oil, 1; YE, 15 | 28, 305 | 6 | (NH₄)₂SO₄, 0.25; MgSO₄, 0.2 | 4 d | 175.20 | [33] |
| Curvularia sp. DHE | d | Wheat bran, 1 | 30, - | 7 | Olive oil, 2; KCl, 0.05; MC, 70 | 7 d | 83.4 (U/g) | [57] |

Note: IS, inoculum size; NaCl, sodium chloride; K₂HPO₄, potassium hydrogen phosphate; KCl, potassium chloride; Na₂S·9H₂O, sodium sulphide; NH₄Cl, ammonium chloride; MgCl₂, 6H₂O, magnesium chloride hexahydrate; CaCl₂, 2H₂O, calcium chloride dehydrate; NaHCO₃, sodium bicarbonate; Na₂CO₃, sodium carbonate; C₂H₃NO₂S, cysteine; (NH₄)₂PO₄, ammonium phosphate; YE, yeast extract; BE, beef extract; a, 10⁴ cells/mL; ECO, emulsified coconut oil; b, 14 h old culture (1.3 × 10⁹ cell/mL); MC, moisture content; SSF, solid-state fermentation; c (10⁶ spores/mL); d (51.27 × 10⁹ spore/mL).
B. coagulans BTS-3 and Staphylococcus aureus was obtained in 48 h using mustard oil as carbon source, probably due to high monounsaturated fats in this oil [42, 60]. TA lipase was also produced by anaerobic T. lipolytica DSM 11003T which was grown in a basal medium containing 0.75% (v/v) yeast extract under nitrogen gas phase (anaerobic condition) [15]. TA lipase production was optimally produced by G. thermoleovorans DA2 in a medium containing dissociacharide galactose (1%, w/v) and ammonium phosphate ((NH₄)₂PO₄, 0.5%, w/v) at carbon/nitrogen ratio of 2:1 [1].

In RSM optimized TA lipase production by T. atroviride 676, a very high yield of TA lipase (101.75 U/ml) could be obtained using optimal concentration of olive oil and yeast extract as carbon and nitrogen source, respectively [33]. Organic nitrogen source like yeast extract improved not only TA lipase production by T. atroviride 676 but also TA lipase production by S. maltophilia and P. gessardii, which may be due to the metabolic suitability and effective mass transfer of vitamins to the microbes [33]. The production of TA lipases from T. atroviride 676, G. steaerothermophilus, and P. gessardii was less enhanced by addition of inorganic nitrogen sources [i.e., ammonium sulfate, (NH₄)₂SO₄] [26, 33]. In one-factor-at-a-time (OFAT) study, a maximum TA lipase from G. steaerothermophilus was produced using xylene (C₈H₁₀O₂) as carbon source, followed by glucose (C₆H₁₂O₆) and sorbitol (C₆H₁₂O₆). Other carbon sources such as galactose (C₆H₁₂O₆), mannitol (C₆H₁₄O₆), starch (C₆H₁₀O₅)n, lactose (C₁₂H₂₂O₁₁), and sucrose (C₁₂H₂₂O₁₁) were not suitable for production of TA lipase from G. steaerothermophilus [26]. Starch concentration above 2% (w/v) reduced the lipase production, which could be attributed to the inhibition at high carbon concentration, viscosity, and low level of dissolved oxygen (DO) [34]. This has been supported by several works on reduction of lipase production by many sugars and complex substrates at higher concentrations [26, 34].

Peptone was found to be the most suitable nitrogen source for TA lipase from G. steaerothermophilus followed by tryptone, gelatine, skim milk, soy protein, and yeast extract [26]. Olive oil is the best source of oil which can act as an inducer for the production of lipase from Bacillus steaerothermophilus, followed by nut oil, sesame oil, sunflower oil, Tween-80, Triton-X-100, and Tween-20 [26]. High TA lipase from B. sonorensis 4R was obtained when glucose (10 g/L) and polysorbate (Tween-80, 10 mL/L) were used as carbon source under static conditions for 96 h fermentation time [4]. On the other hand, high TA lipase yield by B. Pumilus HF544325 can be produced using a mixture of peptone and yeast extract as nitrogen sources and glucose as carbon source [33, 60]. T. harzianum IDM14D was best cultivated using glucose and peptone as carbon and nitrogen sources, respectively, for maximal lipase production (0.24 U/mL) as compared to glucose and yeast extract mixture (0.15 U/mL) at 30°C for 7d fermentation time [47].

Moreover, lipases activity of some other fungi, such as B. cepacia, M. hiemalis, and A. wentii, was also stimulated by addition of glucose into the basal medium [15, 17, 46, 74]. Although TA lipase production from this strain is constitutive, the incorporation of oils in the medium increased its final yield. The yield was greater in a medium containing lipidic substrates such as oils as the carbon sources with an addition of organic nitrogen source, but, sometimes, lipase production was also repressed by polysaturated, long chain fatty acid (LCFA) and esters [17, 46, 74]. Castor oil and sesame oil induced lipase production better as compared to other lipid/oil sources. Castor oil contained about 90% ricinoleic acid (C₁₈H₃₁O₂) which induces and promotes lipase production [27]. RSM optimization for lipase production by Acinetobacter sp. AU07, suggests a significant increase in lipase yield using castor oil as an inducer (2.3%, v/v) to give the maximum lipase activity of 15.84 U/mL [27]. The chain length specificity of the TA lipase gene from T. coremiiforme V3 producing two types of LipA and LipB lipases exhibited an elevated activity with p-nitrophenyl laurate and LCFA glycerides such as trioleate (C18:1) tripalmitin (C16:0) where hydrolysis of ester bonds occurred at 1,3-positions [83]. Lipases show very low activity when substrates are in the monomeric form as lipases are activated at the water-lipid (oil) interface. The activity increases significantly when substrates form emulsions due to interfacial activation. This explains the necessity of emulsions for maximum lipolytic activity for the majority of lipases [42, 83].

5.3. Other Minerals. Additional minerals were required for TA lipase stability and to boost TA lipases production. Inorganic salt like MgSO₄ played a significant role on TA lipase production by T. atroviride 676, yielding an enzymatic activity of 101.75 U/ml [33]. TA lipase production by B. sonorensis 4R was enhanced with the presence of several ions using inorganic salt broth containing 0.001 to 2 g/L of dipotassium phosphate (K₂HPO₄), sodium chloride (NaCl), ammonium sulphate [(NH₄)₂SO₄], manganese chloride (MnCl₂), calcium carbonate (CaCO₃), ferric (II) sulfate (FeSO₄), magnesium sulfate (MgSO₄), zinc chloride (ZnCl₂), and calcium sulfate (CaSO₄) adjusted to pH 9.0 [4]. It was found that lipase stability of B. sonorensis 4R was enhanced when Mg²⁺ and mannitol were added to the fermentation medium [4]. T. lipolytica DSM 11003T lipase was grown in a basal medium containing 0.01% to 0.3% (w/v) NaCl, K₂HPO₄, Na₂CO₃, potassium chloride (KCl), sodium sulphide (Na₂S·9H₂O), ammonium chloride (NH₄Cl), magnesium chloride hexahydrate (MgCl₂·6H₂O), calcium chloride dehydrate (CaCl₂·2H₂O), sodium bicarbonate (NaHCO₃), semiessential proteinogenic amino acid, cysteine (C₅H₉NO₃S), and some vitamin and trace elements [15, 39]. Certain metal ions give positive effect to TA lipases synthesis and stability due to the presence of metal binding motif in its structure. Some other minerals such as phosphate ions and NaCl provide ATP synthesis and osmotic balance for cell growth and TA lipases production.

5.4. Fermentation Temperature. The optimum growth condition (i.e., temperature, pH, etc.) of microbial fermentation may not necessarily be the best condition for highest lipases production even though studies have shown that microbial growth is proportionally increased with TA lipases production and activity. Temperature regulates and gives an effect to the lipase synthesis at mRNA transcriptional regulation of lipase gene and also translation levels of lipase proteins.
This involves several other proteins along the way such as regulatory protein, polymerase (i.e., the expression of gene), and helper protein (i.e., assist in periplasmic lipase folding) [24, 41, 89, 96]. High production of several TA lipases was observed at elevated temperature ranging from 28 to 37°C. It has been reported that TA lipase production was best produced at 30°C by T. harzianum, A. radioresistens, and A. calcoaceticus LP009 [47]. The optimum growth condition and lipase production by T. atroviride 676 and B. Pumilus were at 28°C and 37°C, respectively [33]. Under optimized condition, high lipase production by Acinetobacter sp. AU07 was at temperature of 34°C [27]. Higher temperature for microbes mentioned above was not however favourable for TA lipases production, probably due to denaturation of regulatory peptides and proteins, responsible for lipases production. High temperature may also disturb the stability of cell membranes structure as not all microbes have a heat-tolerant cell membrane [41]. Production of TA lipases at these temperatures (28–37°C) has some advantages such as low energy consumption (i.e., due to moderate temperature). Studies show that optimal temperature for production of TA lipase may not correlate to the temperature of the TA lipases activity, which is normally active and stable at a higher temperature.

Other TA lipases are highly produced at high temperature and this includes TA lipase production by B. steatothromophilus, which was maximally produced at 55°C [26]. A very high TA lipase production by B. sonorensis 4R was reported at 80°C while G. thermodenitrificans AV5 and Bacillus sp. LBN2 were obtained at 50°C [4, 9]. B. thermoleovorans 1D-1, on olive oil (1.5%, w/v) as the sole carbon source, grew very rapidly at 65°C with its specific growth rate (μmax) of 2.5 per hour and maximal lipase activity of 520 U/L [28]. Bacillus sp. strain IHI-91 also grew optimally at 65°C on olive oil with a μmax of 1.0 per hour and maximum lipase productivity of 340 U/L/h [31]. High TA lipase was significantly produced by T. lipolytica DSM 11003T at 60°C [9, 15]. Most of the microbes are thermophiles or hyperthermophiles and capable of withstanding and growing at a very high temperature. High TA lipases at these temperatures are usually related to high kinetic rate and mass transfer rate (MTR) at high temperature [82]. An elevated temperature may influence their secretion, possibly by changing the physical properties of the cell membrane, thus allowing more secretion of extracellular lipases [56].

5.5. Agitation Rate. In shake flask culture (SFC), agitation rates between 100 and 250 rotations per min (rpm) have been reported for the production of TA lipases. For instance, TA lipase production by T. atroviride 676 at agitation rate of 105 rpm and G. thermoleovorans DA2 at agitation rate of 120 rpm can reach a maximum production of 175.20 U/mL and 1021.91 U/mL, respectively [1, 33]. The production of TA lipase Acinetobacter sp. AU07 can be optimized at agitation rate of 150 rpm [3, 27]. Bacillus sp. LBN2 in fermentation medium was agitated at 200 rpm for 48 h to give about the highest TA lipase activity of 16 U/g [9]. The lipase production by B. pumilus and T. harzianum LDM14D was incubated aerobically at 200 rpm and 150 rpm, respectively, at a temperature of 37°C/30°C for 3 to 7 d on a rotary incubator shaker [60]. RSM optimization for lipase production by Acinetobacter sp. AU07 with an agitation rate of 199 rpm gave the maximum lipase activity of 15.84 U/mL [27]. Study of agitation rate in SFC serves as a basis for TA lipases production in bioreactor. Due to geometry difference of shake flask and bioreactor, the effect of agitation rate on lipases production was normally reevaluated in bioreactor. Furthermore, in bioreactor, such as stirred tank bioreactor (STB), agitation rate is controlled by one or more impellers. The effect of different impeller design may give some other effect such as shear stress especially at a very high agitation rate which may damage cells and pulpy state moulds [41].

5.6. Fermentation pH. Medium pH is very significant in nutrients absorption and growth of microorganisms, stimulation of lipase production via signaling pathways, and release of extracellular lipases. In SFC, the effect of pH on the production of TA lipases was solely conducted at initial pH of the fermentation medium and pH was not controlled throughout the fermentation time due to small fermentation size. The initial pH of 6.0 was found to be the optimal TA lipase production by T. atroviride 676 although the optimal TA lipase activity was at pH 8.0 [33]. TA lipase production was also high at initial alkaline fermentation pH by T. lipolytica DSM 11003T (pH 8.2) and B. sonorensis 4R (pH 9.0) [4, 19]. The lipase production of Bacillus sp. LBN2 was found high at alkaline pH range of 8.0 to 10.0 where the maximal production can be obtained at initial pH 9.0 of the fermentation medium [9]. It was suggested by RSM that a significant increase in lipase production by Acinetobacter sp. AU07 could be achieved at fermentation pH 7.8 [27]. The optimum initial pH for maximal production of TA lipases by various microbes is varied maybe due to their nature and habitat where they were isolated. Initial pH of the fermentation medium serves as starting pH for the microbes to grow. Initial pH was usually enough for high production of TA lipases and addition of acid or base to control pH medium at certain value may inhibit growth and production of TA lipases. The microbes will slowly adapt to the changes in fermentation medium as pH drop or rise which explains the reason that medium was only set to an initial pH. In few cases, some TA lipases production can be optimized at controlled pH of the fermentation medium [41].

5.7. Aeration Rate and Dissolved Oxygen Tension. Aeration rate and dissolved oxygen tension (DOT) are important parameters as most microbes reported in literatures were aerobes or facultative anaerobes. The influence of aeration rate cannot be studied in SFC but it can be controlled in bioreactor (i.e STB) system using a rotameter or flow meter. In SFC, aeration rate and dissolved oxygen (DO) are solely determined by agitation rate but this is not the case for bioreactor system where the agitation and aeration rate can be set separately and maintained at a desired value [92]. Moreover, DOT in SFC cannot be measured and its effect on fermentation could not be evaluated. However, in bioreactor system, DOT can be monitored using DO probe and controlled throughout the fermentation [93]. In bioreactor system, the production of
TA lipases was enhanced at aeration rate of 1.5 to 3.0 L/min in 2 L bioreactor which is equivalent to 1.5 to 3.0vvm (volume of air under standard conditions per volume of liquid per minute). Several reported literatures showed that DOT controlled at 20% of saturation during production period can give maximum TA lipases production. Fermentation of Acinetobacter sp. AU07 in 3 L STB at controlled aeration rate of 1.5 vvm increased lipase production considerably to 48 U/mL as compared to SFC (15.84 U/mL) [27]. It has also been reported that DOT was controlled at 30% of saturation for the production of recombinant TA lipase using E. coli BL321 harbouring BTL2 lipase gene (Table 5). These reported studies have demonstrated that the effect of aeration rate and DOT in bioreactor play a major role and responsible for the increase in growth of the aerobes and production of the TA lipases. During growth phase and in a situation where oxygen MTR was limited, moulds cells in the form of mycelia pellet may be prone to autolysis and caused void formation in the interior part of the pellet [41]. The TA lipases production in the nonagitated fermentation culture can also be restricted by MTR where oxygen uptake by the cells was scarce [92, 93].

5.8. Bioreactor Design. TA lipases production in SFC has its own limitation due to the fact of its small size and geometrically not scalable. A successful optimization in small scale fermentation condition was needed for further study in pilot and industrial scales for possible industrial-scale production via bioreactor system (Table 6). Conventional and new bioreactors as well as impeller designs were studied and compared in terms of their efficiency and feasibility for large-scale TA lipases production. Microbial TA lipases may be produced in very different types of bioreactors such as STB, packed beds bioreactor (PBB), fluidized beds bioreactor (FBB), basket bioreactor (BP), and tray bioreactor (TB) [92, 97]. TA lipase production by an anaerobic T. lipolytica DSM 11003T was conducted in 20 L anaerobe bioreactor under nitrogen gas phase where pH of the fermentation was maintained at 7.6 and 9 to give lipase specific activity of 0.15 U/mg and 0.12 U/mg for 18 h and 21 h fermentation time, respectively [15, 39]. On the other hand, submerged fermentation (SmF) of Acinetobacter sp. AU07 in 3 L STB increased growth and lipase production to 3.2 (OD 600) and 48 U/mL, respectively, as compared to SFC (OD 600 = 1; 15 U/mL) under similar fermentation conditions (i.e., temperature, pH, inducer, inoculums size, and agitation) in 16 h fermentation time, which explained the correlation of an excellent microbial growth obtained in STB to an increased lipase production [27]. Moreover, B. cepacia lipase production (50 U/mL) and its specific activity (160 U/mg) in 14 L STB were also higher as compared to the one conducted in SFC (33 U/mL; 112.5 U/mg) under similar experimental condition [i.e., inoculums size, 3% (v/v); temperature, 45°C; uncontrolled pH] [46]. High lipase production and activity from 14 L STB system could be attributed to better nutrients availability and efficient oxygen mass transfer due to the controlled DOT at 25% of saturation with cascade aeration (up to 4 L/min) and agitation rate (between 300 and 1600 rpm) [46, 88].

In SFC, the recombinant E. coli BL321 harbouring BTL2 lipase gene under temperature-inducible kPL promoter enhanced lipase specific activity up to 706 kU/g after temperature-induction (shifting of the temperature from 30°C to 45°C) [95]. Later, E. coli BL321 harbouring BTL2 lipase gene was produced in a 2 L STB via fed-batch mode of operation, which resulted in slight increase of lipase specific activity up to 770 kU/g [93]. Slightly higher TA lipase BTL2 production and activity in STB as compared to SFC may result in a better control and heat transfer in STB, in consequence with the improved response of kPL promoter triggered by 45°C induction temperature [46, 93]. Meanwhile, recombinant P. pastoris X33 harbouring TA lipase gene from T. coremiiforme V3 cloned into plasmid pPICZAa was also studied in STB and SFC [83, 91]. Followed by an induction with MeOH, a maximum V3 lipase production (4 to 5 kU/mL) could be achieved in 5 to 50 L STB via fed-batch mode of operation which was about 27- to 33-fold higher as compared to SFC (0.15 kU/mL) in 7 d fermentation time [91].

Moreover, lipase production by S. warneri EX17 was optimized under optimal volumetric oxygen MTR (kL,a) 38 per hour and at pH 7, via SmF in 2 L batch STB using glycerol (C3H5O3) as a carbon source [82]. Under these conditions, the cell concentration reached its maximal value of 8.0 g/L, and the lipase specific activity reached a very high level, approximately 150 U/g cells, which is about five times higher than that obtained in the SFC after 12 h of cultivation [46, 82].

In SFC, the production of TA lipase from C. freundii IIT-BT L139 was optimized via OFAT [34]. A very high TA lipase IIT-BT L139 activity (8.8 U/mL) can be obtained at optimal temperature, pH, carbon, and nitrogen sources of 40°C, 9.0, starch, and peptone-urea, respectively, as evaluated via OFAT [34, 61]. When TA lipase IIT-BT L139 was produced via 1L and 10L STB (without pH and DOT controlled during fermentation), the activity was found to increase by 36% (12 U/ml) as compared to SFC [34]. STB is a common bioreactor design used for SmF technique while other types of bioreactors such as PBB and TB are more suitable for SSF technique. For instance, lipase production from fungus strain (i.e., Penicillium brevicompactum, Burkholderia sp.) could be produced optimally in TB at temperature of 30°C, moisture content of 70% (w/v), and carbon source concentration (i.e., olive oil and molasses) of 6.25% (g/g) to provide high lipase activity up to 20 U/g [41, 94]. By means of PBB with molasses as carbon source, air superficial velocities of more than 55 cm/min, and temperatures below 28°C, a maximum lipase activity of 26.4 U/g could be achieved, which were 30% higher than that obtained in TB [94]. The lower optimal temperature found using PBB is probably linked to radial heat gradient built inside the PBB [94].

5.9. Solid State and Submerged Fermentation. In industry, cost and simplicity of the production techniques are crucial factors for mass-scales TA production. The feasibility of using various types of fermentation techniques (i.e., SSF, SmF) and modes of operation (i.e., batch, fed-batch, and continuous) on the improvement of TA lipases production have been reported. Nevertheless, when compared to SmF, very few attempts had been made to produce TA lipases using SSF.
| Gene of origin                              | Expression of lipase gene                                      | Medium                        | $T_g$, $T_p$ | Agitation (rpm) | pH | DOT (%) | Medium | Induction | Duration (h) | Activity     | References |
|--------------------------------------------|----------------------------------------------------------------|-------------------------------|--------------|----------------|----|---------|---------|----------|-------------|--------------|-------------|
| **Enterobacter sp. Bn12**                  | E. coli (BL21) pLysS and pET-26(+)                            | LB + antibiotics               | 37, 20       | -              | -  | -       | LB+     | 0.5 mM IPTG (OD$_{600}$: 0.6) | 18          | 2900 (U/mg) | [61]       |
| **Thermomyces lanuginosus (lipase gene Ini)** | Pichia GS115 and expression plasmid vector pPIC9K (Heterologous expression under AOX1 promoter) | BMGY medium                   | 28, 28       | 220            | -  | -       | BMGY   | 1.0% (v/v) MeOH daily (OD$_{600}$: 2.0–4.0) | 168         | 1328 (U/mL) | [66]       |
| **Geobacillus thermocatenulatus**          | E. coli BL32I carrying the PCYTEXPI plasmid                  | Modified LB                   | 30, 45       | 200 to 600     | 7.0| 30, 3   | Modified LB | Using temperature-inducible kPL promoter (OD$_{600}$: 0.4–0.6) | 12          | 770,000 (U/g DCW) | [67]       |

*Note: $T_g$, temperature for growth; $T_p$, temperature for production; BMGY, buffered methanol complex medium; AOX1, alcohol oxidase 1 promoter; LB, Luria-Bertani medium; NaCl, sodium chloride; MeOH, methanol; modified LB medium containing 5 g/L NaCl, 10 g/L Bacto yeast extract, 10 g/L Bacto tryptone, 10 g/L glucose, 10 mM MgSO$_4$, and 40 mM K2HPO$_4$, together with 100 lg/mL of ampicillin; DCW, dry cell weight.*
Table 6: The production of TA lipases in bioreactors.

| Bioreactors (size) | Strain | IS (%) | Medium composition (%), v/v or w/v | Temperature (°C), agitation (rpm) | Aeration rate (L/min), DOT (%) | pH | Other minerals/parameters | Duration (h) | Activity (U/mL) | References |
|--------------------|--------|--------|------------------------------------|----------------------------------|---------------------------------|----|--------------------------|--------------|-----------------|------------|
| 3L                 | Acinetobacter sp. AU07 | 0.5    | Castor oil, 2                       | 30,150                           | 7                               |    | -                        | 12           | 48              | [27]       |
| 2L                 | G. thomodenitrificans  | -      | Waste cooking oil, 2                | 50,400                           | 8                               |    | KH2PO4, 0.1; olive oil, 0.01; vitamin B1, 0.1; MgSO4⋅7H2O, 0.1 NaCl, 0.5; cotton seed oil, 0.75; Tween-80, 0.5; CaCl2⋅2H2O, 0.001 | 18           | 330             | [34]       |
| 2L                 | Antrodia cinnamomea BCRC 35396 | 10     | Glucose, 3; peptone, 0.5; yeast extract, 0.3; malt extract, 0.3 | 28,150                           | 4                               |    | -                        | 18           | 26              | [11]       |
| 20L                | Bacillus sp. RSJ-1     | 0.1    | Peptone, 0.75; yeast extract, 0.75  | 50,350                           | 9.0                             |    | -                        | 10           | 2.13 (U/mg)     | [30]       |
| 1L                 | Citrobacter freundii IIT-BT L139 | 0.1    | Starch, 1 and peptone-urea, 1       | 40, -                            | 9.0                             |    | -                        | 60           | 12              | [12]       |
| 14L                | B. cepacia             | 2      | Glucose, 1; NH4Cl, 0.5; (NH4)2HPO4, 0.24 | 45,300                           | 7                               |    | KH2PO4, 0.1; MgSO4⋅7H2O, 0.01; palm oil emulsified with gum acacia, <1 NaCl, K2PO4, Na2S⋅9H2O, Na2CO3, MgCl2⋅6H2O, KCl NaHCO3, CaCl2⋅2H2O (<1) | 20           | 120 (U/mg)      | [10]       |
| 20 and 100L        | Thermosyntropha DSM    | -      | Yeast extract, 0.75; NH4Cl, <1; cysteine, <1 | 60, -                            | 8.2                             |    | -                        | 15–21        | 0.12–0.15 (U/mg) | [15, 39]  |

Note: STB, stirred tank bioreactor; IS, inoculum size; vvm, vessel volumes per minute; DOT, dissolved oxygen tension; NaCl, sodium chloride; K2HPO4, potassium hydrogen phosphate; KCl, potassium chloride; Na2S⋅9H2O, sodium sulphide nonahydrate; NH4Cl, ammonium chloride; MgCl2⋅6H2O, magnesium chloride hexahydrate; CaCl2⋅2H2O, calcium chloride dehydrate; NaHCO3, sodium bicarbonate; Na2CO3, sodium carbonate; C3H7NO3S, cysteine; (NH4)2PO4, ammonium phosphate; BE, beef extract; ECO, emulsified coconut oil.
Generally, SSF is known as a method where fungus and moulds are grown on a solid medium with limited free liquid phase of the culture [41, 97]. TA lipases production by SSF of Aspergillus sp. and Rhizopus sp. had been reported in several studies. For example, a comparatively high activity of Aspergillus terreus lipases (MW of 46.3 kDa, thermostable at 60°C) was obtained in SSF after incubation at 30°C for 96 h fermentation time using palm oil as a substrate probably due to suitable composition and ratio of the fatty acids present in palm oil as compared to oils of the sunflower, almond, coconut, olive, castor, mustard, and sesame [95]. Meanwhile, the thermotolerant Rhizopus homothallicus produced more lipases (MW of 29.5 kDa) in SSF (10,700 U/mg) as compared to SmF (8600 U/mg) in which maximal activity occurs at 60°C which might be due to different lipases produced from the culture medium as the effect from different fermentation techniques [98]. The lipase production (49.37 U/g) by Aspergillus niger AS-02 using sheanut cake under SSF with addition of 1.0% (v/w) Tween-80, 0.35% (w/w), (NH₄)₂SO₄, and 0.40% (w/w) Na₂HPO₄ [99]. However, the fermentation parameters such as temperature, pH, and DOT are difficult to control in the SSF. In contrast to SmF, the SSF process occurs in very low water content and thus the lipases are strongly adsorbed on the insoluble biomass, which later require an efficient extraction process and lipase recovery. In an economic analysis of the production of Penicillium restrictum lipase in SmF and SSF, it was suggested that SSF techniques offers greater advantage over SmF in terms of raw material, total capital investment, unitary product, cost product selling price, and profitability [41, 100].

Most reports on TA lipases production existing in the literatures are associated with SmF. It is widely used for high performance production of many biomolecules and enzymes including TA lipases. SmF cultures have some advantages over SSF, such as higher homogeneity and more facility to control parameters such as temperature and pH. The growth of aerobes in a SmF culture is greatly affected by the accessibility of substrates, energy (i.e., ATP), and DO. SmF is of a heterogeneous culture, whereby the reactions rates can be restricted by the MTR (i.e., substrates) at a particular interface. Various modes of operations such as batch, fed-batch, continuous, and semicontinuous are also possible in SmF for the improvement of TA lipases production in order to attain an optimal and cost-effective biomanufacturing process. SmF is also suitable for the production of lipases by filamentous fungi and many bacteria where the concentration of the carbon source (i.e., oil) and nitrogen source had a significant effect on lipase production [101]. For instance, B. methylotrophicus PS3, S. pasteurii COM-4A, B. subtilis COM-6B, Aspergillus sp. strain O-8, T. lanuginosus (i.e., GSLMBKU-10, GSLMBKU-13, and GSLMBKU-14), and S. arlettae JPBW-1 [8, 50, 62, 66]. Strain such as Aspergillus sp. strain O-8 lipase produced from the SmF has more stability at higher temperature than SSF [41, 62]. Lipase produced from SmF still retained about 72% of residual activity after one hour of incubation at 90°C. Furthermore, lipase produced from SmF retained 80% of the residual activity at the acidic pH while lipase obtained from the SSF results in residual activity of 60% at the alkaline pH [62]. In SmF, the highest lipase production was at 37°C and pH 7.2 while in SSF optimal temperature and pH are at 35°C and pH 6.0, respectively [41, 62].

Fed-batch culture has been applied for the production of lipases by Acinetobacter radioresistens and Candida cylindracea NRRL Y-17506, where Tween-80 and oleic acid were added intermittently and stepwise feeding to the initial batch fermentation after carbon sources supply was exhausted [24, 102]. However, lipases production may decrease at high specific growth rate in the later part of fed-batch cultures due to the build-up of carbon source oversupplied [102]. Several studies have showed that the growing cells and excessive carbon source may suppress the metabolic pathway of TA lipases production.

6. Fermentation Time

The TA lipases fermentation by many producing strains was classified as a growth-associated process. Growth and fermentation time normally reached the maximum TA lipases production after 24 h up to several days, depending on fermentation optimized condition, mode of operation, type of TA lipases produced, and strains. The TA lipase production was usually maximal when the fermentation reached a maximum growth or at the beginning of the stationary phase and then gradually decreased towards the end of batch fermentation [27]. For instance, a maximal lipase activity Acinetobacter sp. AU07 of 331.16 U (specific activity of 38.64 U/mg) was obtained in SmF at optimal temperature of 30°C and pH 7.0 for 16 h fermentation time when it reached its stationary growth phase [27]. The fermentation time to obtain maximum TA lipase activity by Acinetobacter sp. AU07 was similar to several TA lipase productions like by Acinetobacter sp. BK44 and B. thermoleovorans ID-1 where maximal lipase activity can be observed later than 12 h fermentation time [27, 28]. The TA lipase production by recombinant Pichia pastoris X33 harbouring lipase V3 gene can reach maximum lipase activity in fed-batch cultivation after 168 h induction with MeOH in STB [83, 91]. Meanwhile, TA lipase produced by T. harzianum IDM14D reached its maximum activity (0.24 U/mL) and biomass concentration (1.25 g/L) in SFC at 30°C for 7 d fermentation time [47]. Lipase production from A. cinnamomea BCRC 35396 was enhanced by 0.01% (v/v) olive oil as an inducer in an aerated STB to give final lipase activity of 26 U/ml after 18 d fermentation time [11]. Moreover, the fermentation time of TA lipase IIT-BT LJ39 was comparatively short (60 h) in STB as compared to SFC (70 h) [34, 46]. It has been reported that the lipase production starts when the carbon concentration (i.e., glucose) reduced significantly and cell growth almost ceased and had entered the stationary phase. After optimal fermentation time, there might be an accumulation of fatty acids produced, which has been reported to repress lipase production [34].

On the other hand, continuous culture can be an ideal fermentation process for the production of microbial biomass and other growth-associated process such as TA lipases. Continuous fermentation differs from batch where fermentation...
time is prolonged for certain period of time [41]. Continuous mode of operation is an open system which allows simultaneous addition of nutrients and withdrawal of fermentation broth at a constant rate where the culture is maintained at its exponential phase [41, 92, 103]. For instance, TA lipases of thermoalkalophilic Bacillus sp. strain MC7 and thermophilic Bacillus sp. strain IHI-91 can be produced in continuous mode of operation [103, 104]. In the case for Bacillus sp. strain IHI-91, lipase activity showed a maximum of 340 U/L/h at a dilution rate of 0.4 per hour where its productivity was increased up to 50% as compared to batch fermentation [103].

7. Optimization Approaches

Process optimisation can be performed using technique such as OFAT and statistical tools such as RSM and artificial neural network (ANN) [92, 99, 105]. ANN showed a superior method over RSM for data fitting and estimation capabilities in optimization study of lipase production by recombinant E. coli BL21 and Geobacillus sp. strain ARM [92, 106] Different experimental designs have been employed for lipase production study and optimizations such as central composite design (CCD), Plackett–Burman Factorial Design (PBFD) and Box–Behnken Design (BBD) [41, 99, 101]. The model was later optimized using algorithms such as evolution algorithms (EAs) and Levenberg-Marquardt (LM) algorithm [107]. The standard LM algorithm uses trial-and-error method to estimate the damping factor and is less reliable for large-scale inverse problems [107]. In recent decades, there has been a growing interest in optimization based on the principle of EAs (survival of the fittest) which includes Genetic Algorithm (GA), Differential Evolution (DE), Particle Swarm Optimization (PSO), Evolutionary Programming (EP), Evolution Strategies (ES), and Genetic Programming (GP) [105, 108–111]. EAs have turn out to be very popular as function optimizers, because they are easy to implement and show fair performance for a broad range of functions.

This EAs approach was rarely reported but recent studies showed that GA and PSO approaches experimentally improved the optimization of lipase production [109, 110]. The overall optimized fermentation conditions obtained via PSO approach (96.18 U/gds in 46 generations) have slightly better performance, convergence, and computational efficiency as compared to that of the GA approach (95.34 U/gds in 337 generations) [110]. In another study, the optimization results also indicate that the lipase activity was significantly improved, where PSO (133.57 U/gds in the 27th generation) performs better than GA (132.24 U/gds in the 320th generation), slightly with regard to optimized lipase activity and highly in respective to convergence rate [109]. Therefore, the PSO approach with the minimal parameters tuning is a practical tool for optimization of fermentation conditions of lipases production [110]. The simple structure associated with the effective memory capabilities of PSO has proven to be a superior approach over GA [109].

Alternatively, DE, another type of EAs, is a simple, robust, compact-structured, stochastic direct search approach whose functions based on concepts of the survival of the fittest and natural selection, a common concept of EAs [107]. DE is also better than GA for fine tuning in complex search spaces. DE coupled with RSM has been proven to be a useful approach for the optimization of lipase production by R. oryzae via SSF [105, 107]. The input space of the experimentally validated RSM-model was optimized using this novel DE approach in which the maximal lipase activity of 96.52 U/gds was observed under DE optimal values (temperature, 35.9°C; pH, 5.28; liquid to solid ratio, 1.5; incubation time, 4.8 d) [105]. In another study, a maximal lipase activity of 134.13 U/gds was obtained which was higher than using OFAT approach (36.28 U/gds) under DE-optimized values (DMSO, 25%; buffer, 40 mM; soaking time, 128.5 min; temperature, 35°C) and DE-control parameters (number of population, 20; generations, 50; crossover operator, 0.5; scaling factor, 0.25). The use of DE approach has improved the optimization capabilities and decision speed, resulting in an improved extracellular R. oryzae lipase yield (36.28 U/gds) as compared to the OFAT approach [108]. The developed model and its optimization are generic to biological world, hence, appearing to be practical for the design and scale-up process of the lipase production by R. oryzae via SSF [108].

8. Conclusion

Lipases which are stable at high temperature and resistant to alkaline pH are demanding in biodiesel, detergent, leather, textile, Kraft pulp, and many other industries. The discovery of TA lipases reduced wastes and increased efficiency in such applications. TA lipases are also suitable with combination of surfactant, detergents, chelating agent, and ions for optimum reaction in respective industry. The optimized fermentation process and strategy (i.e., microbes, carbon-nitrogen composition, minerals, temperature, pH, agitation rate, aeration rate, and DOT) are important factors for high production of commercial TA lipases in industry. The development of recombinant and mutant strains showed their advantages over WT strains in producing high amount and stable form of TA lipases. By using correct fermentation techniques and optimization approaches, TA lipases can be produced efficiently and economically.

Conflicts of Interest

The author declares that there are no conflicts of interest in this article.

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References

[1] K. De Godoy Daiha, R. Angeli, S. D. De Oliveira, and R. V. Almeida, “Are lipases still important biocatalysts? A study of scientific publications and patents for technological forecasting,” PLoS ONE, vol. 10, no. 6, Article ID e0131624, 2015.
[32] S. Ugras, “Characterization of a thermophilic lipase from bacillus licheniformis h7? isolated from hayran thermal springs in giresun,” *Romanian Biotechnological Letters*, vol. 22, no. 1, pp. 12297–12306, 2017.

[33] T. Andrade Marques, C. Baldo, D. Borsato, J. Batista Buzato, M. Antonia, and A. P. Celligoi, “Pedrine colabone celligoi, production and partial characterization of a thermostable, alkaline and organic solvent tolerant lipase from trichoderma atroviride 676,” *International Journal of Scientific & Technology Research*, vol. 3, no. 5, 2014.

[34] L. P. Christopher, V. P. Zambare, A. Zambare, H. Kumar, and L. Malek, “A thermo-alcaline lipase from a new thermophile Geobacillus thermodenitrificans AV-5 with potential application in biodiesel production,” *Journal of Chemical Technology and Biotechnology*, vol. 90, no. 11, pp. 2007–2016, 2015.

[35] F. Hasan, A. Shah, S. Javed, and A. Hameed, "Enzymes used in detergents: lipases," *African Journal of Biotechnology*, vol. 9, no. 31, pp. 4836–4844, 2010.

[36] M. Rabbani, F. Shafeeq, Z. Shayegh et al., “Isolation and characterization of a new thermoalkalophilic lipase from soil bacteria,” *Iranian Journal of Pharmaceutical Research*, vol. 14, no. 3, pp. 901–906, 2015.

[37] M. Holmquist, "Alpha/Beta-Hydrolase Fold Enzymes: Structures, Functions and Mechanisms," *Current Protein & Peptide Science*, vol. 1, no. 2, pp. 209–235, 2000.

[38] Dianursanti, M. Delaamira, S. Bismo, and Y. Muharam, “Effect of Reaction Temperature on Biodiesel Production from Chlorella vulgaris using CuO/Zeolite as Heterogeneous Catalyst,” in *Proceedings of the 2nd International Conference on Tropical and Coastal Region Eco Development 2016*, ICTCRED 2016, Indonesia, October 2016.

[39] M. A. Salameh and J. Wiegel, "Effects of Detergents on Activity, Thermostability and Aggregation of Two Alkalithermostable Lipases from Thromosyntha lipolytica," *The Open Biochemistry Journal*, vol. 4, pp. 22–28, 2010.

[40] D. Pérez, F. Kovacić, S. Wilhelm et al., "Identification of amino acids involved in the hydrolytic activity of lipase LipBL from Marinobacter lipolyticus," *Microbiology (United Kingdom)*, vol. 158, no. 8, pp. 2192–2203, 2012.

[41] A. Mehta, U. Bodh, and R. Gupta, “Fungal lipases: a review,” *Journal of Biotech Research*, vol. 8, Article ID 328558, pp. 1944–328558, 2017.

[42] F. A. Raza, A. N. Sabri, A. Rehman, and S. Hasnain, “Characterization of therophilic alkaline lipase produced by staphylococcus aureus suitable for leather and detergent industries,” *Iranian Journal of Science & Technology*, vol. 41, no. 2, pp. 287–294, 2017.

[43] H. A. Sani, F. M. Shariff, R. N. Z. R. Abd Rahman, T. C. Leow, and A. B. Salleh, "The effects of one amino acid substitutions at the C-terminal region of thermostable L2 lipase by computational and experimental approach," *Molecular Biotechnology*, vol. 60, no. 1, pp. 1–11, 2018.

[44] J. Zheng, C. Liu, L. Liu, and Q. Jin, "Characterisation of a thermo-alkali-stable lipase from oil-contaminated soil using a metagenomic approach," *Systematic and Applied Microbiology*, vol. 36, no. 3, pp. 197–204, 2013.

[45] B. L. Zamost, H. K. Nielsen, and R. L. Starnes, “Thermostable enzymes for industrial applications,” *Journal of Industrial Microbiology and Biotechnology*, vol. 8, no. 2, pp. 71–81, 1991.

[46] L. D. Unsworth, J. Van Der Oost, and S. Koutoupiopoulus, “Hypertherophilic enzymes - Stability, activity and implementation strategies for high temperature applications,” *FEBS Journal*, vol. 274, no. 16, pp. 4044–4056, 2007.

[47] A. Kumari, P. Mahapatra, V. K. Garlapati, and R. Banerjee, "Enzymatic transesterification of Jatropha oil," *Biotechnology for Biofuels*, vol. 2, article 1, 2009.

[48] V. Garlapati, R. Kant, A. Kumari, P. Mahapatra, P. Das, and R. Banerjee, "Lipase mediated transesterification of Simarouba glauca oil: a new feedstock for biodiesel production," *Sustainable Chemical Processes*, vol. 1, no. 1, p. II, 2013.

[49] S. Parameswaran, V. K. Dubey, and S. Patra, "In silico characterization of thermostable, alkaline and detergent-stable lipase from a staphylococcus aureus strain," *In Silico Biology*, vol. 10, no. 5-6, pp. 265–276, 2010.

[50] P. Sharma, N. Sharma, S. Pathania, and S. Handa, “Purification and characterization of lipase by Bacillus methylotrophicus PS3 under submerged fermentation and its application in detergent industry,” *Journal of Genetic Engineering and Biotechnology*, 2017.

[51] A. Ben Bacha, N. M. S. Moubayed, and A. Al-Assaf, “An organic solvent-stable lipase from a newly isolated Staphylococcus aureus ALA1 strain with potential for use as an industrial biocatalyst,” *Biotechnology and Applied Biochemistry*, vol. 63, no. 3, pp. 378–390, 2016.

[52] A. Naganthan, M. Masomian, R. N. Z. R. A. Rahman, M. S. M. Ali, and H. M. Nooh, “Improving the efficiency of new automatic dishwashing detergent formulation by addition of thermostable lipase, protease and amylase,” *Molecules*, vol. 22, no. 9, article no. 1577, 2017.

[53] I. A. Rahman, R. N. Z. R. A. Rahman, A. B. Salleh, and M. Basri, “Formulation and evaluation of an automatic dishwashing detergent containing T1 Lipase,” *Journal of Surfactants and Detergents*, vol. 16, no. 3, pp. 427–434, 2013.

[54] D. M. Abol Fotouh, R. A. Bayoumi, and M. A. Hassan, “Production of thermoalkaliphilic Lipase from Geobacillus thermoe polymorans DA2 and Application in Leather Industry,” *Enzyme Research*, vol. 2016, Article ID 9034364, 9 pages, 2016.

[55] C. C. Akoh, G.-C. Lee, Y.-C. Liaw, T.-H. Huang, and J.-F. Shaw, “GDSL family of serine esterases/lipases,” *Progress in Lipid Research*, vol. 43, no. 6, pp. 534–552, 2004.

[56] A. Kumar, K. Dhar, S. S. Kanwar, and P. K. Arora, “Lipase catalysis in organic solvents: Advantages and applications,” *Biological Procedures Online*, vol. 18, no. 1, article no. 2, 2016.

[57] D. H. El-Ghomney, M. S. El-Gamal, A. E. Tantawy, and T. H. Ali, “Extracellular alkaline lipase from a novel fungus Curvularia sp. DHE 5: Optimisation of physicochemical parameters, partial purification and characterisation,” *Food Technology and Biotechnology*, vol. 55, no. 2, pp. 206–217, 2017.

[58] M. Zarini parsaghi, G. Ebrahimipour, and H. Sadeghi, “Lipase and biosurfactant from Ochrobactrum intermedium strain MZV101 isolated by washing powder for detergent application,” *Lipids in Health and Disease*, vol. 16, no. 1, article no. 177, 2017.

[59] S. S. Lokre and D. G. Kadam, “Production and characterization of thermostable lipase from Aerobacillus sp. SSL096201,” *BioScience*, vol. 5, no. 2, pp. 1–7, 2014.

[60] F. Laachari, F. El Bergadi, A. Sayari et al., “Biochemical characterization of a new thermostable lipase from Bacillus pumilus strain,” *Turkish Journal of Biochemistry*, vol. 40, no. 1, pp. 8–14, 2015.

[61] P. Farrokh, B. Yakhchali, and A. A. Karkhane, “Cloning and characterization of newly isolated lipase from Enterobacter sp. Bn12,” *Brazilian Journal of Microbiology*, vol. 45, no. 2, pp. 677–687, 2014.

[62] L. M. Colla, A. M. M. Ficanha, J. Rizzardi, T. E. Bertolin, C. O. Reinehr, and J. V. Costa, “Production and characterization
of lipases by two new isolates of Aspergillus through solid-state and submerged fermentation,” BioMed Research International, vol. 2015, Article ID 725959, 9 pages, 2015.

[63] S. Kumar, K. Kikon, A. Upadhyay, S. S. Kanwar, and R. Gupta, “Production, purification, and characterization of lipase from thermophilic and alkaliphilic Bacillus coagulans BTS-5,” Protein Expression and Purification, vol. 41, no. 1, pp. 38–44, 2005.

[64] A. Balan, D. Ibrahim, R. Abdul Rahim, and F. A. Ahmad Rashid, “Purification and characterization of a thermostable lipase from Geobacillus thermodenitrificans IBRL-nra,” Enzyme Research, vol. 2012, Article ID 987523, 7 pages, 2012.

[65] J. Yang, K. Kobayashi, Y. Iwasaki, H. Nakano, and T. Yamane, “In vitro analysis of roles of a disulfide bridge and a calcium binding site in activation of Pseudomonas sp. strain KW1-56 lipase,” Journal of Bacteriology, vol. 182, no. 2, pp. 295–302, 2000.

[66] B. Sreelatha, V. Koteswara Rao, R. Ranjith Kumar, S. Girisham, L. L. Barton, “Physiological basis for growth in extreme thermophilic and alkaliphilic” Production, purification, and characterization of lipase from Geobacillus thermodenitrificans IBRL-nra,” Enzyme Research, vol. 2012, Article ID 987523, 7 pages, 2012.

[67] S. ¨Ulker, A.¨Ozel, A. C¸olak, and S ¸. Alpay Karaoˇglu, “Isolation, production, and characterization of extracellular lipase by Geotrichum candidum of dairy origin,” Journal of Microbiology and Biotechnology, vol. 44, no. 6, pp. 757–761, 2017.

[68] A. M. Vélez, A. C. L. Horta, A. J. Da Silva, M. R. D. C. Leomar, R. D. L. C. Giordano, and T. C. Zangriolami, “Enhanced production of recombinant thermo-stable lipase in Escherichia coli at high induction temperature,” Protein Expression and Purification, vol. 90, no. 2, pp. 96–103, 2013.

[69] L. L. Barton, “Physiological basis for growth in extreme environments,” in Structural and Functional Relationships in Prokaryotes, pp. 388–393, Springer, New York, NY, USA, 2005.

[70] X. Zhang and L. Xia, “Expression of Talatomeyis thermophilus lipase gene in Trichoderma reesei by homologous recombination at the cbh1 locus,” Journal of Industrial Microbiology and Biotechnology, vol. 44, no. 3, pp. 377–385, 2017.

[71] R. Sangeetha, A. Geetha, and I. Arulpandi, “Concomitant production of protease and lipase by Bacillus licheniformis VSGI: production, purification and characterization,” Brazilian Journal of Microbiology, vol. 41, no. 1, pp. 179–185, 2010.

[72] A. Muhammad, S. Ali, I. Bokhari et al., “Purification and characterization of extracellular lipase by Geotrichum candidum of dairy origin,” Pakistan Journal of Botany, vol. 49, no. 2, pp. 757–761, 2017.

[73] J. Wang, Y.-Y. Li, and D. Liu, “Gene cloning, high-level expression, and characterization of an alkaline and thermostable lipase from Trichosporon coremiiforme V3,” Journal of Microbiology and Biotechnology, vol. 25, no. 6, pp. 845–855, 2015.

[74] S. Ulker, A. Özel, A. Çolak, and Ş. Alpay Karaoğlu, “Isolation, production, and characterization of an extracellular lipase from Trichoderma harzianum isolated from soil,” Turkish Journal of Biology, vol. 35, no. 5, pp. 543–550, 2011.

[75] A. F. B. Lajis, M. Hamid, and A. B. Ariff, “Depigmenting effect of kojic acid esters in hyperpigmented B16F1 melanoma cells,” Journal of Biomedicine and Biotechnology, vol. 2012, Article ID 952452, 9 pages, 2012.

[76] R. Sharma, Y. Chisti, and U. C. Banerjee, “Production, purification, characterization, and applications of lipases,” Biotechnology Advances, vol. 19, no. 8, pp. 627–662, 2001.

[77] R. Sangeetha, I. Arulpandi, and A. Geetha, “Bacterial lipases as potential industrial biocatalysts: An overview,” Research Journal of Microbiology, vol. 6, no. 1, pp. 1–24, 2011.

[78] N. Ishak, A. F. B. Lajis, R. Mohamad et al., “Kojic acid esters: comparative review on its methods of synthesis,” Journal of Biochemistry, Microbiology and Biotechnology, vol. 4, no. 2, pp. 7–15, 2016.
E. coli,” *Turkish Journal of Biochemistry*, vol. 38, no. 3, pp. 299–307, 2013.

[94] L. Toscano, G. Montero, M. Stoytcheva et al., “Lipase production through solid-state fermentation using agro-industrial residues as substrates and newly isolated fungal strains,” *Biotechnology & Biotechnological Equipment*, vol. 27, no. 5, pp. 4074–4077, 2013.

[95] B. K. Sethi, P. K. Nanda, and S. Sahoo, “Characterization of biotechnologically relevant extracellular lipase produced by Aspergillus terreus NCFT 4269.10,” *Brazilian Journal of Microbiology*, vol. 47, no. 1, pp. 143–149, 2016.

[96] J. Krzeslak, G. Gerritse, R. Van Merkerk, R. H. Cool, and W. J. Quax, “Lipase expression in Pseudomonas alcaligenes is under the control of a two-component regulatory system,” *Applied and Environmental Microbiology*, vol. 74, no. 5, pp. 1402–1411, 2008.

[97] B. D. Ribeiro, A. M. De Castro, M. A. Z. Coelho, and D. M. G. Freire, “Production and use of lipases in bioenergy: a review from the feedstocks to biodiesel production,” *Enzyme Research*, vol. 2011, Article ID 615803, 16 pages, 2011.

[98] J. C. M. Diaz, J. A. Rodríguez, S. Roussos et al., “Lipase from the thermotolerant fungus *Rhizopus homothallicus* is more thermostable when produced using solid state fermentation than liquid fermentation procedures,” *Enzyme and Microbial Technology*, vol. 39, no. 5, pp. 1042–1050, 2006.

[99] A. Salihu, M. Bala, and M. Z. Alam, “Lipase production by Aspergillus niger using sheanut cake: an optimization study,” *Journal of Taibah University for Science*, vol. 10, no. 6, pp. 850–859, 2016.

[100] L. R. Castilho, C. M. S. Polato, E. A. Baruque, G. L. Sant’Anna Jr., and D. M. G. Freire, “Economic analysis of lipase production by Penicillium restrictum in solid-state and submerged fermentations,” *Biochemical Engineering Journal*, vol. 4, no. 3, pp. 239–247, 2000.

[101] L. M. Colla, A. L. Primaz, S. Benedetti et al., “Surface response methodology for the optimization of lipase production under submerged fermentation by filamentous fungi,” *Brazilian Journal of Microbiology*, vol. 47, no. 2, pp. 461–467, 2016.

[102] B. S. Kim and C. T. Hou, “Production of lipase by high cell density fed-batch culture of Candida cylindracea,” *Bioprocess and Biotecnology Engineering*, vol. 29, no. 1, pp. 59–64, 2006.

[103] E. Emanuilova, M. Kambourova, M. Dekovska, and R. Manolov, “Thermoalkalophilic lipase-producing Bacillus selected by continuous cultivation,” *FEMS Microbiology Letters*, vol. 108, no. 2, pp. 247–250, 1993.

[104] P. Becker, I. Abu-Reesh, S. Markossian, G. Antranikian, and H. Märkl, “Determination of the kinetic parameters during continuous cultivation of the lipase-producing thermophile Bacillus sp. IH1-91 on olive oil,” *Applied Microbiology and Biotechnology*, vol. 48, no. 2, pp. 184–190, 1997.

[105] V. K. Garlapati and R. Banerjee, “Optimization of lipase production using differential evolution,” *Biotechnology and Bioprocess Engineering*, vol. 15, no. 2, pp. 254–260, 2010.

[106] A. Ebrahimpour, R. Rahman, D. Ean Chng, M. Basri, and A. Salleh, “A modeling study by response surface methodology and artificial neural network on culture parameters optimization for thermostable lipase production from a newly isolated thermophilic *Geobacillus sp.* strain ARM,” *BMC Biotechnology*, vol. 8, article 96, 2008.

[107] B. Subudhi and D. Jena, “Differential evolution and levenberg marquardt trained neural network scheme for nonlinear system identification,” *Neural Processing Letters*, vol. 27, no. 3, pp. 285–296, 2008.