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Improved biodiesel from palm oil using lipase immobilized calcium alginate and *Irvingia gabonensis* matrices

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Abstract

Background: Lipase is an important microbial enzyme and biocatalyst in biodiesel production. The study investigated fuel properties of biodiesel produced from palm oil (PO) using lipase immobilized on *Irvingia gabonensis* and calcium alginate.

Results: Biodiesel yield from PO using free and immobilized lipases was highest at 35 °C and pH 7, with product yield using calcium alginate-immobilized lipase, CAIL (94.42, 96.9%) higher than using *Irvingia gabonensis*-immobilized lipase, IGIL (92.54, 95.8%). Biodiesel produced using immobilized lipases had similar pour point, cloud point, and kinematic viscosity, and they possessed improved fuel properties compared to free lipase biodiesel in terms of densities at 15 °C and flash point. Pour points, flash point, and kinematic viscosity of biodiesel produced using CAIL and IGIL met American and European Standards but density at 15 °C and cloud points are below both standards. CAIL and IGIL biodiesel had similar fatty acid methyl ester (FAME) compounds and consisted more of unsaturated fatty acids (hexadecanoate, 9-octadecenoate, octadecanoate, dodecanoate, and 9,12-octadeca-dienoate) than obtained in biodiesel from free lipase. IGIL and CAIL were re-used in 8 and 12 cycles respectively, with > 90% biodiesel yield achieved in four and 11 cycles.

Conclusions: The study showed that lipase immobilized on *Irvingia gabenensis* and calcium alginate and used in biodiesel production retained high enzyme activity and biodiesel yield in repeated cycles.

Keywords: Biodiesel, Transesterification, Lipase, Immobilization, FAME, *Irvingia gabenensis*, Calcium alginate

1 Background

Biodiesel is a renewable, non-toxic, environmentally friendly, biologically produced diesel fuel alternative to substitute petroleum-based diesel [1–3]. It is produced via enzymatic transesterification of several types of feedstocks such as edible plant oils and animal fats, and in recent times, non-edible oils are also being explored for biodiesel production [4]. The enzymes directly involved in transesterification for biodiesel production are lipases.

Lipases are versatile, industrially important enzymes cheaply produced from microorganisms and serve as biocatalysts in several industrial purposes including food processing technology, fats and oil processing, pharmaceuticals, and biodegradation [5–7]. Microbial lipase is also important as the most common source for biodiesel production by transesterification due to their ease of fermentation and purification steps [8].

Shortcomings of free lipases include slow reaction rate and high cost, therefore, the need for immobilization, while immobilized lipases are advantageous in biodiesel production due to easy product separation and glycerol recovery, potential for continuous production with improved reusability, and reduced cost of lipase production [9–12].

Various techniques and support matrices have been used in lipase immobilization for biodiesel production. This includes different forms of silica and silica gel,

amberlite, sepabeads, sepharose, and resin supports formulated using adsorption, cross-linking and entrapment methods, etc. [13, 14].

Entrapment is a physical restriction of enzymes within a confined space or network [15]. This immobilization technique permits movement of both substrate and product through the matrix and is applicable to a wide range of carriers and lipases for biodiesel production [16]. Different hydrogels have been used as entrapment for lipase [17, 18], while the use of alginates is widely reported in recent times [15, 19–21].

Irvingia gabonensis seeds are thickening agents known to possess hydrocolloid property and have been prepared as immobilization matrix for both microbial cells and lipase [22, 23], therefore, the need to determine its possible use as matrix for lipase immobilization in biodiesel production.

In this study, we investigated comparative biodiesel production from palm oil using lipase entrapped in calcium alginate and *Irvingia gabonensis* matrices based on fuel properties.

2 Methods

2.1 Materials
Lipase-producing *Aspergillus niger* strain from previous study [24] was obtained from a Culture Collection Center. Palm oil (PO) was obtained locally from an oil palm industry, and all chemicals were of analar grade.

2.2 Lipase production and purification assay
Aspergillus niger spores produced lipase on rice bran medium via solid-state fermentation as previously described [22], and enzyme activity was assayed [24]. Sodium phosphate buffer (50 mM; pH 8) was mixed with fermented medium in an orbital shaker operated for 2 h at 150 rpm and 28 °C to obtain crude enzyme. The crude lipase presentin filtrate was further purified by mixing with olive oil emulsion, mixture incubated at 50 °C for 30 min and reaction stopped with absolute ethanol. According to Eq. (1), one unit (U) of lipase activity is the amount of enzyme released from the emulsion substrate 1 μmole of fatty acid per milliliter per minute under specific assay condition.

$$\mu\text{mol fatty acid per ml sample}$$
$$= \frac{((\text{ml NaOH of sample} - \text{ml NaOH of blank}) \times N \times 1000)}{5}$$

$$(1)$$

2.3 Lipase immobilization
Lipase was immobilized on both calcium alginate and *Irvingia gabonensis* as previously described [22, 25]. For immobilization on alginate, lipase was mixed with 2% v/v sodium alginate solution in equal ratio, and the mixture

added drop wise into 0.2 M calcium chloride solution with continuous shaking at room temperature to form lipase-encapsulated calcium alginate beads. Lipase was immobilized on defatted *Irvingia gabonensis* seed powder of adequate gel strength (10% w/v) activated with 2.5% (w/v) glutaraldehyde solution. Beads were formed by adding enzyme-gel mixture drop wise into ethanolic-formaldehyde solution. Beads were washed severally with excess deionizing water and finally with sodium phosphate buffer (pH 7.5), dried, and their lipase activity assayed.

2.4 Optimization of environmental parameters on lipase immobilization
The effect of temperature on biodiesel production from palm oil using lipase immobilized on calcium alginate and *Irvingia gabonensis* was determined by carrying out transesterification between 25 and 45 °C at 5 °C intervals. Similarly, the acidity/alkalinity effect was investigated at pH range 5-9 with 0.5 pH changes considered.

2.5 Enzymatic production of biodiesel (fatty acid methyl ester) by immobilized lipase
Biodiesel production proceeded using the optimized transesterification reaction procedure previously described [26].

Transesterification reaction was carried out in a 250-ml conical flask on rotary orbital shaker as previously described [26]. Briefly, immobilized lipase (2.5%) was added to 40 mL of PO and the mixture was supplemented with methanol (5 ml) in a starting molar ratio 1:1 to the oil, and reaction was completed with stepwise addition to methanol for a final 3:1 molar ratio. Reaction mixture was left for 48 h after which it was separated overnight using a separating funnel. Following separation, two layers were formed: the upper layer being the biodiesel product while glycerin settled at the bottom layer of the separating funnel.

2.6 Biodiesel yield
The fatty acid methyl ester, FAME, yield, or biodiesel yield (% wt), relative to the amount of experimental oil used was calculated by comparing the weight of the upper layer biodiesel to the weight of the crude oil used as described in Eq. (2).

$$\text{Biodiesel yield } (\%) = \frac{\text{Weight of biodiesel produced}}{\text{Weight of crude oil used}} \times 100$$

$$(2)$$

2.7 Characterization of biodiesel
Quality of biodiesel produced from palm kernel using lipase immobilized on calcium alginate and *I. gabonensis* beads was determined via fatty acid methyl ester (FAME)

properties. This was analyzed as previously described [27] using gas chromatography system (Agilent Technologies 7890A model). Fuel properties determined included flash point (FC), cloud point (CP), pour point (PP), kinematic viscosity, and density at 15 °C of transesterified PO. These were compared with American (American Standard of Testing material, ASTM 6751–3) and European (European Union Standard, EN 14214) fuel standards. All properties were determined in triplicate experiments.

2.8 Effect of reusability of immobilized beads on yield in repeated batch cycles
The effect of reusability of immobilized beads on yield in repeated batch production of biodiesel was carried out. Immobilized lipase (2.5% v/v) beads were added to palm oil for esterification as previously described. After each batch of biodiesel production, beads were washed in excess distilled water before re-use.

3 Results
3.1 Effect of temperature and pH on biodiesel production by immobilized lipase
Comparison of the effect of temperature on biodiesel production free lipase and lipase immobilized on both calcium alginate and *Irvingia gabonensis* was presented in Fig. 1. Biodiesel yield was optimum at 35 °C for free and immobilized lipases, while the highest yield was obtained when calcium alginate-immobilized lipase was used for esterification (94.42%) compared to free lipase (94.1%) and *Irvingia gabonensis*-immobilized lipase (92.54%). However, over 90% of biodiesel yield was produced when esterification proceeded with immobilized lipase at 40-45 °C while biodiesel yield between 73 and 81% was produced at similar temperatures by free lipase.

The effect of acidity or alkalinity on biodiesel production by free and immobilized lipases is described in Fig. 2. The highest biodiesel yield was produced at neutral pH 7 using free and immobilized lipases, and biodiesel yield was higher for immobilized lipases than free lipase. Furthermore, lipase immobilized on calcium alginate produced the highest biodiesel yield (96.9%) than *Irvingia gabonensis*-immobilized lipase (95.8%) and free lipase (93.14%). Similarly, over 90% of biodiesel yield was produced at pH 5-6 by immobilized lipases compared to free lipase (< 90%).

3.2 Fuel properties free and immobilized lipase
Using American and European biodiesel standards, the quality of fuel based on physicochemical fuel properties produced by immobilized lipases compared to free lipase are described in Table 1.

Biodiesel produced by immobilized lipases had improved fuel properties compared to that produced by free lipase in terms of its densities at 15 °C and flash point, while both had similar pour point, cloud point, and kinematic viscosity. Furthermore, over 10 °C increase in flash point was observed in biodiesel produced by immobilized lipases compared to free lipase, and a similar increase in density at 15 °C of biodiesel produced by immobilized lipases was also observed. Furthermore, the pour points, flash point, and kinematic viscosity of biodiesel produced by calcium alginate and *Irvingia gabonensis*-immobilized lipase met American and European standards, while the density at 15 °C and cloud points are below both standards.

Chromatogram of biodiesel product from palm oil esterification by calcium alginate and *Irvingia gabonensis*-immobilized lipase in terms of the fatty acid methyl esters (FAME) is described in Figs. 3 and 4, and the FAME
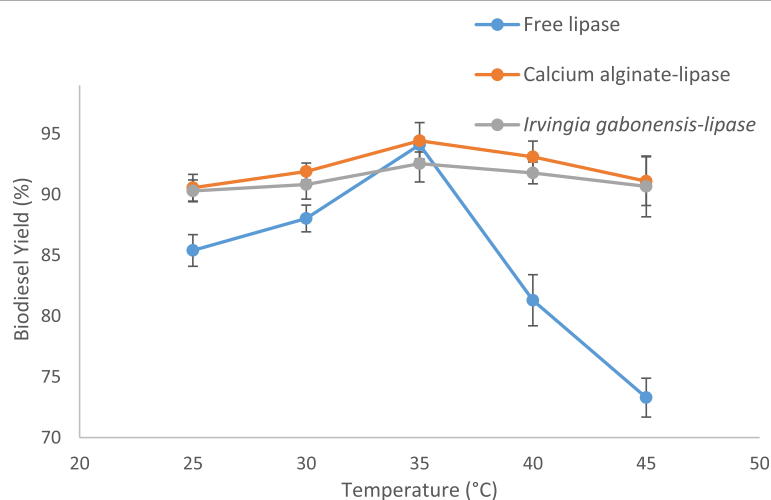

Fig. 1 Effect of temperature on palm oil biodiesel yield using free and immobilized lipases
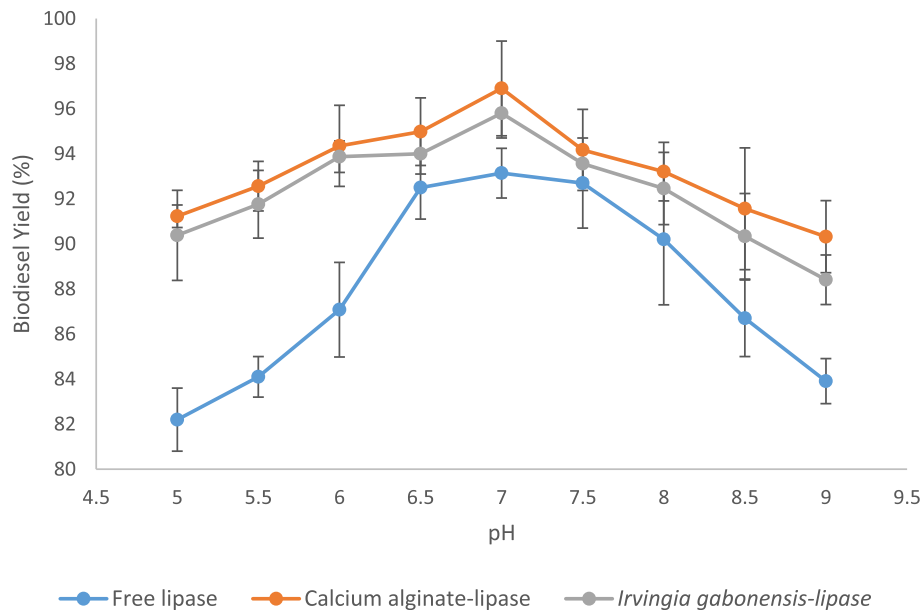
Fig. 2 Effect of pH on biodiesel yield produced using free and immobilized lipases

produced is summarized in Table 2.Biodiesel product from both calcium alginate and *Irvingia gabonensis*-immobilized lipase had FAME compounds hexadecanoate (palmitic acid), 9-octadecenoate (oleic acid), octadecanoate (stearic acid), dodecanoate (lauric acid), and 9, 12-octadeca-dienoate (linoleic acid) in common. However, 13-octadecanoate and 11-octadecanoate were present only in biodiesel produced with calcium alginate-immobilized lipase, while 8-octadecanoate and tetradecanoate were exclusive to biodiesel produced using *Irvingia gabonensis*-immobilized lipase. Furthermore, FAMEs including 13-octadecanoate, 11-octadecanoate, 8-octadecanoate, and tetradecanoate were present in biodiesel from immobilized lipases but not in biodiesel produced by free lipase.

3.3 Effect of bead reusability on immobilized lipase production of biodiesel
Re-usability of immobilized lipase in the production of biodiesel based on product yield is described in Fig. 5. Calcium alginate-immobilized lipase retained activity in

twelve cycles, while *Irvingia gabonensis*-immobilized lipase was re-useable in eight cycles. Furthermore, over 50% of biodiesel yield was achieved in 11 cycles by calcium alginate-immobilized lipase while a similar yield was maintained in seven (7) cycles for *Irvingia gabonensis*-immobilized lipase. Also, > 90% yield was achieved in four (4) and eight (8) for *Irvingia gabonensis*- and calcium alginate-immobilized lipases respectively.

4 Discussions
Temperature is an important factor in transesterification that can influence enzyme activity which affects reaction rate and FAME yield. Optimum temperature of 35 °C has previously been reported in biodiesel produced using alginate-immobilized lipase [28]. It is however lower than previous studies that reported optimum biodiesel production at > 40 °C using lipase immobilized on calcium alginate and silica [29, 30]. Furthermore, optimum reaction at 35 °C indicates esterification would reduce energy costs, and prevent evaporation of alcohols. Decreased biodiesel yield

Table 1 Comparison of fuel properties of biodiesel produced from palm oil using free lipase and calcium alginate and *Irvingia gabonensis*-immobilized lipase based on American and European biodiesel standards

Fuel properties	Calcium alginate lipase	*Irvingia gabonensis* lipase	Free lipase	ASTM D6751 standard	EN 14214 standard
Pour point (°C)	5.8	6.5	6.7		(−15)-10
Flash point (°C)	282	280	270	> 100-170	> 130
Density (at 15 °C)	820	823	813	880	860-900
Cloud point (°C)	16.9	15	16.8		
Kinematic viscosity (mm^2/s)	4.9	4.9	4.9	1.9-6.0	3.5-5.0

Fig. 3 Gas chromatogram of biodiesel produced by esterification of palm oil using calcium alginate-immobilized lipase

Fig. 4 Gas chromatogram of biodiesel produced by esterification of palm oil using *Irvingia gabonensis*-immobilized lipase
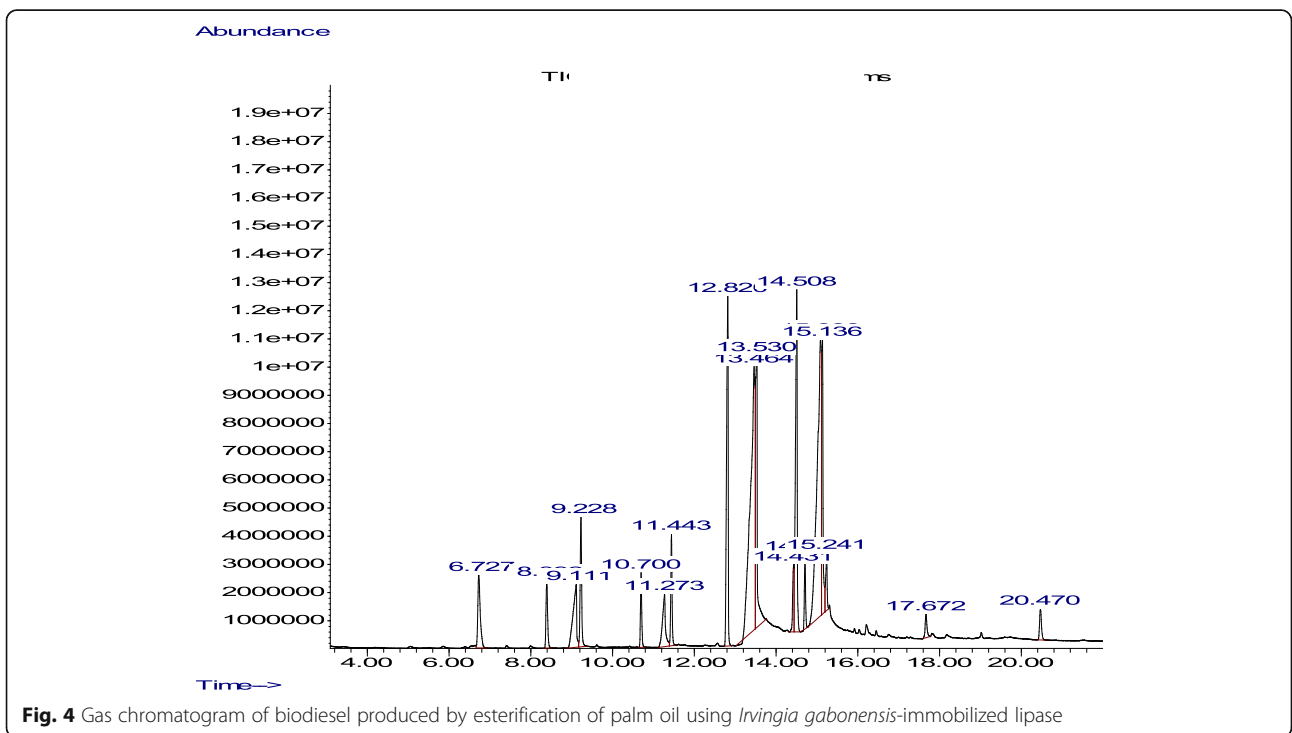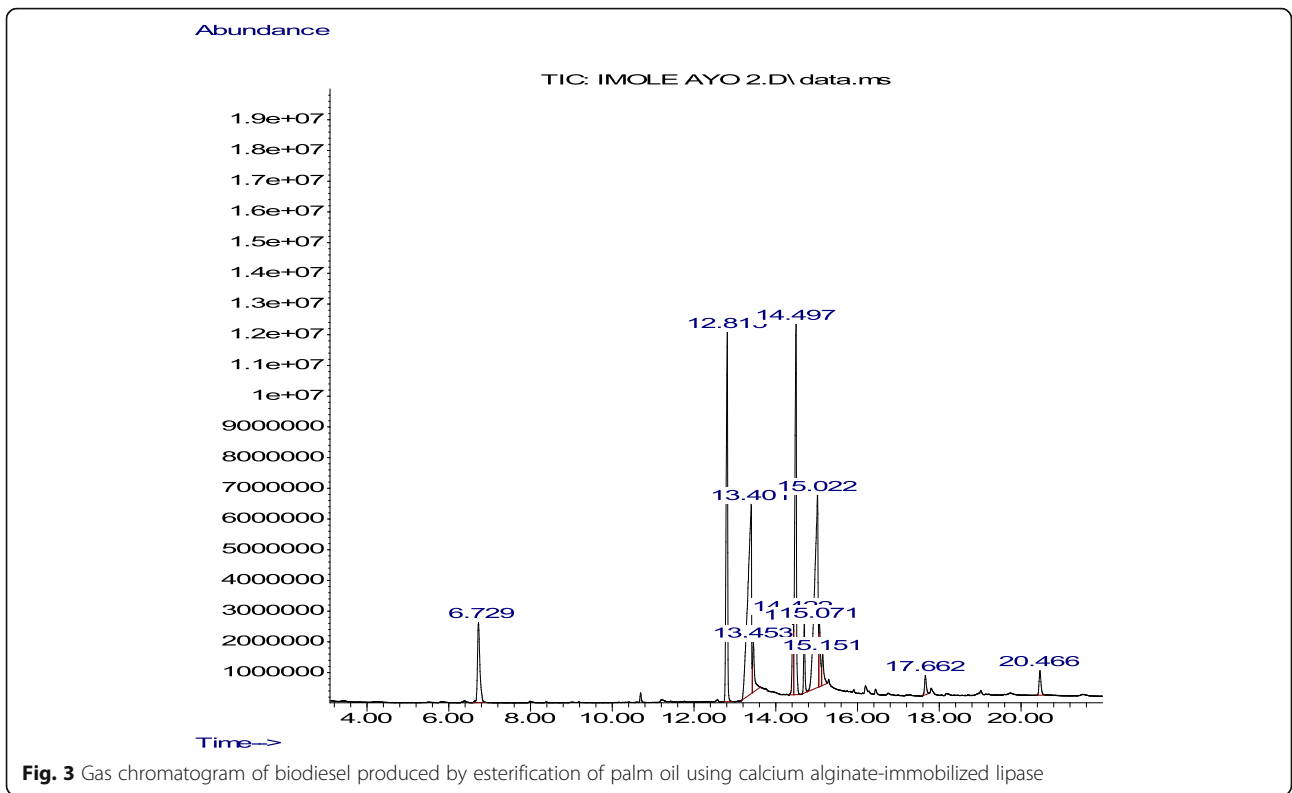
Table 2 Comparison of gas chromatogram summary of fatty acid methyl ester product of biodiesel produced from palm oil transesterified by free lipase, and alginate and *l. gabonensis*-immobilized lipase

| Methyl esters	Calcium alginate lipase	*Irvingia gabonensis* lipase	Free lipase
Hexadecanoate (palmitic acid)	17.12	9.5	13.7
9-Octadecenoate (oleic acid)	15.13	14.7	15.61
Octadecanoate (stearic acid)	2.59	1.44	2.34
Dodecanoate (lauric acid)	18.17	18.2	18.32
9,12-Octadeca-dienoate (linoleic acid)	3.3	2.13	0.8
13-Octadecanoate	18.44		
11-Octadecanoate	3.14		
8-Octadecanoate		1.43	
Tetradecanoate		2.46	

above 35 °C in this study could be due to poor lipase stability at elevated temperatures.

Optimum biodiesel yield at pH 7 for both free and immobilized lipase indicates entrapment in alginate, and *Irvingia gabonensis* matrices did not influence the ionic property of the enzyme. Also, the optimum activity of lipase at neutral pH is similar to the report of biodiesel production by resin-immobilized lipase [31, 32].

Fuel properties determine the quality of produced fuel and influence several processes during usage. Pour point (PP) is the temperature at which the produced biodiesel starts melting from solid to liquid, and a lower PP temperature in biofuel produced via immobilized lipase indicates formation earlier melting of fuel compared to fuel from free lipase. In the same vein, cloud point (CP) is the temperature at which biodiesel begins to form cloud, and CP determined in the study is lower than previous reports for edible and inedible tallow methyl ester (15-16 °C) [33]. This indicates produced biodiesel would be suitable for use in temperate regions, thereby

reducing the possibility of clogging injectors, filters, and fuel pipelines [30]. Kinematic viscosity influences the fuel injection process and atomization of fuel [34], and values determined in this study might be due to the presence of glycerol content in adequate concentration [35]. Furthermore, similar kinematic viscosity of biodiesel produced using both free and immobilized lipases is also in agreement with the previous report [24]. In addition, the density of biodiesel from immobilized lipases was higher than obtained from free lipase biodiesel, indicating an improved esterification process. Pour point of both biodiesels agrees with both American and European standards, while cloud point and flash point were higher than standards.

Unsaturated fatty acids are responsible for the FAME properties of the biodiesel product and their increased presence in biodiesel produced using immobilized lipases makes it a better biodiesel product [36]. It also indicates improved esterification process due to immobilization which increased the surface area of
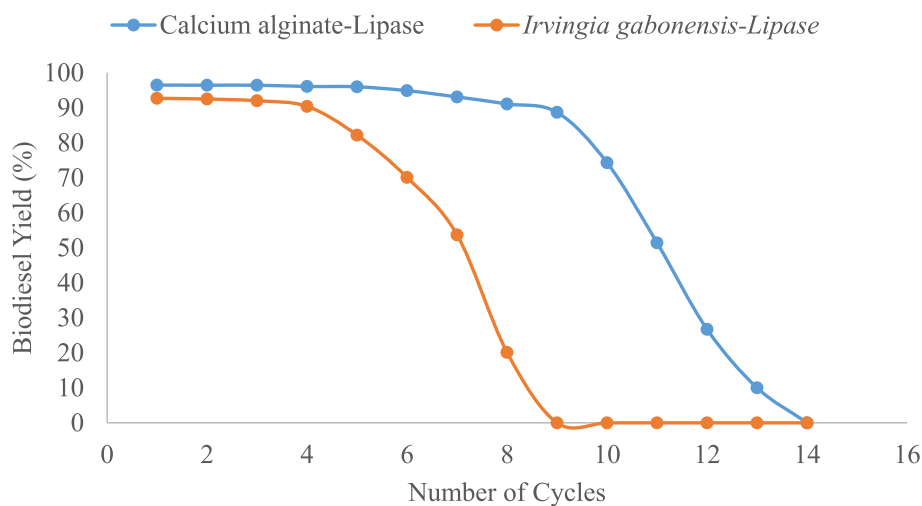
Fig. 5 Reusability of calcium alginate and *Irvingia gabonensis*-immobilized lipase in biodiesel production from palm oil

enzyme-substrate interaction. Furthermore, minor differences in FAME compounds produced by the different immobilized enzymes could be due to the influence of matrix parameters.

Reusability of immobilized lipase in over seven cycles and high biodiesel yield in 11 cycles using alginate-immobilized lipase agrees with the previous report of using *Irvingia gabonensis* as lipase immobilization matrix [23]. Reduced efficiency of *Irvingia gabonensis* matrix in this study however disagrees with the report, and this could be attributed to matrix interference in esterification reaction and leakage of enzyme from the support matrix due to weak binding forces [23, 37].

5 Conclusions

The study showed that using lipase immobilized on *Irvingia gabonensis*and calcium alginate matrices resulted in better biofuel (FAMEs and physicochemical fuel properties) compared to free lipase biofuel due to possibly improved enzyme-substrate interaction. Also, calcium alginate matrix conferred high potential for lipase reuse and maintain high biodiesel yield, while reusability of *Irvingia gabonensis* matrix could be further improved. This study showed that with further improvement, the use of immobilized lipase in transesterification for biodiesel production could improve product properties and lead to cost-effectiveness in industrial usage due to continuous use.

Abbreviations
CAIL: Calcium alginate-immobilized lipase; FAME: Fatty acid methyl esters; IGIL: *Irvingia gabonensis*-immobilized lipase; ASTM: American Standard of Testing Material; PO: Palm oil; FC: Flash point; CP: Cloud point; PP: Pour point

Authors' contributions
KSO and EIF co-designed the study, carried out the research, and wrote the manuscript draft; SAB and OAK contributed to the study design, analyses, and manuscript writing; OSO contributed to microbiology-related investigation and co-wrote the manuscript. All authors have read and approved the manuscript.

Availability of data and materials
Not applicable

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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