Vol. 1 (2) (2025) 45-51

IJBAHR

PRODUCTION OF LIPASE FROM *BACILLUS SP*. AND ITS APPLICATION IN THE MAKING OF BIODIESEL FROM USED COOKING OIL

Shahana Rasheed Qureshi[1*], Sana Ahmad[2], Aliya Riaz[1], Isha Arif[1], Ayesha Usman[1] and Laiba Noor[1]

Department of Biochemistry, Jinnah University for Women, Karachi, Pakistan[1]
Bahria Medical College and University, Karachi, Pakistan[2]

Abstract: There is a pressing need for affordable and environmentally responsible fossil fuel substitutes because of the depletion of fossil fuels, rising greenhouse gas emissions, and the growing demand for renewable energy. Utilizing waste cooking oil (WCO) to produce biodiesel reduces the environmental risk associated with inappropriate oil disposal while producing a sustainable energy source. With benefits over chemical catalysis, lipase-catalyzed transesterification has emerged as a productive method for the synthesis of biodiesel. With an emphasis on critical factors that affect enzyme activity and substrate conversion efficiency, this study attempts to maximize lipase production and assess its use in biodiesel synthesis from WCO. Lipase was produced by microbial fermentation. Thorough research was done on the effects of temperature, carbon and nitrogen sources, incubation time, tolerance to organic solvents, and substrate concentration. Under ideal circumstances, the biodiesel yield from WCO was measured. According to the study's findings, lipase activity peaked at 40°C after a 24-hour incubation period. The best sources of carbon and nitrogen are ammonium phosphate and olive oil, respectively. The enzyme demonstrated exceptional resistance to the organic solvent methanol. Studies on substrate concentration revealed that 0.1% was the ideal level. Under these favorable conditions, WCO was converted to biodiesel in an efficient and significant manner.A lipase-catalyzed method of producing biodiesel from spent cooking oil shows promise as a sustainable and eco-friendly substitute for traditional diesel. The goal of ongoing research and development is process optimization, tackling issues with lipase stability and scalability.

Key words: Lipase optimization, *Bacillus sp*., Waste cooking oil, Fatty acid methyl esters, Biodiesel

INTRODUCTION

The depletion of fossil resources, environmental pollution, and rising greenhouse gas emissions are posing previously unheard of challenges to the global energy sector. Petroleum-based conventional diesel fuels greatly increase air pollution and climate change, highlighting the urgent need for environmentally friendly, renewable, and sustainable alternatives (Liu *et al*. 2011; Wang & Azam, 2024). Renewable energy sources that can lessen reliance on fossil fuels, cut harmful emissions, and promote environmental sustainability; biodiesel has emerged as a promising reply to these problems (Ogunkunle & Ahmed, 2021). In addition to its nutritional value, starch is an essential industrial raw material that is used in food, medicine, textiles, fermentation, adhesives, and paper (Akinfemiwa, *et al*. 2023). Fatty Acid Methyl Ester (FAME), one of the different types of biodiesel, attracted special attention because of its advantageous physicochemical characteristics and suitability for use with current diesel engines and infrastructure. Triglycerides made from vegetable oils, animal fats, or waste cooking oil (WCO) are transesterified with short-chain alcohols like methanol to create FAME, typically with the aid of a catalyst (Meher *et al*., 2006; Wcisło & Labak, 2017).WCO addresses two significant problems simultaneously: the environmental hazards associated with inappropriate disposal of used cooking oils and the rising demand for renewable energy (Avagyan & Singh, 2019). By converting a common waste product into a useful energy source, WCO-based biodiesel production not only provides an environmentally friendly fuel alternative but also aids in effective waste management. Although the production of biofuel has increased globally in recent decades, it still only makes up less than 3% of the fuel used for transportation worldwide. This highlights the urgent

need for sustainable feedstock and enhanced production systems in order to meet future energy demands (Wang & Azam, 2024; Meher et al., 2006).Waste cooking oil (WCO) has become more popular as an inexpensive and plentiful source of feedstock for the production of biodiesel. WCO addresses two significant problems simultaneously: the environmental hazards associated with inappropriate disposal of used cooking oils and the rising demand for renewable energy (Avagyan & Singh, 2019). By converting a common waste product into a useful energy source, WCO-based biodiesel production not only provides an environmentally friendly fuel alternative but also aids in effective waste management. Although the production of biofuel has increased globally in recent decades, it still only makes up less than 3% of the fuel used for transportation worldwide. This highlights the urgent need for sustainable feedstock and enhanced production systems in order to meet future energy demands (Wang & Azam, 2024; Meher *et al*., 2006). When different edible vegetable oils are used to cook food in residences, hotels, restaurants, and other catering establishments, WCO is typically produced. In most developed countries, vegetable oils make up 15-20% of total caloric intake and are primarily triacylglycerols (88-98%), making them an important component of a balanced diet. Vegetable oil consumption increased from 150 million metric tonnes (MMT) in 2013–14 to over 200 MMT in 2020–21. By 2027, the global WCO market is anticipated to have grown from $5.50 billion in 2019 to $8.48 billion. (Meher *et al,* 2006).

At commercial scales, the production of FAME with traditional chemical catalysts, like sodium or potassium hydroxide, has shown effectiveness; however, it is often linked to the production of unwanted by-products, like soaps, and requires lengthy purification procedures. Additionally, FAME biodiesel presents operational challenges that could compromise fuel quality and storage, including oxidative instability, microbial contamination, and moisture absorption (Benjumea *et al*., 2008).The stability and longevity of FAME as a sustainable fuel can be ensured by utilising antioxidants,el iminating water, and adhering to appropriate storage procedures (Longanesi *et al*., 2022).

Enzyme-catalyzed biodiesel production has become a viable and effective way to get around these limitations. Compared to chemical catalysts, lipases, which are hydrolase enzymes capable of catalysing both hydrolysis and esterification processes, offer a number of benefits. They can use low-quality feedstock's, like WCO with a high concentration of free fatty acids, and they limit the production of unwanted byproducts while facilitating the synthesis of biodiesel under mild reaction conditions (Fernando *et al*., 2007; Atadashi *et al.,* 2012). Microbial lipases particularly those made by bacteria and fungi like *Bacillus sp*., *Aspergillus niger*, *Candida rugosa*, and *Rhizomucor miehei* are utilised extensively due to their high yield, stability, and genetic manipulation tolerance (Chandra *et al*., 2020; Ali *et al*., 2023) (Table 1). For industrial applications, lipases can be immobilised on a range of supports to enhance stability, recovery, and reuse (Meinert & Meierhenrich, 2012).

Table 1: Source of lipases used in industrial and biodiesel applications

BACTERIUM	REFERENCES
hrobacter sp.	dey *et al.* 1999
illus sp. Strain DVL 2	mar *et al.,* 2012
illus sp. RN 2	njanavas *et al*., 2010
illus steorothermophilus	ssedah *et al*., 2011
illus subtilis Pa 2	h *et al*., 2011
illus Coagulans ZJU318	nghua *et al*.,2005
illus sp. MPTK 912	kesh *et al*., 2012
erococcus faecalis	*et al.* 1996
robacterium luteolum	eph *et al.,* 2012
raxella sp.	ger et al. 1999
udomonas aureofaciens	itala *et al.* 1987
phylococcus sp.	nbhurkar *et al.,* 2012

Immobilisation increases thermal stability, enhances product separation, and shields enzymes from solvent deactivation. Titrimetric assays enable precise reaction condition adjustment by measuring free fatty acids generated during catalysis and tracking enzyme activity (Ray, 2012). According to Bisen *et al.* (2010), lipase-catalyzed processes offer a number of benefits, such as low environmental impact, high conversion efficiency, selectivity, and gentle working conditions. Beyond the production of biodiesel, lipases have numerous industrial uses. They reduce denim streaking and increase fabric absorbency in the textile industry (Sarmah *et al.,* 2018).Lipases alter fats and oils in food and baking, enhance flavour, and help create products like mayonnaise (Mehta *et al*., 2021).In order to improve analytical precision in the food, pharmaceutical, and clinical sectors, lipases are also used as biosensors to measure triacylglycerols (Sarmah *et al*., 2018). By removing grease at lower temperatures, they help the detergent industry reduce its environmental impact and power consumption. Their versatility and commercial potential are further demonstrated by additional applications such as wastewater treatment, pulp and paper processing, tea fermentation, cosmetics and perfumery, and more (Wcisło & Labak, 2017; Fahim *et al*., 2024; Hu *et al*., 2018).

A sustainable and economical method of producing biodiesel is the incorporation of Bacillus sp. lipase to convert waste cooking oil (WCO) into FAME (Patade *et al*., 2018). According to Sarmah *et al*. (2018), using WCO lowers feedstock costs, lessens environmental pollution, and follows the circular economy. Microbial enzyme catalysis, efficient immobilisation, and reaction tuning can be used to boost FAME yield, enhance fuel quality, and create scalable biodiesel production systems with negligible negative environmental impacts (Verma *et al*., 2013). The purpose of this work is to determine whether lipase-catalysed transesterification with *Bacillus sp*. as a biocatalyst is feasible for converting waste cooking oil into fatty acid methyl esters

(FAME). The goal of the research is to improve catalytic efficiency and yield by optimising key process parameters such as temperature, incubation time, carbon and nitrogen sources and solvent tolerance. This will help to advance the production of sustainable biodiesel that satisfies industrial quality standards

.

MATERIALS AND METHODS

Isolation of the enzyme

The biological process of separating a specific enzyme from a complex mixture is known as enzyme isolation. Since we were able to isolate the lipase enzyme, any plant, animal, or microbial tissue could be the source.

Preparation of Nutrient Broth

Add 1.30 gm of Nutrient Broth to 100 ml of deionized water. Then the media was autoclaved for 2 hours.

Screening of isolated culture

Add 1.30 gm of nutrient broth and 4.0gm of Agar agar in 100 ml deionized water. The slants were autoclaved, and the tube was tilted for a full day at room temperature to solidify.

Media Optimization

For maximum lipase production, culture conditions were optimized. Temperature optimization was done over a range of 30°C to 60°C, and the impact of incubation time was evaluated at 24, 48, and 72 hours. Various carbon sources (olive oil, coconut oil, mustard oil, castor oil, starch, and ghee) and nitrogen sources (Urea, Yeast extract, Peptone, Ammonium sulphate and Ammonium phosphate) were added to the production medium in order to assess the impact of nutrient availability. Furthermore, to ascertain their function in promoting or impeding lipase activity, the effects of organic solvents such as ethanol, methanol, acetone, and n-butanol were examined.

Preparation of Phosphate buffer

A 0.2 M solution of monobasic sodium phosphate (0.35 gm. in 250 ml) and a 0.3 M solution of dibasic sodium phosphate (0.67 gm. in 250 ml) were combined to make the buffer.

Preparation of Olive Oil substrate

Olive oil (1%) and Triton (0.1 ml) were added to 100 ml of Phosphate buffer.

Preparation of Lipase producing media

The lipase production medium was prepared with calcium chloride (1.0 gm), potassium dihydrogen phosphate (0.01 gm), yeast extract (2.0 gm.), olive oil (1.0 ml), and Tween-80 (0.10 gm) as essential ingredients to support microbial growth and enzyme induction.

Enzyme assay

The titrimetric method, which measures the amount of fatty acid released by hydrolysing olive oil at pH 7.0, was used to determine the lipase activity isolated from *Bacillus sp*. The scheme shows the steps involved in the titrimetric assay used to measure lipase activity. The activity was calculated by titration with 0.005M sodium hydroxide (NaOH) using phenolphthalein as a marker until the final product was achieved.

$$\text{Enzyme Activity (uM/ml/min)} = \text{T-C} * \frac{0.005}{60/5} \times 1000 \times 1000$$

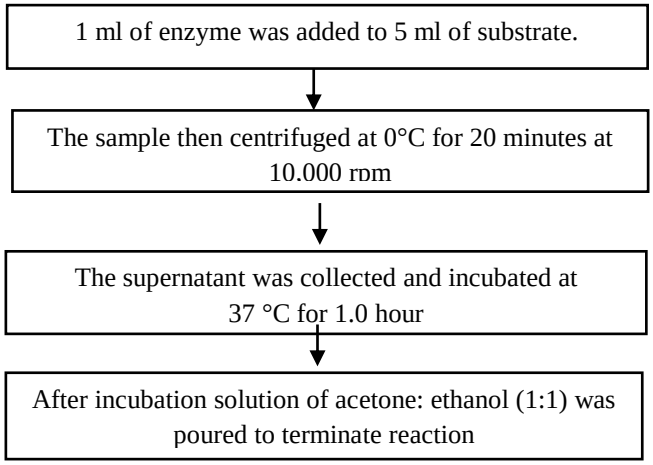

Figure 1: Schematic of the titrimetric assay to measure the lipase activity by hydrolysing an Olive oil substrate.

RESULTS

Effect of carbon source on lipase production

The production of lipase was significantly impacted by the carbon source selection (Figure 2). Olive oil was the most appropriate of the different carbon sources. Though they can be regarded as the next best carbon sources for enzyme induction, the presence of starch and ghee in the middle also affects the progression. On the other hand, mustard oil only moderately supported activity, and coconut and castor oils were less effective**.**

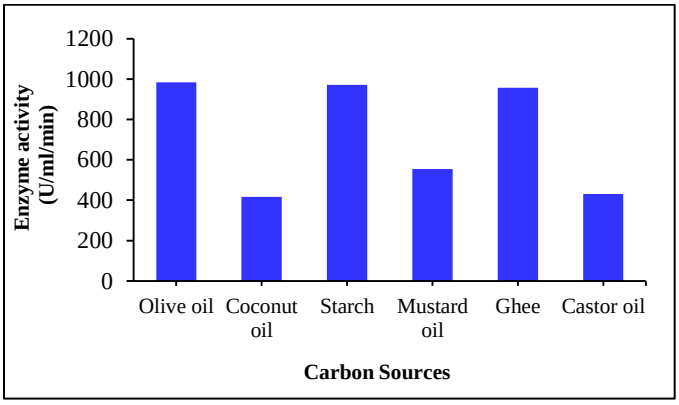

Figure 2: Effect of carbon sources on Lipase production

Effect of nitrogen source on lipase production

Nitrogen sources allow bacteria to grow quickly and produce large amounts of cells compared to inorganic nitrogen sources. Nitrogen is often the most limiting factor for crop productivity because it often affects the amino acid composition of proteins, which in turn affects their nutritional value. Other inorganic and organic sources were less effective than ammonium phosphate, which was the most

effective of the sources studied and promoted the maximum amount of enzyme synthesis (Figure 3).These results highlight how crucial it is to choose the right nitrogen source in order to optimize microbial growth and lipase yield.

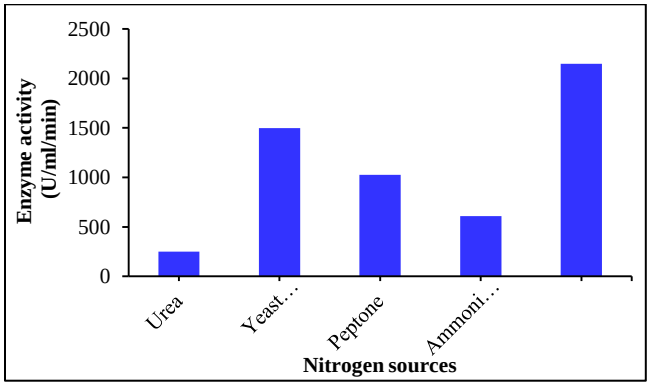

Figure 3: Effect of various nitrogen sources on lipase production

Effect of incubation time on Lipase production

The longer a bacterium is incubated with its substrate, the more products it produces. Nevertheless, there is no simple linear relationship between the incubation time and the rate of product production. All proteins undergo denaturation over time, which reduces their catalytic activity. The effect of the time period was assessed in this study for 24, 48, and 72 hours, with the activity peaking at 24 hours and then declining (Figure 3).

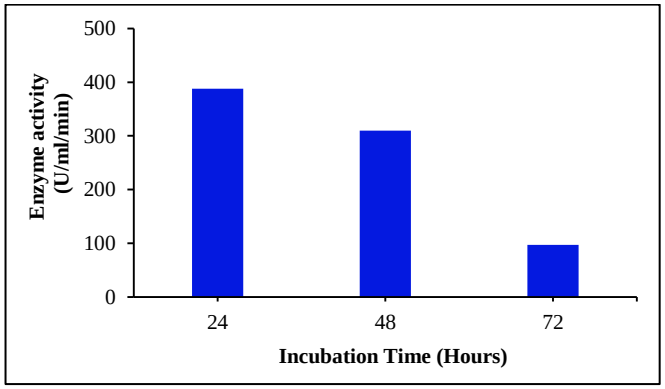

Figure 3: Effect of time on lipase production

Effect of temperature on Lipase production

Throughout the fermentation process, temperature always has a significant impact on the production of lipases. The current study found that the production of lipase by the bacterial strain peaked at 40 °C and then decreased as the temperature increased to 60°C (Figure 4). Some researchers believe that different Bacillus species' ability to produce enzymes is influenced by the amount of dissolved oxygen in the fermentation medium (Qader *et al*., 2006).The amount of oxygen available for the fermentation medium's enzyme is limited by high temperatures because they make oxygen less soluble (Campbell and Pace, 1968).

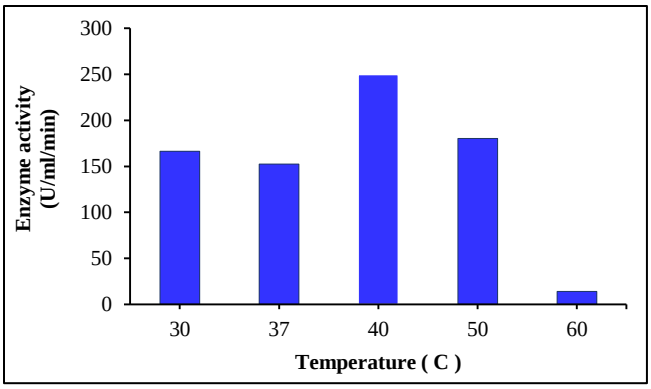

Figure 4: Effect of temperature on lipase production

Effect of Organic Solvents Tolerance

Bacteria that can survive organic solvents are not common among extremophilic microbes. This is principally due to novel adaptations such as fast membrane repair mechanisms, cis-trans isomerisation of membrane fatty acids, and toluene efflux pumps. The results suggested that Lipase from *Bacillus sp*. has the best tolerance to methanol and acetone, while ethanol and n-butanol have poor tolerances (Figure 6).

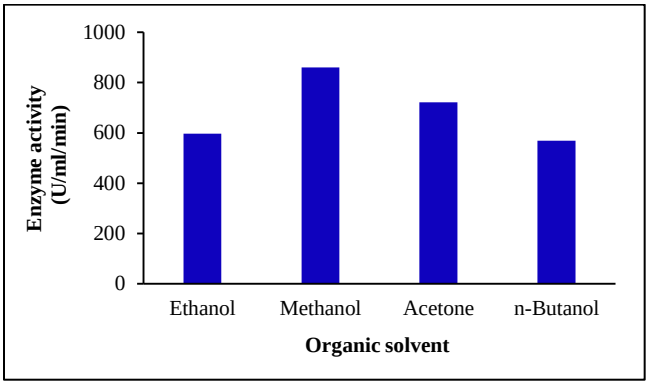

Figure 6: Effect of organic solvents on Lipase activity

Effect of concentration on waste cooking oil (WCO)

The impact of concentration was clear because, in accordance with the law of mass action, higher reactant concentrations increased the rate of reaction by encouraging more frequent successful collisions. Phenolphthalein, which turns red in low acidity and appears colorless in high acidity, was used as an indicator to track pH variations. The current study found that 0.1% was the ideal concentration for converting waste cooking oil (Figure 7). However, enzyme activity dropped above this threshold, suggesting that excessive concentrations might inhibit lipase activity and reduce the total production of fatty acid methyl ester.

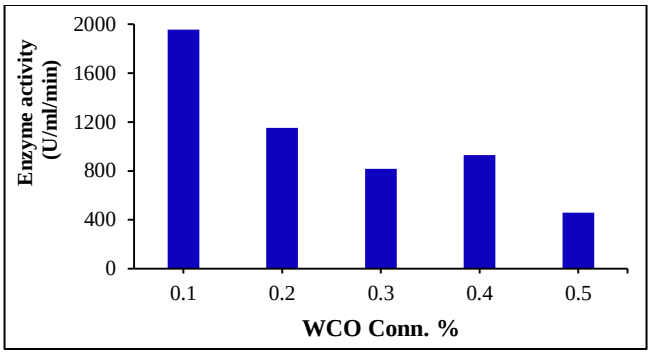

Figure 7: Effect of concentration on waste cooking oil (WCO).

DISCUSSION

According to the research by Kattatheyil *et al*. (2024), the maximum and ideal lipase activity was noted following 96 hours of incubation. Conversely, after additional incubation, the lipase activity from *Bacillus cereus* I13 from Arctic sediments declined. Mazhar *et a*l. (2018) found similar results, evaluating the samples under different incubation periods ranging from 24 to 120 hours and observing the highest production of lipolytic enzymes at 72 hours. When taken as a whole, these results show that enzyme activity is highly time-dependent and that extended incubation reduces catalytic potential, perhaps as a result of denaturation and protein instability (Mumtaz *et al.,* 2017).

Another important factor influencing lipase activity is temperature. Ji *et al.* (2010) found that 40 °C was the ideal temperature for lipase activity, which is in line with research on lipase from the Pseudomonas genus. After one hour of incubation at 60 °C, the enzyme maintains 64% of its activity and is stable below 50 °C (Longanesi *et al.,* 2022; Ji *et al*., 2010). In a similar vein, Ma et al. (2021) showed that the maximum lipase activity was observed at 35 °C, with activity rising from 25°C -35 °C. However, an additional temperature increase up to 65°C markedly reduced enzyme activity. These results are consistent with the previously documented ideal temperature range of 30 to 60 °C for the majority of bacterial lipases (Grahame & Schlesinger, 2010). According to da Cunha *et al*. (2009), enzyme catalysis usually rises with temperature; however, at higher temperatures, enzyme activity falls because of structural changes in the active site (Felizardo *et al.,* 2006).

Numerous studies have also been conducted on the effect of carbon sources on lipase synthesis. Using palm oil as the carbon source produced the highest enzyme activity, according to Sirisha *et al.* (2010), when olive oil was substituted with palm oil, ghee, coconut oil, groundnut oil, sunflower oil, and mustard oil at a final concentration of 1% (w/v), while maintaining the same values for the other parameters. Similar to this, Kattatheyil *et al*. (2024) found that glucose is the most effective carbon source for increasing lipase activity, followed by fructose, out of lactose, sucrose, glucose, maltose, and fructose. Furthermore, biofilm development rates were found to be inversely proportional to carbon content by Fazal *et al*. (2011). This suggests that the availability of carbon has a significant impact on the metabolism of microorganisms and the biosynthesis of enzymes.

According to a study by Boonchaidung *et al*. (2013), yeast extract was the most effective organic nitrogen source for Candida sp. growth and lipase synthesis, while peptone, casein acid, tryptone, and ammonium nitrate were the most inorganic sources. Similar findings were reported by Savalia and Dungrechiya (2022), who demonstrated that tryptone was the maximum lipase activity. Microorganisms typically show higher lipase yields when using organic nitrogen sources because they often affect the amino acid composition of proteins, which in turn affects enzyme yield and functionality (Gouveia & Maisonet, 2006; Fernando *et al.,* 2007).

In addition to their resistance to carbon and nitrogen sources, lipases' resistance to organic solvents has a major impact on their industrial application. According to Ugur *et al.* (2014), the LipSB 25-4 enzyme was stable even at high methanol (above 80%) and ethanol (above 74%) concentrations, but it was activated by acetone, acetonitrile, n-heptane, isooctane, chloroform, and ethyl acetate. Moreover, in the presence of various organic solvents, immobilised lipase provides enhanced stability and maintains 92-93% of its initial activity (Rmili *et al.* 2022).These findings suggested that immobilization improves the stability of enzymes in the presence of solvents. Studies on toluene-tolerant *Pseudomonas* strains have led to a comprehensive understanding of solvent tolerance mechanisms, including toluene efflux pumps, cis-trans isomerization of membrane fatty acids, and rapid membrane repair (Grahame & Schlesinger, 2010). Additionally, the rate of a chemical reaction is directly proportional to the reactant concentration, as per the law of mass action. This implies that the reaction rate rises as the reactant concentration rises and falls as the reactant concentration falls. Chemical indicators like phenolphthalein have two different structures: one in a high-acid environment and another in a low-acid environment. When the concentration of hydrogen ions is high, the compound is colorless; when the concentration is low, it turns red (Carrapiso & García, 2000).

According to the results, *Bacillus sp*. lipase optimization can significantly boost the production of biodiesel from used cooking oil; however, improving enzyme stability and reducing production costs are crucial for economic viability. Improvements in immobilization methods, protein engineering, and recombinant DNA technology offer promising avenues for creating lipases that are more effective, reusable and process design and feedstock diversification can aid in industrial scaling. Such developments in enzymatic catalysis have the potential to expand sustainable energy options without escalating competition for food resources, as global biofuel production is steadily rising but still only meets a small percentage of the demand for transportation fuels.

CONCLUSIONS

A lipase-catalyzed method of producing biodiesel from spent cooking oil shows promise as a sustainable and eco-friendly substitute for traditional diesel. The goals of ongoing research and development are process optimization, efficiency enhancement, and resolving issues with lipase stability and scalability. According to this study, *Bacillus sp*. lipase is an effective and sustainable biocatalyst for converting used cooking oil into fatty acid methyl esters (FAME). Under ideal circumstances, the process not only produces high yields but also solves waste disposal issues and produces sustainable energy. These results highlight enzyme-based biodiesel production as a viable substitute for traditional chemical processes, providing industrial and environmental benefits for the development of sustainable fuels.

Conflict of interest

Authors declare no conflict of interest.

REFERENCES

Akinfemiwa, O., M. Zubair, and T. Muniraj (2023) Amylase. StatPearls. StatPearls Publishing;Treasure Island.

Ali, S., S.A. Khan, M. Hamayun and I.J. Lee (2023) The recent advances in the utility of microbial lipases: A review. *Microorganisms*. 11, 510-535.

Atadashi, I.M., M.K. Aroua, A.A. Aziz and N.M. Sulaiman (2012) Production of biodiesel using high free fatty acid feedstocks. *Renew. Sustain. Energy Rev.*, 16, 3275-3285.

Avagyan, A.B. and B. Singh (2019) Biodiesel from plant oil and waste cooking oil. InBiodiesel: Feedstocks, Technologies, Economics and Barriers: Assessment of Environmental Impact in Producing and Using Chains. pp. (15-75). Springer: ISBN 978-981-13-5745-9

Benjumea, P., J. Agudelo and A. Agudelo (2008) Basic properties of palm oil biodiesel–diesel blends. *Fuel*. 87, 2069-2075.

Boonchaidung, T., T. Papone and R. Leesing (2013) Effect of carbon and nitrogen sources on lipase production by isolated lipase-producing soil yeast. *J. Life Sci. Technol*. 1, 176-179.

Campbell, L. L. and B. Pace (1968) Physiology of growth at high temperatures. *J. Appl. Bact.*31, 24-35.

Carrapiso, A.I. and C. García (2000) Development in lipid analysis: some new extraction techniques and in situ transesterification. *Lipids.* 35, 1167-1177.

Chandra, P., Enespa, R. Singh and P.K. Arora (2020) Microbial lipases and their industrial applications: a comprehensive review. *Microb. Cell Fact.*19, 169-210.

da Cunha, M. E., L. C. Krause, M. S. Moraes, C. S. Faccini, R. A. Jacques, S. R. Almeida, M. R. Rodrigues, and E. B. Caramão (2009) Beef tallow biodiesel produced in a pilot scale. *Fuel Proces. Technol*. 90, 570-575.

Fahim, Y.A., A.M. El-Khawaga, R.M. Sallam, M.A. Elsayed and M.F. Assar (2024) A review on lipases: sources, assays, immobilization techniques on nanomaterials and applications. *BioNanoScience*. 14, 1780-1797.

Fazal, M. A., A. S. Haseeb and H. H. Masjuki (2011) Biodiesel feasibility study: an evaluation of material compatibility; performance, emission and engine durability. *Renew. Sustain. Energy Rev*. 15, 1314-1324.

Felizardo, P., M. J. Correia, I. Raposo, J. F. Mendes, R. Berkemeier and J. M. Bordado (2006) Production of biodiesel from waste frying oils. *Waste management*. 26, 487-494.

Fernando S., P. Karra, R. Hernandez and S.K. Jha (2007) Effect of incompletely converted soybean oil on biodiesel quality. *Energy*. 32, 844-851.

Fernando, S., P. Karra, R. Hernandez and S. K. Jha (2007) Effect of incompletely converted soybean oil on biodiesel quality. *Energy*. 32, 844-851.

Gouveia, N.C. and M. Maisonet (2005) Health effects of air pollution: an overview. Air quality guidelines: Global update.87-109.

Grahame ,T. J. and R. B. Schlesinger (2010) Cardiovascular health and particulate vehicular emissions: a critical evaluation of the evidence. *Air Qual. Atmos. Health*. 3, 3-27.

Hu, J., W. Cai, C. Wang, X. Du, J. Lin and J. Cai (2018) Purification and characterization of alkaline lipase production by *Pseudomonas aeruginosa* HFE733 and application for biodegradation in food wastewater treatment. *Biotechnol. Biotechnol. Equip*. 32, 583-590.

Ji, Q., S. Xiao, B. He and X. Liu (2010) Purification and characterization of an organic solvent-tolerant lipase from *Pseudomonas aeruginosa LX1* and its application for biodiesel production. *J. Mol. Catal. B Enzym.* 66, 264-269.

Kattatheyil, H., V. Sajeela, S.S. Kabeer, A.P. Ezhuthanikkunnel and K.K. Padinchati, M.H. Ammanamveetil (2024) Screening, optimization, and molecular characterization of cold-active lipase producing *Bacillus cereus* I13 from Arctic sediments. *Biologia.* 79, 1041-1055.

Liu, X., X. Ge, H. Zhang and C. Lin (2011) Lipase-catalyzed biodiesel production from waste cooking oil using a stepwise addition of methanol strategy. *Bioresour. Technol.*, 10, 468-472.

Longanesi, L., A. P. Pereira, N. Johnston and C. J. Chuck (2022) Oxidative stability of biodiesel: recent insights. Biofuel. Bioprod. Biorefin. 16, 265-289.

Longanesi, L., A.P. Pereira, N. Johnston and C.J. Chuck (2022) Oxidative stability of biodiesel: recent insights. *Biofuels, Bioproducts and Biorefining*. 16, 265-289.

Mazhar, H., N. Abbas, T. Zamir, Z. Hussain and S.S. Ali (2018) Optimization study of lipolytic enzyme from *Bacillus cereus*, PCSIR NL-37. *Punjab Univ. J. Zool*. 33, 217-224.

Meher, L.C., D.V. Sagar, and S.N. Naik (2006) Technical aspects of biodiesel production by transesterification—a review. *Renewable and sustainable energy reviews*. 10, 248-268.

Mehta, A., S. Guleria, R. Sharma and R. Gupta (2021)The lipases and their applications with emphasis on food industry. InMicrobial biotechnology in food and health. pp. 143-164, Academic Press.

Meinert, C., and U.J. Meierhenrich (2012) A New Dimension in Separation Science: Comprehensive two-dimensional gas chromatography. *Angew. Chem. Int. Ed*. 51, 10460-10470.

Mumtaz, M.W., Adnan, A., Mukhta, H., Rashid, U. and Danish M. (2017) Biodiesel production through chemical and biochemical transesterification: Trends, technicalities, and future perspectives. In Clean energy for sustainable development pp.465-485 Academic Press.

Ogunkunle O., and N. A. Ahmed (2021) Overview of biodiesel combustion in mitigating the adverse impacts of engine emissions on the sustainable human–environment scenario. *Sustainability*. 13, 5465.

Qader, S. A. U., S. Bano, A. Aman, . N. Syed and A. Azhar (2006) Enhanced production and extracellular activity of commercially important amylolytic enzyme by a newly isolated strain of *Bacillus. sp.* AS-1. *Turk. J. Biochem*. 31,135-140.

Ray, A. (2012) Application of lipase in industry. *Asian J. Pharm. Tech.*. 2, 33-37.

Rmili, F., B. Hadrich, M. Chamkha, A. Sayari and A. Fendri (2022) Optimization of an organic solvent-tolerant lipase production by *Staphylococcus capitis* SH6. Immobilization for biodiesel production and biodegradation of waste greases. *Prep. Biochem. Biotechnol.* 52,108-122.

Sarmah, N., D. Revathi, G. Sheelu, K. Yamuna Rani, S. Sridhar, V. Mehtab and C. Sumana (2018) Recent advances on sources and industrial applications of lipases. *Biotechnol. Prog*. 34, 5-28.

Savalia, H. J. and A. Dungrechiya (2022) Identification and optimization study of lipase producing bacteria isolated from municipal waste and Bio-deteriorated Waste. *J. Pure Appl. Microbiol*. 16, 2592-2600.

Sirisha, E., N. Rajasekar and M. L. Narasu (2010) Isolation and optimization of lipase-producing bacteria from oil-contaminated soils. *Advances in Biological Research*.. 4, 249-252.

Ugur, A., N. Sarac, R. Boran, B. Ayaz, O. Ceylan and G. Okmen (2014) New lipase for biodiesel production: Partial purification and characterization of LipSB 25-4. ISRN Biochem. Article ID 289749, 7 pages.

Verma, M.L., C.J. Barrow and M. Puri (2013) Nanobiotechnology as a novel paradigm for enzyme immobilization and stabilization with potential applications in biodiesel production *Appl. Microbiol. Biotechnol.* 97, 23-39.

Wang, J. and W. Azam (2024) Natural resource scarcity, fossil fuel energy consumption, and total greenhouse gas emissions in top top-emitting countries. *Geoscience frontiers*. 15, 101757.

Wcisło, G. and N. Labak (2017) Determination of the impact of the type of animal fat used for production of biofuels on the fractional composition of AME. *ECONTECHMOD:* An *Interna Quarter. J. on Econ. of Technol. Modelling Proce*.;6.

Corresponding Author:
Shahana Rasheed Qureshi
Department of Biochemistry,
Jinnah University for Women, Karachi, Pakistan
Email: shahana.bch@gmail.com

Submitted on 22-09-2025
Revised on 18-10-2025
Accepted on 10-11-2025