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Research Article

Lipase Production from *Aspergillus niger* Used as a Biocatalyst for Transformation of Used Cooking Oil into Biodiesel

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Keywords
Biodiesel, Molasses, *Aspergillus niger* EFRL-FC-024, Waste cooking oil, Transesterification

Abstract | *Aspergillus niger* lipase was employed in transesterification of used cooking oil into biodiesel. Fungus cultivated in molasses mineral medium with tryptone as the nitrogen source for 5 days at 37 °C and 6.0 pH, strain produced a maximum lipase concentration of 29.39 (U/mL). Effect of metal ions, pH, pH stability, temperature, and thermostability on lipase activity were examined. Transformation of used oil into biodiesel was carried out by employing lipase produced in the current work. Maximal fatty acid methyl ester formation was noted after 18 h, 45 °C, and a 1:05 oil-to-methanol ratio. Agitation speed and enzyme concentration also enhanced biodiesel yield. Biodiesel production reached 92% under optimal conditions. Biodiesel production is simplified by using on-site produced lipase as catalyst. These findings could be significant advancement in biodiesel production from its commercialization prospects.

Novelty Statement | Utilization of onsite produced lipase from locally isolated strain to produce biodiesel from waste cooking oil.

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Introduction

Today's societies are increasingly turning to the renewable energy in order to reduce environmental pollutions and more affordable cost, as fossil fuel reserves are depleting and the price of oil and oil derivatives

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continue to increase at the same time. As a result, biofuels are widely accepted (Gonçalves *et al.*, 2021; Yahya and Aghel, 2021). One possible replacement for fossil fuels is fatty acid methyl ester (FAME) (Salinas *et al.*, 2018). The fact that it has a high flash point, contains no sulfur, is naturally lubricating, is biodegradable, and is safe for use around organisms has contributed to its expanding popularity (Baskar and Soumiya, 2016; Fan *et al.*, 2016; Uppar *et al.*, 2022). As well as, these benefits, biodiesel is considered a green fuel because its combustion produces negligible levels of pollutants such as sulfur oxides (SOx), carbon monoxide (COx), and nitrogen oxides (NOx) as well as suspended solid particles (Salinas *et al.*, 2018). When it comes to liquid fluids that can be produced from lipid material, biodiesel is by far the most plentiful available source. Diesel engines are compatible with biodiesel because of their similarities to those of fuels generated from petroleum (Tran *et al.*, 2016).

Biodiesel has been produced from renewable resources, for instance, edible oils, animal fats, used cooking oil, lipids from microalgae and microorganism (Tran *et al.*, 2016). Producing biodiesel from edible oils is more expensive than producing it from other sources since the expenditure of basic precursor materials comprises between 60% to 80% of the entire expenditure of biodiesel production. For this reason, it is not cost-effective to make biodiesel from edible oils (Foroutan *et al.*, 2019) because the biodiesel market competes with the market for human consumption of oils. Consequently, is anticipated that in the near future, efforts will emphasize on using low-cost, non-edible biological resources to cut biodiesel processing cost while securing the environment (Foroutan *et al.*, 2019).

Worldwide, the food sector, restaurants, retailers, and homes emit enormous volumes of WCO every year (Ashok *et al.*, 2018). Oil oxidation is an unavoidable consequence of the food industry's widespread habit of reusing cooking oil to cut costs (Gunnepana and Nawaratne, 2015). When oil is repeatedly heated, oxygen is driven out of the mixture (Goh *et al.*, 2020). Increased rates of cancer and heart diseases have been linked to the practice of recycling oxidized and hazardous cooking oil in food production (Ganesan *et al.*, 2018). When discarded inefficiently, waste cooking oil (WCO) can represent environmental hazards (Lombardi *et al.*, 2018). Released oxidation products have negative effects on ecosystems. WCO output has skyrocketed due to severe laws limiting the reuse and disposal of cooking oil, resulting in a variety of new management difficulties. About 16.5 million tons of WCO is produced annually by the food manufacturing and restaurant industries (Khodadadi *et al.*, 2020), the majority of which is often thrown in landfills (Yaakob *et al.*, 2013) or flushed down the toilet (Ortner *et al.*, 2016). Energy security, waste and pollution reduction, food supply protection, and progress toward a circular bioeconomy and environmental sustainability are all possible outcomes of transforming WCO to renewable energy (Zhao *et al.*, 2021). Transformation of used cooking oil into bioenergy cut 60-90% cost according to a 2018 study by Sahafi *et al.* (2018), and doing so could aid in the movement toward "Zero Hunger" by minimizing the need to choose between fuel and food (Hosseinzadeh-Bandbafha *et al.*, 2022).

Used cooking oil and alcohol undergo a transesterification reaction that produces biodiesel in the presence of a catalyst. To boost biodiesel production yield, homogeneous or heterogeneous catalysts can be utilized in the transesterification reaction (either alkaline or acidic) (Arumugamurthy *et al.*, 2019). Since the transesterification reaction is about 4,000 times faster in the presence of alkali including NaOH or KOH than in the presence of acids (Malhotra and Ali, 2019), this process is routinely applied for biodiesel production. The creation of soap and water, for example, requires a washing step to remove the catalyst from the biodiesel after it has been made using an alkali catalyst (Esmaeili and Foroutan, 2018; Seffati *et al.*, 2019). Because of their low toxicity, tolerance to organic solvents, reusability, and insensitivity to free fatty acids, lipases have been the subject of extensive study as a biocatalyst in biodiesel production (Kalita *et al.*, 2022). In our previous study (Ali *et al.*, 2017) lipase from *Pseudomonas aeruginosa* FW_SH-1 was employed in biodiesel production. Microorganisms are excellent source of enzymes including protease (Jatt *et al.*, 2022), cellulase (Tunio *et al.*, 2024); lipases (Ali *et al.*, 2016).

Lipases are hydrolytic enzyme that can catalyze many different chemical reactions. These reactions include alcoholysis, acidolysis, amynolysis, and hydrolysis. Lipases can be produced by plants, animals, and even microorganisms. Specificity and hydrolysis rate of lipases also differ between strains and are pH and temperature dependent (Maldonado *et al.*, 2016). The active site of lipases consists of the amino acid a combination of serine, histidine, and aspartate (Melani *et al.*, 2020). One of the most impressive uses of lipase is in biodiesel production. Mittelbach (1990) was the first to report that lipase increased biodiesel synthesis. In transesterification reactions, lipases frequently produce fatty acid methyl esters via a two-step process (Ping-Pong Bi Bi mechanism) (Alzuhair *et al.*, 2007). In the present study, lipase produced from *Aspergillus niger* was used as a biocatalyst to facilitate the transesterification of WCO, a feedstock for biodiesel, with methanol.

Materials and Methods

Microorganism

Aspergillus niger EFRL-FC-024 was available and stored in IBGE laboratory was used for lipase production. Strain was stored at -40 °C. Fungal culture was activated by

spreading on agar plates. Synthetic medium was composed of 20 g/L of glucose, 10 g/L of peptone, and 20 g/L of agar.

Fermentation conditions

Glucose (10 g/L), peptone (10 g/L), magnesium sulfate (1.0 g/L), and potassium phosphate (2.0 g/L) were the components of the fermentation medium and this media was used in all fermentation experiments. 50 mL of the fermenting media was placed in a 250 ml conical flask, initial pH was set at 5.0 using dilute acid/base. Cotton plugs were used to seal off the flasks before they were autoclaved for 20 min at 121°C. For inoculation, the sterilized media was kept in a laminar air flow cabinet at room temperature. 2.0% v/v of *Aspergillus niger* culture was added to the medium and incubated for 7 days at 37 °C. Samples were obtained at regular interval of 24 h, samples were centrifuged to separate the fungal biomass. The cells were oven-dried until their weight was consistent, and the broth was kept for biochemical testing. Experimental scheme is shown in Figure 1.

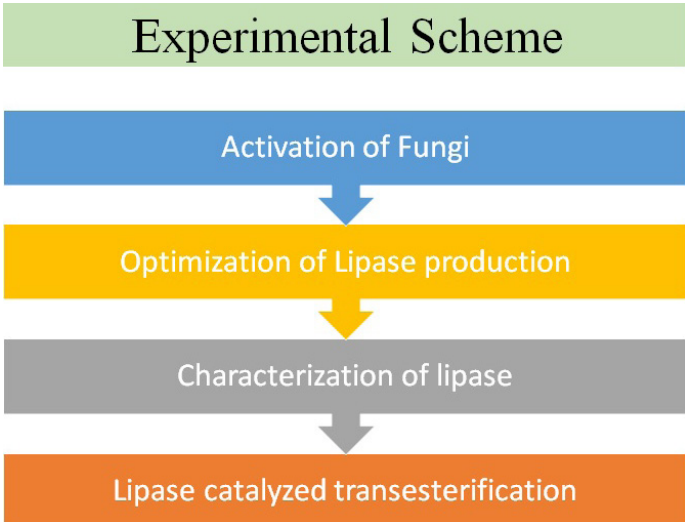

Figure 1: Step-wise experimental scheme.

Optimization of fermentation conditions

Sugars including molasses (agroindustry waste) and pure glucose, galactose, fructose, starch, and maltose were added to the fermentation medium to stimulate mycelial growth and increase extracellular lipase synthesis. Incubation of the inoculated medium at 37 °C for 7 days was performed. Tryptone, casein, ammonium chloride, potassium nitrate, and sodium nitrate were used in place of peptone in the fermentation medium. The fermentation process completed after seven days. Researchers looked at how altering the fermentation medium's initial pH from 3 to 12 using diluted acid or base affected microbial growth and lipase production. After fermentation completed, the pH was checked to see where it had settled.

Lipase activity

Lipase activity was measured using a technique established by Winkler and Stuckman (1979), same method was adopted in previous study (Ali *et al.*, 2016).

Characterization of lipase

Researchers looked into the effects of temperature, metal ions, reaction time, substrate concentration, enzyme concentration, pH, and pH stability on lipase activity. By varying the substrate concentration from 5.0 to 45 g/L and the enzyme dose (0.5 to 5.0 mL), we were able to characterize the effects of substrate and enzyme concentrations on lipase activity. Lipase activity was checked at a range of pH values (4 to 11) and incubation temperatures (30 to 80 °C) to determine their effects. In order to assess the pH stability of the crude enzyme, enzyme was incubated (0.5 mL) for 1 h then 2.5 mL of substrate solutions was mixed and reaction incubated at above mentioned conditions then measured the percentage of the original activity that was still present. One-h incubation at temperatures between 30 and 80 °C were used to measure the thermal stability of the enzyme. Lipase activity was measured using the aforementioned test conditions. To see how different chemicals affected lipase activity, we incubated a 0.5 mL enzyme solution with 0.5 mL of metal ions and 2.5 mL of substrate at 37°C.

Transesterification and determination of biodiesel

Ali *et al.* (2017) procedure was adopted for transesterification process. Briefly, vials of 100 mL capacity, sealed with screws, using a solvent-free operation. To the pretreated waste cooking oil, crude lipase and methanol were added in separate vials. The mixture was shaken for 36 h while transesterification occurred at 40 °C. The reaction was stopped at the 6-h by adding n-hexane to the mixture; as a result, two layers separated; the biodiesel was in the upper layer. A similar volume of n-hexane was blended with the isolated top layer before being introduced to gas chromatography (GC). The FAME yield was then calculated by submitting 100 uL aliquots to GC analysis. Gas chromatography (GC) was used to quantify biodiesel output, as described by Ali *et al.* (2017).

Results and Discussion

The rising global need for energy makes it imperative to research and develop renewable energy sources such as biofuel. Renewable fuel biodiesel is typically utilized in diesel vehicles. Since chemically catalyzed transesterification methods generate more fatty acid alkyl esters in shorter time, they are currently used for the great majority of biodiesel synthesis. Downstream processing costs and environmental concerns about biodiesel and its byproducts have motivated researchers to look for more cost-effective production alternatives. Making biodiesel using enzymatic transesterification with lipases has become increasingly common in recent years because to its high purity and ease of glycerol removal. More lipase is required when the lipase concentration is raised (Amini

et al., 2017). Despite extensive research into lipases and their verified strong catalytic activity, much about the enzymatic reaction remains unknown. This gap can be bridged by the use of reaction modeling and simulations, as well as the design and deployment of reactors for large-scale production, by figuring out what proportions of alcohol, oil, water, time, and temperature will yield the best results in a given reaction. For this reason, studies on lipase production and its application in making biodiesel from waste cooking oil are now being done.

Optimization of Lipase production from Aspergillus niger *EFRL-FC-024*

Table 1 displays the findings of an analysis of how fermentation period (from 1-7 days) affected microbial growth and lipase production. The maximum concentration of lipase (3.11 U/mL) was obtained after 5 days of fermentation, when both microbial growth and lipase concentration had increased. The shift in pH, the accumulation of byproducts in the fermentation medium, and the depletion of nutrients are likely causing reduction in lipase production after 5 days. Table 1 shows how different carbon sources influence lipase synthesis and fungal growth. Fungal growth and lipase production were examined using a variety of sugars such as glucose, galactose, fructose, starch, molasses, and maltose. Triplicate flasks were kept at 37 °C for several days. The molasses mineral medium had the highest lipase content of 13.44 U/mL. Molasses superior effect may originate from the abundance of vitamins, minerals, and nitrogenous substances it contains. These nutrients promote metabolic rate and help microbes thrive. Previous research showed that the use of molasses mineral medium resulted in the highest concentration of amylase from Bacillus BCC-01-50 (Simair *et al.*, 2017). By switching out peptone for tryptone, casein, sodium nitrate, potassium nitrate, and ammonium chloride, we were able to examine the impact of these alternative nitrogen sources on microbial growth and lipase production and cultured at pH of 5 and 37 °C. Tryptone, when compared to other nitrogen sources, has the highest lipase content (22.89 U/mL). The outcomes of experiments using organic nitrogen sources are superior to those using inorganic nitrogen sources. Simair *et al.* (2017) also found highest enzyme activity when peptone was used as organic nitrogen source as compared to inorganic nitrogen sources. Using *Aspergillus niger* and *Aspergillus flavus* as lipase producing strains, Colla *et al.* (2016) tuned different fermentation parameters, such as nitrogen sources and their concentrations, inducer and its concentration and pH, to get the highest feasible lipase titer. When 45 g/L of yeast extract was added to the fermentation medium, the highest concentration of lipase was achieved. Oliveira and Lima (2014) obtained 4.22 ± 0.35 U/mL of lipase from Fusarium sp. under optimized fermentation conditions. Response surface methodology noted best nutrients and their concentrations as 1 g/L of yeast extract, 15 mL/L of chicken fat, 15 g/L of Triton X-100 and 4.5 g/L of ammonium sulfate. The pH ranges from 3 to 12 was studied for its potential effects on fungal growth and lipase production. Up until pH 6 (29.39 U/mL), lipase activity increased with increasing pH. However, at higher pH values, lipase activity declined, likely because of the acidic nature of the microbe. The genes responsible for producing enzymes and any disruptions to the active center of the enzyme's structure determine its optimal pH, or the point at which the enzyme is at its most active. Oliveira *et al.* (2017) investigated production of lipase from *Aspergillus ibericus* MUM 03.49 under solid state fermentation conditions. Lipase concentration reached to 127±17 U/g when only palm kernel oil cake was used as

Table 1: Optimization of lipase production from *Aspergillus niger* EFRL-024.

Time period (days)	**1**	**2**	**3**	**4**	**5**	**6**	**7**			
Lipase[a] (U/mL)	1.06±0.07	1.97±0.05	2.44±0.08	2.75±0.16	3.11±0.09	2.72±0.04	2.14±0.03			
DCW[b] (g/L)	1.12±0.13	1.37±0.16	2.49±0.12	2.87±0.09	3.33±0.11	2.74±0.13	2.31±0.09			
Carbon sources	Glucose	Galactose	Fructose	Starch	Molasses	Maltose				
Lipase (U/ml)	3.11±0.09	1.87±0.04	2.70±0.06	1.92±0.05	13.44±0.15	1.93±0.07				
DCW (g/L)	3.33±0.11	1.64±0.13	2.22±0.42	2.13±0.21	5.67±1.23	1.26±0.22				
Nitrogen sources	**Peptone**	**Tryptone**	**Casein**	**Sodium Nitrate**	**Potassium Nitrate**	**Ammonium chloride**				
Lipase (U/mL)	13.44±0.15	22.89±0.84	12.44±0.21	0.70±0.02	1.21±0.04	1.91±0.07				
DCW (g/L)	5.67±1.23	8.93±1.34	5.73±0.99	1.03±0.11	1.32±0.15	2.04±0.16				
pH	3	4	5	6	7	8	9	10	11	12
Lipase (U/mL)	3.73±0.12	8.21±0.27	22.89±0.84	29.39±1.38	24.13±0.89	12.90±0.11	8.81±0.07	5.21±0.06	3.14±0.12	2.06±0.19
DCW (g/L)	3.22±0.93	4.97±1.12	8.93±1.34	11.14±1.45	9.67±2.38	5.3±1.44	4.63±1.13	4.08±1.4	3.78±0.56	2.23±0.82

[a] final lipase activity; [b] dry cell weight.

carbon source (PKOC). However, lipase concentration increased to 460±38 U/g employing a substrate consisting of 0.45 grams of PKOC and 0.55 grams of sesame oil cake fermented for 6 days at a moisture content of 57.0 %. Facchini *et al.* (2016) investigated fermentation conditions for four fungal strains. The response surface method was tested to optimize the best combination of growth medium parameters. Fermentation was operated in liquid medium under submerged conditions at 30°C for 72 h with 100 rpm. The lipase activity was boosted by a factor of two when corn oil was used as the carbon source in conjunction with Tween 80. The experimental design resulted 3.5-fold increase than the original medium. New medium composition was 0.5% corn oil, 0.012% of magnesium sulphate, 0.015 g of KH_2PO_4, and 0.05 g of NH_4PO_4. Plants are excellent source of natural bioactive compounds with antimicrobial properties (Rahu *et al.*, 2021).

Characterization of lipase produced from Aspergillus niger

Figure 2a displays the findings of a study that investigated the influence of reaction time (range: 5-120 min) on the crude lipase activity of *Aspergillus niger*. Lipase activity was found to be higher for shorter reaction times up to 30 min, after which it dropped. This happened probably due to denaturation of the enzyme at prolong incubation Lipase performance was checked by substrate and enzyme incubation at 37 °C for varying time to assess the impact of reaction time on the enzyme activity. After incubation at optimal conditions for 10, 20, 30, 40, 50, 60, 70, 80, and 90 min, the enzyme's activity was measured. After 30 min of incubation, enzyme activity reached its peak and then gradually declined with continued incubation (Sethi *et al.*, 2016). Figure 2b shows the effect of lipase dosage (0.5 to 5.5 mL) on lipase activity. Until to a concentration of 3 mL of enzyme, lipase activity increased; after that, it declined. Figure 2c shows the influence of substrate concentration from 0.5 to 4.5 g/L on lipase activity. After reaching a maximum at a substrate concentration of 3.0 g/L, lipase activity thereafter reduced with additional increases in substrate concentration. Using olive oil as a substrate for lipase hydrolysis, Sethi *et al.* (2016) tested a range of concentrations (0.25-2.5% v/v), finding that lipase activity was highest at 1.5% (v/v).

Measurements of pH stability were performed across a wide range of pH values (from 4 to 11) and buffer systems (to control for shifts in pH), as shown in Figure 3a. The acidic nature of lipase was demonstrated by the fact that its activity increased up to a pH of 5.5 and then reduced when the pH was raised higher. Stability of crude lipase was observed in the acidic pH range and marked decrease in the alkaline pH range. pH 6 was shown to be optimal for lipase activity and stability. Lipase activity was maximum for *Burkholderia ubonensis* SL-4 at an alkaline pH of 8.5, and it was maintained at greater than 50% from a pH range of 7.5 to 10 (Yang *et al.*, 2016). Lipase activity was analyzed from 30 to 80°C for its effect on thermostability (Figure 3b). Up until 45 °C, lipase activity increased with temperature, but then it dropped off. This was due to the enzyme was denaturing at the extreme temperature. Preincubating the enzyme for 24 h at varying temperatures allowed to determine the thermostability of the crude lipase. The enzyme's stability ranged from 30 to 60 °C, and it gradually dropped with increasing heat. Its thermostability and optimal temperature range have been studied. A temperature of 65 °C was shown to be optimal for lipase activity. The activity of purified lipase was maintained at 62.34 % after 12 h of treatment at 60 °C, 61.24% after treatment at 65 °C, and 56.02 % after treatment at 70 °C Yang *et al.* (2016). Many chemicals and metal ions were tested to see how they affected lipase activity. As can be seen in Figure 2c, the addition of iron resulted in the highest lipase activity relative to the other chemicals tested. Researchers looked into how various metal ions affected enzyme activity in their purest form. The 50 mM phosphate buffer was used for all the solutions (pH 8.0). Pre-incubating the enzyme with the ions of interest, then adding the substrate, and measuring the resulting lipase activity was used to examine the impact of metal ions on lipase activity.

Biodiesel production

Biodiesel from WCO is a renewable energy source that can be used to produce liquid fuels. Economic, environmental, and waste management benefits are among those offered by biodiesel production by the recycling of WCO and methanol in the presence of a lipase biological catalyst (Ali *et al.*, 2017). Several methods, including

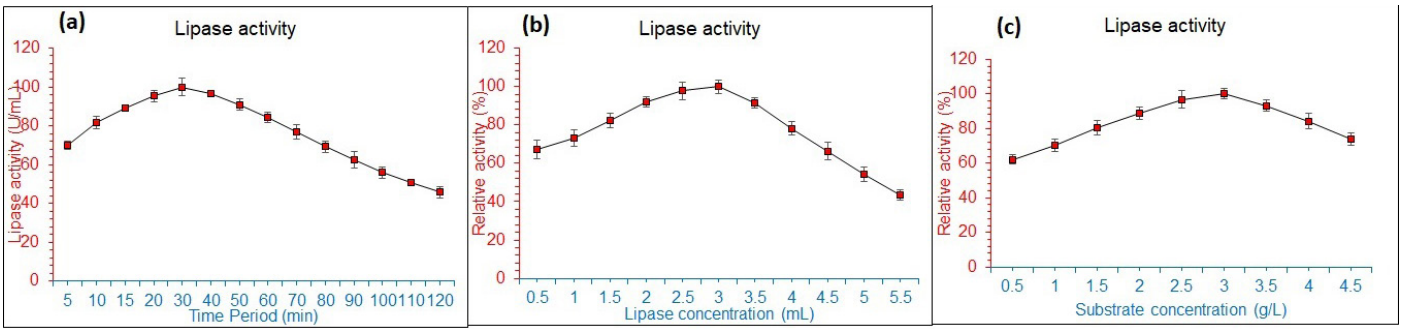

Figure 2: Characterization of crude lipase (a) reaction time; (b) lipase concentration; (c) substrate concentration.

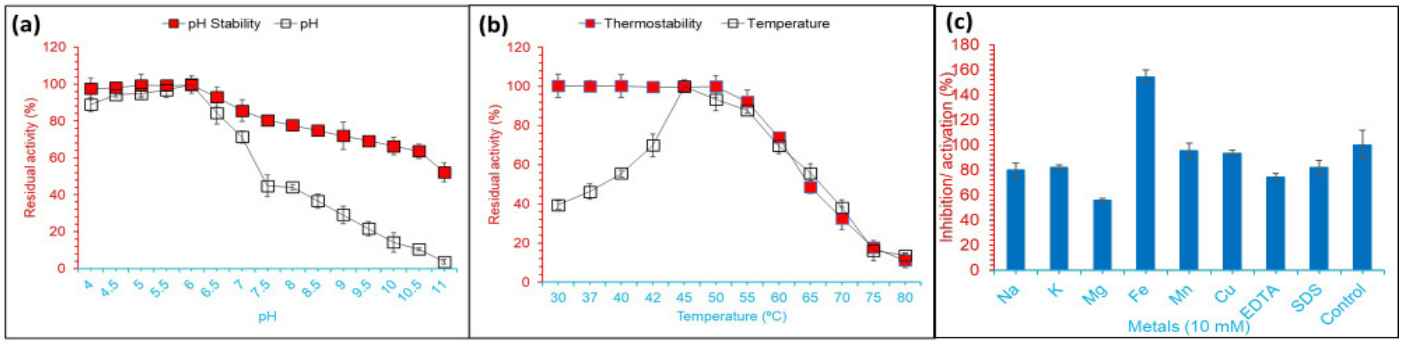

Figure 3: Characterization of crude lipase (a) pH and pH stability of lipase; (b) temperature and thermostability; (c) effect of metal ions on lipase activity.

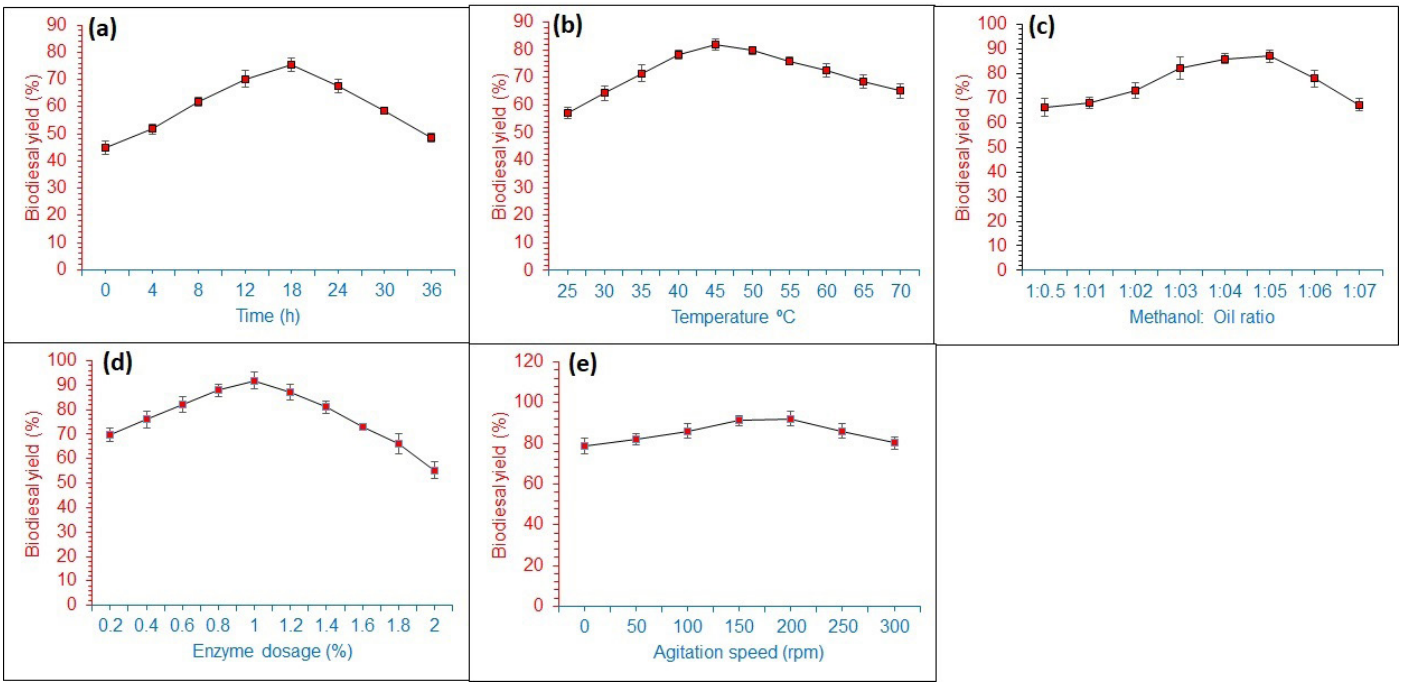

Figure 4: Biodiesel production from waste cooking oil using crude lipase produced by A. niger. (a) reaction time; (b) reaction temperature; (c) methanol to oil molar ratio; (d) enzyme dosage; (e) agitation speed.

hydrotreating (Bezergianni *et al.*, 2010), gasification (Tamosiunas *et al.*, 2019), pyrolysis (Lam *et al.*, 2016), and transesterification (Cordero-Ravelo and Schallenberg-Rodriguez, 2018), have been studied in the past to convert WCO into energy and fuel. Transesterification is one of the most well-known technology for converting WCO into biodiesel since it is both economical and environmentally benign (Tabatabaei *et al.*, 2019). No adjustments to the engine are necessary when using WCO biodiesel either neat or combined with diesel (Sadaf *et al.*, 2018). WCO biodiesel is favored over diesel because it is superior in many ways, including biodegradability, combustion efficiency, intrinsic lubricity, cetane number, flash point, and sulfur and aromatic content (Demirbas, 2007). Furthermore, it has been shown that the combustion of WCO biodiesel produces fewer hazardous emissions than diesel, including unburned hydrocarbons, particulate matter, and carbon monoxide (Aghbashlo *et al.*, 2018). In this article, we focus on the lipase catalyzed transesterification of used cooking oil into biodiesel.

Figure 4 demonstrates biodiesel production and effects of time, temperature, methanol to oil molar ratio, agitation speed, and enzyme dose. A variety of response times, from instantaneous to 36 h, were examined first. Production of biodiesel was high for the first 18 h (Figure 4a), but then it started to taper off. Our previous studies demonstrated that 5 h of reaction time using waste cooking oil as a substrate for biodiesel could provide an 84% biodiesel yield, albeit with a higher catalyst loading of 6% animal bone waste. The maximum yield of FAME was achieved when utilized cooking oil was transesterified at the ideal time for lipase catalyzed transesterification (Ali *et al.*, 2018).

Figure 4b shows the effect of temperature from 25 to 70 °C on transesterification reaction. Biodiesel production was maximized up to 45 °C, but began to decline at higher temperatures, possibly due to enzyme denaturation. The effect of changing the oil to methanol molar ratio on biodiesel production is shown in Figure 3c for molar

ratios of 1:0.5, 1:1, 1:2, 1:3, 1:4, 1:5, and 1:7. The yield was determined by varying the oil-to-methanol ratio from 1:0.5 to 1:7 at a constant 45 °C for an 18-h reaction time. As can be seen in Figure 4c, the biodiesel yield is strongly affected by the oil-to-methanol molar ratio. The production of biodiesel is maximized by increasing the molar ratio from 1:0.5 to 1:5. With a biodiesel production of 87%, the optimum oil to methanol molar ratio was found to be 1:5. The development of methoxy species on the surface of the catalyst is necessary to increase the methanolysis rate, hence an excess of methanol must be found. The formation of biodiesel will become more favorable as a result of this change. When the molar ratio of oil to methanol was greater than 1:5, the biodiesel output decreased somewhat. The product's quality as biodiesel fuel is diminished by the presence of excess alcohol, which also lowers the fuel's viscosity, density, and flash point. Glycerol is produced during the transesterification process. When there is too much methanol present, glycerol dissolves easily and then block the methanol from reacting with the reactants and the catalyst. As a result, the separation of glycerol from the product becomes extremely difficult, and the equilibrium shifts in the opposite direction, reducing the biodiesel production (Degfie *et al.*, 2019).

Figure 4d revealed that an increase in catalyst loading concentration from 0.1 to 1.0% w/w leads to a rise in biodiesel yield, while an increase in catalyst loading concentration beyond this point leads to a decline in biodiesel production. As a result, a 92% biodiesel yield was observed with a 1% w/w catalyst loading. Due to an increase in reaction soap formation brought on by the extra catalyst amount, the biodiesel yield has decreased marginally (Prasertsit *et al.*, 2014; Degfie *et al.*, 2019). The effect of catalyst dosage on enzymatic transesterification was investigated by Parandi *et al.* (2022) in the range of 0.2–2 g. Increases in enzyme concentration and catalyst loading were both responsible for the gradual increase in biodiesel production from 21% to 96% as biocatalyst mass was increased in the reaction medium from 0.2 g to 1 g (Lv *et al.*, 2021). Figure 4e shows the effect of agitation on the biodiesel production in the range of 0-300 rpm, biodiesel yield increased upto 200 rpm then declined. Under optimal conditions (44.2 °C, 3.05:1 methanol to oil ratio, 0.782 g of lipase, and 170 rpm), Ali *et al.* (2017) observed an 86% biodiesel output.

Conclusions and Recommendations

This study tested *Aspergillus niger* lipase for biodiesel generation. Lipase catalysed the waste cooking to biodiesel. Fungus cultivated in molasses mineral medium with tryptone as nitrogen source and initial pH of 6.0 attained maximum lipase concentration of 29.39 U/mL after 5 days at 37 °C. Crude lipase was tested for pH, pH stability, temperature, thermostability, and metal ions (10 mM). Lipase was active in acidic pH range from 4-6 and preserved 100% after 1 h at 50 °C. This technique produced biodiesel from leftover frying oil using lipase. At 45 °C and 1:05 oil-to-methanol ratio, biodiesel synthesis peaked at 18 h. Enzyme dose and agitation speed also affected biodiesel production. Biodiesel production exceeded 90% under ideal conditions. Onsite lipase manufacture simplifies biodiesel production and reduces enzyme acquisition costs. These findings suggest the biodiesel biorefinery can be improved and commercialized.

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Conflict of interest

The authors have declared no conflict of interest.

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