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Review

Production and applications of microbial lipases: A review

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Lipases (triacylglycerol ester hydrolases, EC 3.1.1.3) are biotechnologically relevant enzymes that find immense applications in food, detergent and pharmaceutical industries. They catalyze the breakdown of fats and oils as well as various synthetic reactions such as esterification, transesterification and interesterification in organic solvents. Microbial lipases stand out as the major sources of the enzyme because of their diversity in catalytic activity, high yield and low cost of production, as well as relative ease in genetic manipulation. This review describes the major factors that affect the lipase production, indicating some of the strategies used to enhance the production. Also, the potential industrial applications that make lipases to be the biocatalysts of choice for the present and future have been presented. This information helps in understanding the major aspects of lipase production as well as the applications.

Key words: Lipases, industrial applications, enzyme, production.

INTRODUCTION

Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) are carboxylesterases that catalyze the hydrolysis of triglycerides at the water-liquid interface. In non-aqueous conditions they catalyze the reverse reaction (such as esterification, interesterification and transesterification) producing glycerides from glycerol and fatty acids (Saxena et al., 2003; Sharma et al., 2001). Thus, lipases catalyze the hydrolysis and synthesis of long-chain acylglycerols. Although, there is no strict definition available for the term “long-chain” but glycerol esters with an acyl chain length of >10 carbon atoms can be regarded as lipase substrates, with trioleoylglycerol being the standard substrate (Jaeger et al., 1999).

Lipase has α/β-hydrolase fold, a conserved catalytic triad (Ser, His, Asp/Glu) and usually a consensus sequence (Gly-x-Ser-x-Gly) is found around the active site serine (Gupta et al., 2004). This triad is buried completely beneath a short α-helical segment, which opens upon contact of the lipase with an interface. The enzyme’s structural rearrangement during catalysis creates an electrophilic region (the oxyanion hole) around the serine residue. This stabilizes the transition state intermediate by exposing the hydrophobic residues and by burying the hydrophilic ones (Stamatis et al., 1999). The presence of features in lipases like a lid and an amphiphilic loop covering the active site differentiates them from esterase.

Villeneuve et al. (2000) indicated that the current interests in unprecedented use of lipases in biotechnology, manufacture of pharmaceuticals and pesticides, single cell protein production, biosensor preparation and in waste management, among others (Vakhlu and Kour, 2006) can be linked to their potentials to catalyze both hydrolytic and synthetic reactions. Based on this, it is noteworthy that lipases are endowed with substrate and reaction specificities that surpass those of any other known enzymes, with application potentials that are

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literally boundless (Gandhi, 1997).

Among the hydrolases, lipases account for about 5 to 10% of the enzyme market but considering their extremely high versatility (Gandhi, 1997) with respect to their unique properties (such as chemoselectivity, regioselectivity, stereoselectivity), non-requirement of cofactors and stability in organic solvents (Diaz et al., 2006); it would not be surprising if lipases take the top position in the enzyme application area in the near future. This could be attributed to the amount of information reported in the literature, ranging from production, purification, biochemical catalysis, industrial applications to medical relevance. Thus, this review intends to describe the lipase production (especially the upstream processes) that can aid in understanding the major aspects of production as well as the potential applications.

Lipases

Sources of lipases

Lipases can be obtained from animals mainly from fore-stomach tissue of calves or lambs and pancreatic tissues of hogs and pigs. Among the disadvantages of using animal lipases include presence of trypsin in pig pancreatic lipases, which results in bitter tasting amino acids, presence of residual animal hormones or viruses as well as their undesirable effects in the processing of vegetarian or kosher diets (Vakhlu and Kour, 2006). Plant lipases are also available but not exploited commercially because of the yield and the processes involved. Thus, microbial lipases are currently receiving more attention because of their technical and economic advantages, where the organisms are cultivated in medium containing appropriate nutrient composition under controlled conditions (Srivastava, 2008). Also, lipase production by microorganisms varies according to the strains, the composition of the growth medium, cultivation conditions, pH, temperature, and the kind of carbon and nitrogen sources (Gupta et al., 2004; Souissi et al., 2009; Treichel et al., 2010). Generally, bacteria, fungi, yeast and actinomycetes are recognized as preferred sources of extracellular lipases, facilitating the enzyme recovery from the culture broth: although *Candida, Pseudomonas, Mucor, Rhizopus* and *Geotrichum* sp. stand out as the major commercially viable strains (Ertugrul et al., 2007).

Properties and reactions of lipases

Besides the conventional ability of lipases to catalyze hydrolytic reactions, they can catalyze synthetic reactions such as esterification and transesterification in form of acidolysis, alcoholysis and interesterification in the presence of small amount of water. Unlike other enzymes, oil-water or air-water interfaces activate the lipases (Shimada et al., 2005; Villeneuve et al., 2000). Divakar and Manohar (2007) grouped the reactions catalyzed by lipases into three important types:

i. Hydrolysis: This occurs in aqueous media, when there is high amount of water, cleavage of ester bonds is the dominant reaction. This technology is currently employed in the production of fatty acids, diglycerides, monoglycerides, flavouring agents for dairy products and detergents for laundry and household uses.
ii. Esterification: This reaction occurs under low water conditions such as in nearly anhydrous solvents; a high yield of the esterified products is obtained under controlled conditions. Production of oleic acid esters of primary and secondary aliphatic and terpenic alcohols is among the commonest example. Others are the formation of geranyl and menthyl esters from butyric acid and geranol or lauric acid and menthol (Marlot et al., 1985).
iii. Transesterification: this involves the exchange of acid moiety between two or more compounds (if the acyl donor is a free acid the reaction is called acidolysis, whereas the reaction is called interesterification if the acyl donor is an ester; in alcoholysis, the nucleophile alcohol acts as an acyl acceptor) (Macrae, 1985).

FACTORS AFFECTING MICROBIAL LIPASE PRODUCTION

Microbial lipases are mostly extracellular and their production is greatly influenced by medium composition as well as physicochemical factors such as temperature, pH, and dissolved oxygen. These enzymes are generally produced in the presence of lipid substrates such as oils or any inducers in form of triacylglycerols, fatty acids, hydrolysable esters, Tweens, bile salts, and glycerol (Gupta et al., 2004; Sharma et al., 2001).

Effect of nutritional factors

Carbon sources

Carbon sources serve as important substrates for energy production in microorganisms. Lipidic carbon sources serve as inducers and are generally essential for obtaining a high lipase yield. Benjamin and Pandey (1996) showed that *C. rugosa* lipase production was proportionally increased with the increase in concentration of olive oil and maximum production was achieved at 10% (v/v) olive oil concentration. Production of a thermostable lipase from thermophilic *Bacillus* sp. strain Wai 28A 45, in the presence of tripalmitin at 70°C, was described by Janssen et al. (1994). Media with tripalmitin, tristearin, and trimyristin carbon sources were

tested, and tripalmitin was found to be the best inducer of lipase activity. Enzyme activity was not detected when glucose was the sole carbon source, confirming that the presence of an inducer is necessary for *Penicillium aurantiogriseum* to produce lipases (Lima et al., 2003). However, the requirement for sugar as a carbon source in addition to lipids varies with the microorganism. But generally, media supplemented with glucose along with triglycerides stimulate the lipase production in *Rhizopus nigricans* as reported by Ghosh et al. (1996). Both olive oil and Tween-80 stimulated the production of extracellular lipase in *Penicillium citrinum* at 0.1 and 0.7% (v/v), respectively (Maliszewska and Mastalerz, 1992). Dalmau et al. (2000) showed that Tween-80 stimulates both lipase biosynthesis and its secretion in *C. rugosa.* The yeast *Pseudozyma hubeiensis* HB85A lipase was strongly stimulated by 150.8% in the presence of Tween-80 compared to non-Tween-80 supplemented media (Bussamara et al., 2010).

Nitrogen sources

Both organic and inorganic nitrogen sources have been traditionally used for lipase production. In *Aspergillus wentii*, *Mucor racemosus* and *R. nigricans*, lipase yield was enhanced by adding peptone in the production medium at the concentration of 20 g/L (Ghosh et al., 1996). However, for lipase production by *Rhodotorula glutinis*, inorganic nitrogen sources, such as ammonium phosphate appear to favour lipase production (Papaparaskevas et al., 1992). Salleh et al. (1993) obtained maximum production of extracellular lipase by thermophilic fungi, *R. oryzae* when the medium was supplemented with peptone as the nitrogen source. Rajendran and Thangavelu (2009) found that both peptone and yeast extract were required by *R. arrhizus* for lipase production. However, inorganic nitrogen source in form of NH_4Cl was reported to be the best for *C. cylindracea* NRRL Y-17506 (Brozzoli et al., 2009) and *P. citrinum* (Miranda et al., 1999). Nitrogen sources such as corn steep liquor and soybean meal stimulated *P. citrinum* lipase production to a lesser extent than peptone; while urea and ammonium sulfate inhibited the lipase synthesis (Sztajer and Maliszewska, 1989).

Inorganic minerals

Different microorganisms require different inorganic minerals for their growth and lipase production. Inorganic salts in form of $MgSO_4$, $(NH_4)_2SO_4$, NaCl, K_2HPO_4, $BaCl_2$ are required for maximum lipase production by *Hendersonula toruloidea* (Odibo et al., 1995). In case of *Candida* sp. 99-125, culture medium containing (w/v) soybean oil, 4.187%, soybean powder 5.840%, K_2HPO_4 0.284%, KH_2PO_4 0.1%, $(NH_4)_2SO_4$ 0.1%, $MgSO_4$ 0.05% and Span 60 0.1% was found to be the optimum for lipase production and absence of any of these components affect the organism's growth and lipase activity (He and Tan, 2006). Generally, Magnesium salt is required by most microorganisms due to its ability to play some regulatory functions associated with increased adenosine triphosphate metabolism and nucleic acid synthesis (Bankar et al., 2009). Others needed for microbial growth include potassium in yeast strains, which was found to be essential for osmoregulation. Irons are essentially used for heme and cytochrome synthesis (Venkateshwar et al., 2010), and calcium was found to be necessary for effective lipase stabilization and activity in *Acinetobacter* sp. (Snellman and Colwell, 2004).

Effects of physical factors

The physical parameters such as pH, temperature, agitation, aeration and inoculum have great influence on the lipase production. Some of these parameters are better controlled in bioreactors, which are mechanical vessels in which microorganisms are cultivated under controlled conditions.

Temperature and pH

Most lipase producing organisms are mesophilic in nature (growing in moderate temperature typically between 25 and 40℃). However, some psychrophilic and thermophilic organisms have been reported in the literature. Lipase production by a wild-type Brazilian strain of *P. simplicissimum* showed an activity of 90 U/g after 72 h incubation in the presence of abundant residue of babassu oil industry. The enzyme was found to have high activities at 35 to 60℃ (Gutarra et al., 2009). Also, some psychrophilic strains reported to produce lipases include *Acinetobacter* sp*., Achromobacter lipolyticum, Aeromonas hydrophila, Bacillus sphaericus, Photobacterium lipolyticum, Morexella* sp*., Pseudomonas fluorescens, Pseudomonas fragi, Psychrobacter okhotskensis, Serratia marcescens* and *Staphylococcus epidermidis* (Joseph et al., 2007). Generally the temperature required for lipase production corresponds with the growth conditions of most microorganisms. Thus, some of the physical conditions reported for enhanced lipase production by different organisms are represented in Table 1.

pH being a measure of acidity or basicity of a medium, plays an important role in determining the type of organisms that can colonize a particular substrate. Fifty-nine lipase-producing fungal strains were isolated from Brazilian savanna soil by employing enrichment culture techniques. The most productive strain identified as *Colletotrichum gloesporioides*, showed lipase activities between 27.7 and 27.4 U/ml when cultured in shaken

Table 1. Fermentation conditions required by different microorganisms for lipase production.

Microorganism	pH	Temperature (°C)	Fermentation time (h)	Agitation speed (rpm)	Reference
Bacillus sp.	6	30	64	100	Etugrul et al. (2007)
Pseudomonas sp.	7.5	25 to 28	24	150	Haba et al. (2000)
Burkholderia multivorans	7	37	36	250	Gupta et al. (2007)
Aspergillus sp.	7	30	144	120	Colla et al. (2010)
Rhizopus chinensis CCTCC M201021	6	30	72	150	Pagori et al. (2008)
Rhizopus homothallicus	6.5	40	22	170	Diaz et al. (2006)
Serratia marcescens	7	30	12	120	Long et al. (2007)
Penicillium citrinum	7	22	144	150	Maliszewska and Mastalerz (1992)
P. aurantiogriseum	7	29	48	120	Lima et al. (2003)
Candida sp.	6	26	120	220	He and Tan (2006)
C. cylindracea	6.5	27	120	160	Haba et al. (2000)

liquid medium under alkaline conditions at pH range of 7.4 to 8.4 (Colen et al., 2006).

Geon-Ho et al. (2007) reported that lipase from *Y. lipolytica* NRRL Y-2178 was produced under alkaline conditions at pH 9.0. Brozzoli et al. (2009) monitored lipase production in a 3-L stirred tank reactor to assess the effects of medium pH on the *C. cylindracea* enzyme activity. At constant pH of 6.5, lipase production was low (1.8 U/ml), but significant increase was seen up to 18.7 U/ml with uncontrolled pH and was maximum (20.4 U/ml) when the pH was allowed to vary freely below pH 6.5.

Most lipases that are active at extremely acidic pH (pH 1.5 to 2.0) are mainly from mammalian sources e.g. gastric lipase. However, *Aspergillus niger* NCIM 1207 shows high level of extracellular lipase when cultured at pH 2.5 (Mhetras et al., 2009). Also, high lipase producing micro-organisms such as *Rhizopus* sp., *Aspergillus* sp., *Penicillium* sp., *Geotrichum* sp., *Mucor* sp., and *Rhizomucor* sp. (Treichel et al., 2010), *Bacillus* sp., *Pseudomonas* sp., *Burkholderia* sp. (Gupta et al., 2004), *C. cylindracea* and *Yarrowia lipolytica* (Vakhlu and Kour, 2006) grow and produce lipases at pH ranges of 6 to 8.

Aeration and agitation

Aeration and agitation are among the physical factors that are important in enhancing and optimizing the lipase production. Thus, growth of microorganisms and enzyme production are affected by agitation and aeration based on oxygen supply during the lipase production process especially in bioreactors. Fickers et al. (2006) studied the lipase production by *Y. lipolytica* in a 2000-L bioreactor containing glucose 1.5%, whey powder 3%, yeast extract 3%, corn steep liquor 1%, olive oil 0.5%, and $(NH_4)_2SO_4$ 0.8% as the optimum medium with a working volume of 1100 L. It was found that stirring speed of 120 rpm and an air flow of 0.7 vvm at 29°C led to a lipase activity of approximately 1100 U/ml after 53 h of fermentation.

The effects of both media and process parameters (aeration and agitation) on lipase production using *Rhodotorula mucilaginosa* MTCC 8737 in 1.5-L stirred tank reactor with molasses as sole production medium was studied by Potumarthi et al. (2008). Various aeration (1, 2, and 3 vvm), agitation speeds (100, 200, and 300 rpm) and molasses concentration (1.0, 1.5 and 2.0%) were explored and the maximum lipase activity of 72 U/ml was obtained after 96 h of fermentation at 2 vvm, 200 rpm, pH 7, and 25±2°C temperature using 1% molasses. Sokolovska et al. (1998) used 2-L batch fermentor to study the effects of aeration, nature and concentration of substrates on extracellular lipase production of *C. cylindracea* CBS 6330. Oxygen saturation above 20% favored the lipase production, thus a combined flow control of air (84 L/h) and oxygen (8.4 to 50.1 L/h) was used at a stirring rate of 500 rpm, temperature of 30°C and non-controlled pH using 10 g/L of olive oil to achieve the best production.

Alonso et al. (2005) studied the lipase production by a Brazilian wild strain of *Y. lipolytica* at different agitations (100 and 400 rpm) and air

flow rates (0.8 to 2.5 vvm) in 2-L bench fermenter. Maximum lipase production was achieved at agitation and aeration of 200 rpm and 0.8 vvm respectively. Based on this, it was concluded that higher stirring speeds may cause mechanical and/or oxidative stress to the microorganism, while lower stirring speeds may affect the oxygen levels and uptake. On the other hand, an increase in the availability of oxygen at higher air flow rates lead to faster substrate uptake with anticipation of enzyme release into the culture medium.

Inoculum concentration

The amount of inoculum present during the fermentation process can affect the lipase production in many microbial strains. Thus, high inoculum sizes might not necessarily result in higher lipase yield and often lead to oxygen and nutrient depletion in the culture media and thus affecting the overall productivity (Rahman et al., 2005). In order to improve the lipase production in *Serratia marcescens* ECU1010, fermentation experiments were carried out in 250 ml flasks containing 50 ml of medium comprising (w/v) of dextrin 1.5%, beef extract 1.0%, Tween-80 1.0%, $(NH_4)_2SO_4$ 0.2%, KH_2PO_4 0.2%, NaCl 0.1% and $MgSO_4$ 0.05%. The process conditions that resulted in highest lipase production were 30°C, 200 rpm and 10% (v/v) of the seed culture (Zhao et al., 2010). Dominguez et al. (2005) reported the use of 5% (v/v) inoculum of *Thermus thermophiles* HB27 for lipase production in a 5-L stirred tank bioreactor (Biostat B, Braun, Germany), containing 3-L of medium, operating without pH control at an agitation speed of 200 rpm. This led to a maximum lipase production of 158 U/g cells. Ten microorganisms, six strains belong to *Penicillium* sp. (E-01PC, E-04PC, E-06PC, E-07PC, E-08PC and E-10PC), *Mucor hemalis*, *Rhizopus oryzae*, *Candida cylindracea* (ATCC 14830) and *Penicillium citrinum* (ATCC 42799) were screened by Salihu et al. (2011a) for their potential to produce lipase in palm oil mill effluent (POME)-based medium. The most promising strain was found to be *C. cylindracea* (ATCC 14830) at 2% (v/v) inoculum concentration with appreciable activity both on solid and liquid cultures. However, Brozzoli et al. (2009) showed that 0.2% inoculum of *C. cylindracea* (equivalent to 1×10^6 cells) was found to be adequate for lipase production in olive-mill media in both shake flasks and 3-L bioreactor, and the maximum activities of 10 and 20.4 U/ml were realized in shake flasks and 3-L bioreactor, respectively.

LIPASE PRODUCTION USING STATISTICAL EXPERIMENTAL DESIGN

Initial development of an effective medium for lipase production involves selection of carbon source, nitrogen source, inorganic salts, trace elements and growth factors. With recent technological advancement, it is common for the appropriate nutrients and optimal concentrations to be established after the initial formulation of the medium (Ooijkaas et al., 2000). This technique is found to be suitable to increase the productivity of any bioprocess techniques (Dominguez et al., 2003), and thus regarded as optimization process. Media optimization is one of the best strategies used for enhancing the production of any product of interest, which is achieved by studying the composition of production medium. Statistical approach that involves the use of Plackett–Burman (PB) design and Response Surface Methodology (RSM) has been widely used for optimization processes, so that interactions as well as effects of various physico-chemical parameters can be determined using a minimum number of experiments (Gupta et al., 2007). Several conventional medium and renewable agro-industrial residues used for lipase production have been optimized using this approach. This is achieved by sequential steps of screening using PB design, optimizing the important components and verifying the statistically developed medium that lead to the best possible response through RSM (Gohel et al., 2006). The RSM evaluates the effects of several process variables and their interactions on response variables. It helps in the design of experiments, building of models, evaluating the effects of factors, and analyzing optimum conditions of factors for desirable responses. Therefore, RSM is faster and more informative than the empirical method of classical one-factor- at-a-time approach (Li et al., 2007; Ozddemir et al., 2008).

Thus, PB design is a well-established factor screening experiment widely used for selecting the medium components in shake flask cultures. In order to come up with the best media components influencing the lipase production by *Pseudomonas aeruginosa*; Ruchi et al. (2008) screened 11 media components for lipase production (peptone, tryptone, NH_4Cl, $NaNO_3$, yeast extract, glucose, glycerol, xylose, gum arabic, $MgSO_4$, and NaCl) using PB design. Based on their findings, the most significant factors affecting the production (gum arabic, $MgSO_4$, tryptone, and yeast extract) were optimized by the RSM. The results revealed that the optimized media led to 5.58-fold increase in lipase production (4580 IU/ml) over the un-optimized one.

Also, Salihu et al. (2011b) studied the effects of eleven medium components using POME as the main substrate by *P. citrinum* (ATCC 42799) on lipase production. Preliminary screening using Plackett–Burman (PB) design was carried out and the most significant components affecting lipase production were found to be Tween-80, peptone, yeast extract, malt extract and $NaNO_3$ at $P < 0.05$. In case of *C. cylindracea* lipase production, maximum activity of 20.26 U/ml was realized based on sequential steps involving the use of PB design, one-factor-at-a-time (OFAT) method and response surface methodology (RSM). The developed

Table 2. Selection of media components influencing the lipase production through statistical techniques.

Media component		Microorganism	Lipase activity (U/ml)	Reference
PB design	**RSM**			
Maltose, Olive oil, Peptone, K_2HPO_4, Agitation, Inoculum size, Fermentation volume, pH	Inoculum size, Olive oil, Fermentation volume, Peptone	*Rhizopus chinensis* CCTCC M201021	13.875	Teng and Xu (2008)
Sunflower oil, Glucose, Peptone, Agitation, Incubation period	Sunflower oil, Glucose, Peptone, Agitation, Incubation period	*Aspergillus carneus*	12.7	Kaushik et al. (2006)
pH, Temperature, Agitation, Olive oil, Glucose, Peptone, Tween-80, NaCl	Temperature, Glucose, Olive oil	*Bacillus brevis*	5.1	Rajendran and Thangavelu (2010)
Soybean oil, $(NH_4)_2SO_4$, K_2HPO_4, KH_2PO_4, Soybean powder, $MgSO_4$, Span 60	Soybean powder, Soybean oil, KH_2PO_4	*Candida* sp. 99-125	6230	He and Tan (2006)

optimum medium contains 0.45% (w/v) peptone, 0.65% (v/v) Tween-80 and 2.2% (v/v) inoculum concentration (Salihu et al., 2011c). Statistical optimization strategies involving the use of PB and RSM to enhance the lipase production using different substrates and microorganisms are represented in Table 2.

BIOREACTOR SYSTEMS

Fermentation processes have been conducted in different types of bioreactors using batch, repeated-batch, fed-batch, and continuous mode. In most cases the choice of mode of operation is to a large extent, dictated by the characteristics of the product of interest (Treichel et al., 2010).

Lipase production by *Y. lipolytica* W29 and *C. cylindracea* CBS 7869 on olive mill wastewater supplemented with ammonium chloride and yeast extract was studied in batch and fed-batch mode of operation in 2-L bioreactor at pH 7.2, 500 rpm, with constant or variable aeration. Batch operation was found to be more appropriate for lipase production than fed-batch for both strains but the difference was more pronounced in *C. cylindracea* CBS 7869 (Goncalves et al., 2010).

Deive et al. (2009) studied the lipolytic enzymes by *Thermus thermophilus* HB27 in 5-L laboratory scale stirred-tank and airlift bioreactor to determine the most suitable reactor system for the production. Stirred-tank configuration showed the highest extracellular lipolytic enzyme, which was 2.3-fold higher than those found in the airlift bioreactor. The results revealed that lipase production in stirred tank system was less dependent on aeration than agitation rate based on their relationship with mass transfer coefficient. Thus, mechanical stirring of the bioreactor affects the rotund bodies found on the membranes of thermophilic microorganisms, thereby increasing the extracellular enzyme production.

In case of *Geotrichum candidum,* lipase production in both 3-L stirred tank and airlift bioreactors were compared by Burkert et al. (2005). Using the optimum conditions of agitation and aeration of 300 rpm and 1 vvm respectively in stirred tank bioreactor, the lipase activity reached 20 U/ml after 54 h of fermentation. However, similar lipase activity was obtained after 30 h of fermentation in the airlift bioreactor at the maximum aeration condition of 2.5 vvm. Based on this, airlift bioreactor was found to be two-fold higher in terms of productivity than the stirred tank reactor. Kim and Hou (2006) used oleic acid as an inducer and carbon source for extracellular lipase production by *C. cylindracea* NRRL Y-17506. The highest lipase activity obtained in flask culture was 3 U/ml after 48 h of fermentation. Fed- batch cultures (intermittent and stepwise feeding) were carried out in a 7.5-L bioreactor to improve the cell concentration and lipase activity. For the intermittent feeding, the final cell concentration was 52 g/L and the lipase activity reached 6.3 U/ml after 138.5 h of fermentation. However, using stepwise feeding to investigate the effects of specific growth rate on cell growth and lipase production, the highest cell concentration of 90 g/L was obtained when the set point of specific growth rate was 0.02 h^{-1} and the highest lipase activity was 23.7 U/ml at 179.5 h.

The influence of temperature, agitation and

aeration was investigated in a 2-L stirred-tank bioreactor by Salihu et al. (2011d). The optimum temperature, aeration and agitation rates were found to be 30℃, 1.0 vvm and 400 rpm respectively, with a maximum activity of 41.46 U/ml after 36 h of fermentation. Thus, this study confirmed that POME can be used as a fermentation medium for lipase production and the process aids in chemical oxygen demand (COD) removal throughout the fermentation period.

APPLICATION OF LIPASES FOR INDUSTRIAL PROCESSES

Lipases are forecasted to be among the fastest growing enzymes based on their applications in organic synthesis, pharmaceuticals, and by expanded penetration into the detergent industry and biofuel production. The total industrial enzyme market in 2009 was estimated to be about US $2.4 billion and it is expected to have 4 to 5% average annual growth rate (Hasan et al., 2006; Snellman and Colwell, 2004). Lipases have been extensively utilized in production of various products of industrial importance as indicated below:

Lipase in detergency

New detergents with different enzyme formulations especially from United States, Europe and Japan, have been emerging continuously for different purposes utilizing different enzyme systems such as lipase, protease, amylase and cellulase. Stability at higher pH and temperature is required for the enzyme to be used in detergent formulation. Thus, alkaline lipases and proteases are used in the formulation (Khoo and Ibrahim, 2003).

Novo Nordisk identified a lipase from *Humicola* strain capable of dissolving fatty stains. Several strategies to increase the yield proved unsuccessful using conventional and traditional methods but through molecular techniques, the gene coding for this lipase was cloned and inserted into *A. oryzae,* which produced the enzyme in commercially relevant yields and aided in better washing performances with energy savings (Hasan et al., 2006). *Bacillus* sp. B207 and *Pseudomonas paucimobilis* produce alkaline lipases that can be used as additives in the formulation of detergent (Khoo and Ibrahim, 2003). Both lipases showed excellent pH and temperature stability in the range of 7.0 to 9.0 and 30 to 50℃, respectively. Jaeger and Reetz (1998) showed that about 1000 tonnes of lipases are added annually to approximately 13 billion tonnes of detergents produced.

Food industry applications

Modification of fats and oil based on their structure and composition is of great importance in the food industries. The ability of lipolytic reactions to occur in both aqueous and non-aqueous media has been indicated as a novel approach for the production of several products of importance in food industries. The products obtained from lipase catalyzed reactions are considered to have wide application in flavour synthesis, wines, baked foods, emulsifiers, supplements and dairy products (Rajendran et al., 2009). Also, lipase catalyzed reactions can be used to modify and upgrade cheap oils into a nutritionally important structural triacylglycerols; such as low caloric triacylglycerols, PUFA-enriched and oleic acid enriched oils (Gupta et al., 2003).

Flavour development

Low molecular weight esters such as ethyl, isobutyl, amyl and isoamyl acetates have been widely used for flavour development in the food industry. Flavour substances such as S-methyl butanethioate and S-methyl 3-methyl butanethioate are important components of the dairy aromas, especially cheese aroma and of fruit aromas like strawberry and banana (Shieh and Chang, 2001; Rajendran et al., 2009). All these can be produced by lipase catalyzed reactions based on their unique specificity, high reaction rate even at low molar fractions and activity in organic solvents.

Transesterification of hexanol and tributyrin by immobilized lipase (Lipozyme IM-77) from *Rhizomucor miehei* led to the formation of excellent flavour and fragrance. Esterification of citronellol and geraniol with short-chain fatty acids are widely applicable in beverages production (Chang et al., 2003).

Cocoa butter equivalents

Lipase catalyzed interesterification has been used particularly in the production of cocoa butter-type triacylglycerols, exploiting the microbial lipase that are 1, 3 regio-specific (Macrae and Hammond, 1985). The interesterification by 1,3 regio-specific lipases has been used to enrich low-cost fats such as palm-oil fractions into 1, (3) palmitoyl, 2-oleoyl, 3(1) stearoylglycerol and 1(3) stearoyl, 2-oleoyl, 3(1) stearoylglycerol, which have immense applications as confectionary fats. Chocolate contains 30% cocoa butter, which gives it a required crystallization properties as well as melting characteristics. However cocoa butter tends to be very expensive. Thus, an alternative source from fat mixtures was developed that requires an initial mixing of palm mid fraction and stearate ester; followed by dehydration and lipase enzymatic reactions. Further processes such as distillation and solvent fractionation are necessary for the required product formation. This process has been used extensively in commercial production of cocoa butter equivalent by Loders Croklaan of the Uniliver Group in

Wormerver, Netherlands (de Castro and Anderson, 1995).

Resolution of racemic acids and alcohols

The stereospecificity as a unique feature of lipases is widely employed in identification of racemic organic acid mixtures in immiscible biphasic systems via esterification and transesterification reactions (Klibanov, 1990; Sharma et al., 2001). Tsai and Dordick (1996) studied the characteristics of both pure and crude lipases isolated from *C. rugosa* in aqueous and organic solvents. The purified enzyme was found to be less active than the crude enzyme in organic media, whereas presence of small quantity of water stimulated the activity of the purified enzyme by several folds in the esterification of racemic 2-(4-chlorophenoxy) propanoic acid with *n*-butanol. Lipases have been found in resolving racemic mixtures especially in enantiomers such as non steroidal anti-inflammatory drugs that are pharmacologically active mainly in the (S)-enantiomeric form. Thus, pure (S)-ibuprofen, which is the active form is obtained using lipase-catalyzed kinetic resolution via hydrolysis and esterification (Ducret et al., 1998; Xie et al., 1998). The (S)-ibuprofen [(S)-2(4-isobutylphenyl) propionic acid] is 160 times more active than its antipode in inhibiting prostaglandin synthesis.

Lipases in oleochemical industry

Alcoholysis, acidolysis, hydrolysis and glycerolysis are the common reactions associated with oleochemical industry. These reactions are energy intensive with high temperature requirement of 240 to 260℃ and high pressure. However, utilization of lipases can minimize the energy consumption (Bornscheuer, 2000, Sharma et al., 2001). For instance, commercial use of *C. cylindracea* lipase in production of soaps was reported. The enzymatic method resulted in a superior product at lesser cost than the conventional chemical process (Saxena et al., 1999). The current trend in the oleochemical industry involves the use of immobilized lipases to initiate various reactions (hydrolysis, alcoholysis, and glycerolysis) using mixed substrates. Thus, the use of immobilized enzyme ensures high productivity as well as continuous running of the processes. This offers a greatest hope for successful fat splitting/modification without substantial investment in expensive equipment as well as in expenditure of large amounts of thermal energy (Saxena et al., 1999).

Biodegradation of plastics

The use of biodegradable plastics is widely employed as a clean technology measures to curtail environmental problems; however, there are biodestructible plastics, which are used interchangeably despite their differences. The main difference between biodestructible and biodegradable plastics is the extent and rate of degradation, where the former requires further treatment unlike the latter. de Castro and Anderson (1995) reported that Fermentation Research Institute Tsukuba, Japan devised strategies using lipases for complete destructibility of plastics. This is based on the ability of lipases to degrade polycaprolactone (an aliphatic polyester), which can be mixed with plastics to enhance their rate of degradation.

Lipases in fatty acids unsaturation

Regulation of synthesis of unsaturated fatty acids (UFAs) depends on the environmental and nutritional changes in microorganisms, plants and animals. Unsaturation in fatty acid chains plays significant structural and functional roles in cell membranes; thus a proper ratio of saturated to UFAs contributes to membrane fluidity. Alterations in this ratio have been linked to various disease states including cardiovascular diseases, immune disorders, cancer and obesity (Mansilla et al., 2008).

He and Shahidi (1997) reported that acylglycerols containing EPA (all-*cis*-5,8,11,14,17-eicosapentaenoic acid) and DHA (all-*cis*-4,7,10,13,16,19-docosahexaenoic acid) can be synthesized using glycerol and ω-3 fatty acid concentrates from seal blubber oil through esterification reaction of Lipase LP-401-AS, with a maximum yield of 94.3% under standardized assay conditions. Several products are now studied to enhance their nutritional quality by incorporating omega-3-poly-unsaturated fatty acyl content of bulk oil; this is important in using enzyme processing techniques for production and protection of the omega-3 oils against oxidation which affect the process operation (Ibrahim et al., 2008).

Biodiesel production

The process of production of biodiesel as an alternative fuel using natural oils and fats is environment friendly since these substrates are free of nitrogen and sulphur compounds. This process will markedly reduce the greenhouse effect and air pollution produced by the fossil fuels (Li et al., 2009). The biodiesel production can be achieved by chemical or enzymatic methods. The conversion of oils to methyl- or other short-chain alcohol esters can be obtained in a single transesterification reaction using lipases in organic solvents (Jaeger and Eggert, 2002). Thus, several problems associated with chemical production that impede its continued growth, such as glycerol recovery and the need to use refined oils and fats as main substrates can be overcome by

enzymatic transesterification reactions (Kramer, 1995).

Haas and Foglia (2005) reported that residual oil from soy, rapeseed, and palm oil refining waste extracted and recovered from hexane when subjected to methanolysis by *R. oryzae* lipase in the presence of water content and methanol; highest conversion to methyl esters was observed in palm oil with about 55% yield after 96 hr reaction. Thus, several strategies are now suggested to harness some of the production problems especially in increasing the enzyme's stability upon repeated use by immobilization (Iso et al., 2001) and overproduction of lipases in the target organisms by genetic manipulation for efficient methanolysis in a solvent-free reaction system (Matsumoto et al., 2001).

CONCLUSION

Lipases have diverse applications in food (flavour modification), fine chemicals (synthesis of esters), detergent (hydrolysis of fats), fat modification, biodiesel production, etc. The worldwide rising demand on the use of renewable energy showed that lipases have tendency to dominate the global enzyme market in the near future. This calls for detailed understanding of lipase production using conventional and renewable raw materials by different fermentation techniques as well as identification of potential microorganisms and factors that can improve the production. The use of experimental design techniques presents a more balance alternative to one-factor-at-a-time approach typically employed in fermentation studies. Using some of these strategies in producing this biocatalyst may help in alleviating some of the challenges affecting the large scale production. Thus, in order to meet up with these challenges, the focus should be on getting the right microbial strain through screening and modification, selection of appropriate medium components, identification of physical parameters and finally the intended applications of the produced lipases. Thus, any production strategies that put these into consideration will be highly effective.

Some of the problems affecting lipase production in developing countries may be related to economic barriers, issues associated with design, process, control and mechanism of catalytic reactors. Based on the information available in the literature, these challenges are achievable in the near future.

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