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Gülşah Saç, Gökmen Arabacı, Aleyna Çağan, Sevan Ağdamar, Müfit Özuluğ & Yasemin İspirli Doğaç

Sustainable biodiesel from an invasive fish using immobilized lipase on magnetic nanogels as a novel management approach

Gülşah Saç[1,*], Gökmen Arabacı[2], Aleyna Çağan[3], Sevan Ağdamar[4], Müfit Özuluğ[1] Yasemin İspirli Doğaç[5]

[1] Department of Biology, Faculty of Science, İstanbul University, İstanbul, Türkiye

[2] Department of Molecular Biology and Genetics, Graduate School of Natural and Applied Sciences, Muğla Sıtkı Koçman University, Muğla, Türkiye

[3] Department of Autopsy Assistant, Vocational School, Doğuş University, İstanbul, Türkiye

[4] Department of Forestry, Bayramiç Vocational School, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye

[5] Chemistry and Chemical Processing Technology Department, Muğla Vocational School, Muğla Sıtkı Koçman University, Muğla, Türkiye

Correspondence: gulsah.sac@istanbul.edu.tr

GS: 0000-0001-9988-1116
GA: 0000-0002-5848-6117
AÇ: 0000-0001-7688-6379
SA: 0000-0002-1268-0379
MÖ: 0000-0002-1437-3890
YİD: 0000-0001-8616-0280

Abstract

An alternative strategy to mitigate the ecological and economic challenges posed by invasive fish species is to transform these organisms into valuable resources, generating economic benefits while simultaneously addressing ecosystem-related concerns. However, while the search for sustainable feedstocks continues, the specific potential of highly resilient invasive fish species for high-yield biodiesel production has not yet been evaluated. The aim of this study was to address this research gap by producing high-yield biodiesel using the invasive fish *Carassius gibelio* as an oil source. In this context, lipase immobilized $MnFe_2O_4$- polyhydroxymethyl methacrylate magnetic nanogels were prepared and the production system conditions (lipase amount, methanol/oil molar ratio, and temperature) were optimized. Optimal conditions were obtained using a 4000 U lipase amount, a 5:1 methanol/oil molar ratio and a temperature of 55 °C. A 97.45% biodiesel yield was achieved with this system prepared under optimum conditions, and this prepared biocatalysis system was able to produce biodiesel with at least 50% yield 13 times. This study is the first to use the highly invasive *C. gibelio* as a sustainable raw material for biodiesel production. It introduces a novel biocatalytic approach by integrating invasive fish species utilization with a reusable $MnFe_2O_4$-pHEMA nanogel system, achieving both high efficiency and operational stability.

Keywords: Bioeconomy, *Carassius gibelio*, Renewable energy, Transesterification, Biocatalysis

Introduction

The heavy reliance on fossil fuels for transportation and industry has led to increased environmental concerns, such as pollution and carbon dioxide emissions, prompting a shift toward more sustainable energy alternatives [1-3]. Renewable energy sources—including clean options of energy such as the solar, wind, hydro and geothermal energy—have received a growing interest and support. Biomass energy, derived from plant or animal biomass through the biodegradation of organic materials, is considered and applied as an alternative renewable energy source due to its easy accessibility and low cost. These characteristics present significant opportunities for the energy sector [1, 4]. To this end, the concept of the circular bioeconomy has been developed in this direction to produce sustainable products (e.g., biofuel or food, cosmetic and pharmaceutical products) using renewable natural resources and their wastes. This approach ensures environmental sustainability while achieving economic benefits, and it is evaluated as a kind of industrial symbiosis where the waste/surplus (material or energy) of one process serves as an input for another [3, 5].

The rapid growth of the world's population has also increased the volume of organic waste. The accumulation of this waste in nature negatively impacts on the environment through the emission of greenhouse gases and the disruption of natural biological cycles [6, 7]. The reuse of organic waste for energy production is an important development in providing sustainable and cost-effective energy. Researchers have classified biodegradable materials into first, second, third, and fourth generations according to their production source and, have begun to produce biofuels such as biodiesel, bioethanol, biomethanol, biogas, and biohydrogen from them [8]. First-generation biofuels are derived from food crops such as wheat, barley, maize, rice, potatoes or sugar cane grown on cultivable land. Agricultural crops grown for biomass production require cultivable land and also serve as food for humans and animals, making this category questionable. In contrast to the first-generation group, second-generation biofuels are produced from non-edible agricultural crops or their residues and were developed to overcome the limitations of the first-generation biofuels. The third-generation focuses on the production of biofuels from photosynthetic microalgae and other aquatic biomass. This production can be considered one of the most sustainable, environmentally friendly, and economically viable fuels, as it does not require arable agricultural land and reduces greenhouse gas emissions. Fourth-generation biofuels are a complementary generation that provide a higher degree of output energy than input energy from feedstock and aim to produce high-quality biofuels from inexhaustible feedstock that are cheap and readily available globally [8, 9]. Oils derived from animal waste from the meat and fish processing industries, tanneries and slaughterhouses are considered suitable feedstock for biofuel synthesis due to their good calorific value compared to vegetable sources [10-14]. In today's world, where there is a growing interest in transitioning to alternative industrial raw materials and green processes [4], could the assessment of invasive species—which cause high ecological/economic damage—emerge as a new-generation approach?

Biological invasions, which are increasing because of the introduction and establishment of non-native species outside their native ranges, are a major driver of environmental degradation worldwide, resulting in significant ecological and economic impacts [15, 16]. Copp et al. [17] defined invasive species as native or alien/non-native species that have spread, with or without human assistance, into natural or semi-natural habitats, causing significant change in composition, structure or ecosystem processes, or severe economic losses to human activities. At the ecological level (biodiversity and ecosystem functions), the main negative impacts (with direct and indirect interactions) of invasive species in new habitats include predation (e.g., infection, herbivory, or parasitism), competition with native species for resources (food and habitat), hybridization, habitat modification, and the alteration of nutrient cycles [18, 19]. As a result, they can cause irreversible damage to local fauna and flora—particularly to endemic or economically important species—negatively impacting ecosystem services and functioning, and ultimately radically altering the socio-economic structure of the environment they enter [20-23]. Increasingly recognized as a major form of biological pollution [24-26], invasive species are undermining ecosystems and social welfare. The negative impacts of invasive species on biodiversity and the economy remain unclear in many countries. However, the costs of control, research, prevent and monitoring, as well as the damage caused to sectors as agriculture, livestock, fisheries, forestry and human health, are proving to be quite high [27-29].

Carassius gibelio is an invasive species that has become widely distributed across most European countries during the 20th century due to its introduction and translocation [30-32]. Its unique reproductive mode—specifically involving allogynogenetic and/or gonochoristic biotypes (or bioforms)—its resilience to adverse environmental conditions and its negative ecological impact (e.g., competition and sperm parasitism on native fishes, negative effects on water quality, and benthic fauna) on both ichthyofauna and habitats have attracted significant interest from aquatic biologists in recent years [30, 33-37]. Risk assessment studies have documented *C. gibelio* as a species with a high or very high invasion risk in many countries and ecosystems [38-43]. Researchers are now revealing the economic cost of biological invasions that threaten the stability of ecosystems and societies [44-46]. The total cost of invasive species in Europe between 1960 and 2020 amounts to US$ 140.20 billion (or € 116.61 billion), the majority (60%) of which is damage-related and affects several sectors [46]. Lastly, the total economic cost attributed to invasive species in Türkiye has been estimated at US$ 4.1 billion between 1960 and 2022, and *C. gibelio* is reported to be one of the top ten most costly invasive species, significantly affecting the fisheries sector in terms of damage costs [29]. Therefore, both its wide distribution and high bio-ecological tolerance, which allow it to be sustainably accessed, will make it possible to reduce ecological and economic damage by integrating this fish into the economy.

To reduce the ecological/economic burden caused by invasive species, an important approach, in terms of both ecosystem and economic policies, is to convert these species into opportunities by using them in different areas and generating economic benefits. For this purpose, this study aims to first use the invasive fish *C. gibelio* (gibel carp), which is costly to ecosystems and economies, as a feedstock in biodiesel production and to include it in the ecological carbon cycle. The second objective of the study is to demonstrate the feasibility of an alternative method to obtain the highest biodiesel yield using a reusable system. To achieve this goal, the lipase enzyme was used by immobilizing it on magnetic nanogels using an adsorption and cross-linking method. Waste oils or oils that are not consumed as food can be used to produce biodiesel fuel through the biocatalysis of lipase enzymes [47]. Lipases are enzymes that catalyze the hydrolysis of the ester bonds of lipids, i.e., they hydrolyze triacylglycerols to diacylglycerol, monoacylglycerol, free fatty acids, and glycerols [47-49]. Furthermore, biodiesel production using biocatalysts offers advantages over other biodiesel production methods because of their high selectivity, prevention of by-product formation, and easier product recovery [50, 51]. The outputs of the present study are also linked to the United Nations Sustainable Development Goals 7 (energy) and 14 (life under water). The novelty of this study lies in its dual innovation: the first-time use of invasive *C. gibelio* as a feedstock for biodiesel production and, the implementation of a reusable magnetic nanogel-based biocatalyst system.

Materials and Methods

Materials

2-hydroxyethyl methacrylate (99%, for synthesis) (HEMA), N,N′-Methylenebis(acrylamide) (99%) (MBA), Porcine pancreatic lipase (triacylglycerol lipase, E.C.3.1.1.3), glutaraldehyde solution (25% (v/v) in water) (GA), Triton X-100 (laboratory grade) were purchased from Sigma-Aldrich. Methanol (99%, suitable for GC/MS) and chloroform (99%, suitable for GC/MS) were purchased from Supelco.

Fish sampling

Carassius gibelio specimens were collected in February 2024 using gill nets (mesh size 60×60 mm) in the Büyükçekmece Reservoir (İstanbul, Türkiye). Fish samples were measured to the nearest 0.01 g for body mass and then were stored in a deep freezer (-18 °C) until the biodiesel procedures were performed.

Laboratory procedures

The step-by-step procedures for extracting biodiesel from *C. gibelio* are outlined below. A summary of the laboratory processes is illustrated in Fig. 1.

Fish oil extraction from Carassius gibelio

Lipid extraction was performed using a modified version of the conventional solvent method based on the Bligh and Dyer protocol and microwave-assisted extraction [52]. For oil extractions, a total of five whole-body fish samples (146.56 – 158.69 g) were used. Fish were dried in a drying oven (MEMMERT, UN260) at 55-60 ºC and powdered. Lipid extraction was performed by the conventional solvent and microwave-assisted methods. In the conventional solvent method, chloroform (10 mL), methanol (20 mL), and distilled water (8 mL) were mixed with 10 g biomass for 24 h. Microwave-assisted lipid extraction was performed at 600 W power with a 100% duty cycle at 80 °C for 10 min. The lipid layer was separated using a separatory funnel, and the solvents were evaporated with a rotary evaporator (YAMATO SCIENTIFIC, RE-801 Model).

Lipase immobilization on $MnFe_2O_4$-polyhydroxymethyl methacrylate (pHEMA) magnetic nanogels

Photopolymerization was used to prepare the magnetic nanogels. The sequential adsorption-crosslinking strategy was adopted for lipase immobilization based on our previous work, which demonstrated that this two-step approach effectively preserves the catalytic orientation of the enzyme while providing high structural stability [53]. This strategy was specifically selected to combine the high protein loading capacity of pHEMA-based magnetic nanogels with the robust stabilization offered by covalent crosslinking. Accordingly, a 50 mL aqueous solution containing 1% (v/v) HEMA and 5 mg MBA was stirred and degassed under a nitrogen gas environment for 30 min to eliminate oxygen inhibition. 1 mL of 10 mg/mL $MnFe_2O_4$ magnetic nanoparticles was added and the photopolymerization reaction was carried out under UV-light (UVP, C15G Model) for 10 min. The formed $MnFe_2O_4$-pHEMA magnetic nanogels were washed several times. To determine the morphology of the magnetic nanogels, samples prepared with 10 mg $MnFe_2O_4$, and 1% (v/v) HEMA were analyzed using a Scanning Electron Microscope (SEM) (JEOL, JSM-7600F).

A sequential strategy involving adsorption (1 h) and then covalent cross-linking with 2% glutaraldehyde (2 h) were used to immobilize the lipase enzyme on the magnetic nanogels. 3000 U, 4000 U or 5000 U of the lipase enzyme was first mixed with 0.01 g/mL support and then stirred for 1 h at 4 °C to allow physical adsorption onto the nanogel surface. Lipase activity was measured in the supernatant obtained after centrifugation (HETTICH, ROTANTA 460 R Model) at 8000 rpm for 5 min. A cross-linking reaction with 2% glutaraldehyde was then carried out with stirring for 2 h to prevent enzyme leaching and enhance stability. Finally, the immobilized lipase was washed three times with ultrapure water and separated magnetically. The average immobilization efficiency of the replicate experiments was calculated to be 94.35% and the average protein loading capacity was calculated to be 89.97%. The immobilization efficiency (IE%) and protein loading capacity (LC%) were calculated based on the difference between the initial amount of protein and the amount of protein remaining in the supernatant after the immobilization process, determined by the Bradford method. The equations used for these calculations are as follows:

$$IE\% = \frac{C_i - C_f}{C_i}.100$$

$$LC\% = \frac{(C_i - C_f).V}{W_p}.100$$

where C_i is the initial lipase concentration (mg/mL), C_f is the final concentration of lipase in the supernatant after immobilization (mg/mL), V is the total volume of the protein solution (mL), and W_p is initial weight of protein added to the system (mg).

The measurement of the immobilized lipase activities was carried out using an oil emulsion as the substrate under appropriate standard pH and temperature conditions.

Application of the biodiesel production process

The optimization of biodiesel production with immobilized lipase was carried out in a 250 mL screw-capped flask. The performance of lipase immobilized on $MnFe_2O_4$-pHEMA magnetic nanogels was investigated by mixing 15 g of *C. gibelio* oil and immobilized lipase solution (3000 U, 4000 U or 5000 U) and 10 µl of Triton X-100. The process was continued at 250 rpm, 50, 55, 60 or 65 °C, at a specific methanol/oil molar ratio (3:1; 5:1; 7:1 or 10:1) for 24 h. At the end of each process, the product obtained was separated by centrifugation at 9500 rpm for 30 min, and the biodiesel obtained was kept in the separating funnel for 2 h.

The biodiesel yield was determined as the percentage ester mass/oil mass. The fatty acid methyl esters were analyzed by gas chromatography-mass spectrometry (GC-MS) (THERMO SCIENTIFIC, ISQ with TRACE 1300 GC-MS). GC-MS conditions: initial temperature 100 °C for 5 min, 5.5 °C/min up to 170 °C, 2.5 °C/min up to 250 °C for 10 min, carrier gas helium, injector and detector temperatures 250 °C and 235 °C, respectively. To compare the activity of the immobilized lipase, the activity of the free lipase was also determined under the same conditions.

Determination of the reusability performance of the biodiesel production system prepared with immobilized lipase

The reusability of the system obtained by immobilizing lipase on $MnFe_2O_4$-polyhydroxymethyl methacrylate (pHEMA) magnetic nanogels was evaluated by removing it from the medium after each transesterification process. To ensure the removal of any residual biodiesel, glycerol, or unreacted feedstock that could block the active sites of the enzyme, the recovered biocatalysts were washed three times with ultrapure water and subsequently conditioned by incubation in the reaction buffer at 4 °C for 15 min before the next cycle. The cycles were continued until the activities were completely lost. The total biodiesel yield of the first reaction was taken as 100%, and the yields of the successive processes were calculated accordingly.

Additionally, control experiments were performed using blank (enzyme-free) magnetic nanogels under the same optimized conditions to investigate the potential catalytic effect of the support material.

Statistical Analysis

All experimental assays were performed in triplicate (n = 3), and the results were expressed as mean ± standard deviation (SD). The statistical significance of the differences between the free and immobilized lipase performances was evaluated using the two-tailed Student's t-test with unequal variance (heteroscedastic). A *p*-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

Results and Discussion

SEM analysis of magnetic nanogels

Magnetic nanogels are inorganic-polymer composites with different sizes, shapes, and surface areas. The photochemical method was used for the preparation of $MnFe_2O_4$-pHEMA magnetic nanogels, and their morphological properties were determined by scanning electron microscopy (SEM) analysis. Figure 2 shows the SEM images of the magnetic nanogel prepared using 10 mg $MnFe_2O_4$ and 1% HEMA. Examination of the SEM images revealed an excess of inhomogeneous rough structure on the surface of the pHEMA magnetic nanogels. This roughness on the surface increases the surface area, which allows more enzyme molecules to adsorb on the surface during enzyme immobilization.

Fatty acid composition of C. gibelio fish oil

Oil extraction from the fish mass yielded 3.61% of the total sample weight. The major fatty acid composition of *C. gibelio* fish oil was determined by GC/MS and the results are presented in Table 1. As shown in Table 1, the major fatty acid composition of the fish oil was found to consist of 26.98 wt.% palmitic acid (C16:0), 22.14 wt.% oleic acid (C18:1), 16.86 wt.% linoleic acid (C18:2), 7.75 wt.% palmitoleic acid (C16:1), 6.72 wt.% stearic acid (C18:0), 5.48 wt.% ▯-linoleic acid (C18:3), 4.56 wt.% docosahexaenoic acid (C22:6), and 2.92 wt.% myristic acid (C14:0). Evaluating the fatty acid profile enables the prediction of some properties of the obtained biodiesel. The presence of the saturated fatty acids palmitic acid (C16:0) (26.98%) and stearic acid

(C18:0) (6.72%) gives biodiesel high oxidative stability. However, if the ratios of these saturated fatty acids are too high, the fluidity properties of the biodiesel may deteriorate in cold environments. The presence of the monounsaturated fatty acids oleic acid (C18:1) (22.14%) and palmitoleic acid (C16:1) (7.75%) as well as the polyunsaturated fatty acids linoleic acid (C18:2) (16.86%) and α-linoleic acid (C18:3) (5.48%) improves the flow properties of the biodiesel in cold conditions. This profile is desirable in biodiesel production but may shorten its shelf life. The presence of the polyunsaturated fatty acid docosahexaenoic acid (C22:6) (4.56%), which is specific to fish oils, also contributes to the flow properties in cold conditions. The presence of myristic acid (C14:0), a short-chain saturated fatty acid, improves the combustion characteristics [54-56]. Also, the fuel properties of the produced *C. gibelio* biodiesel were evaluated based on its FAME profile (Table 1) in accordance with EN 14214 standards. The composition consists of 36.62% saturated fatty acids (SFA), primarily palmitic acid (26.98%), which contributes to a high cetane number (typically >51) and ensures efficient combustion. The total unsaturated fatty acid (UFA) content was 56.79%, including a significant portion of oleic acid (22.14%). According to the literature [57], a higher degree of unsaturation improves the cold flow properties, such as the cloud point and pour point. However, the presence of polyunsaturated acids like linoleic (16.86%), linolenic (5.48%), and docosahexaenoic acid (4.56%) can influence the iodine value and oxidation stability. Based on the calculated mass percentages, the iodine value is estimated to be well within the European standard limit of <120 g I_2/100 g. Furthermore, the balanced SFA/UFA ratio suggests that the biodiesel meets the density requirements (0.86–0.90 kg/m^3) and provides a robust alternative to conventional diesel fuel.

The observed oil yield of 3.61% from *C. gibelio* is consistent with the general lipid ranges reported for freshwater fish, yet it highlights the inherent biochemical variability and physiological fluctuations characteristic of the species. The lipid ratio of fish is known to vary significantly depending on the species, ontogenetic development, sex, and specifically the habitat. Previous studies have documented a wide spectrum of lipid ratios in various fish, ranging from as low as 0.78% to as high as 8.2% [58]. This variation is often linked to habitat-related differences in lipid reserves, as fish adapt their biochemical composition to environmental conditions. Furthermore, the specific fatty acid profile observed in this study, which includes high levels of palmitic (26.98%) and oleic acid (22.14%), can be attributed to the species' ecological interactions and nutrient availability in its environment. Different freshwater habitats have been shown to cause significant comparisons in the lipid contents and fatty acid profiles of fish, confirming that the biochemical composition of *C. gibelio* is not static but fluctuates according to the specific characteristics of the water body it inhabits. Consequently, while seasonal and habitat-based factors may influence the precise lipid yield, the overall high biomass and sustainable availability of this invasive species render it a robust candidate for consistent biodiesel production [58-60].

Application of the biodiesel production process

The main factors affecting the transesterification process are the amount of biocatalyst, the alcohol-to-oil molar ratio, and the reaction temperature. In the present study, to determine the biodiesel yield produced using lipase immobilized on $MnFe_2O_4$-pHEMA magnetic nanogels, the effects of the amount of immobilized lipase, methanol/oil molar ratios, and temperature were determined.

The effect of the amount of lipase on biodiesel production was also investigated, and the amount of lipase was varied to 3000, 4000, and 5000 U. Throughout the experimental trials, the reaction temperature and the methanol/oil molar ratio were maintained at constant values of 55 °C and 7:1, respectively. The graph shows that the highest biodiesel production efficiency (96.78%) was achieved with the immobilized lipase using 4000 U of lipase (Fig. 3). It was also found that immobilized lipases had a higher efficiency than free lipase at all lipase amounts. Statistical analysis indicated that the differences in activities between free and immobilized lipase were significant for all enzyme loadings tested (3000-5000 U), with p-values ranging from 0.0036 to 0.0195 ($p < 0.05$).

In addition to the studies using different methodologies, there are some studies on biodiesel production by enzymatic transesterification of fish oil with lipase [52, 61]. The major obstacle to the use of lipase in biodiesel production is its high production cost. However, this cost can be reduced by using immobilized lipase enzymes because they offer many advantages such as reusability, thermal and pH stability, and reaction control [62-64]. Although there are many techniques for enzyme immobilization, adsorption, cross-linking or entrapment methods are preferred due to their ease of application, cost and activity preservation properties [65]. In this study, adsorption followed by the cross-linking method was the preferred immobilization technique and proved to be an applicable method because of its high efficiency (97.45%). The use of lipase enzyme in the immobilized form for biodiesel production has been investigated using various methods, and the studies have resulted in high yields. In the literature, a similar biodiesel production yield (96.5%) was reported for lipase immobilized on polyhydroxybutyrate [66], a biodiesel production yield of 97.2% obtained with lipase immobilized on amino functional silica material [67] and a yield of

92.66% for lipase immobilized on $CaCO_3$ [68]. In addition, in this study, the use of magnetic polymers (magnetic nanogels: $MnFe_2O_4$-polyhydroxymethyl methacrylate) enabled both fast and easy separation for enzyme recovery. As this process can be multi-step for other supports, the use of magnetic polymers is more attractive in terms of energy, cost, and efficiency. The use of magnetic particles in bioprocessing has many advantages. These particles are easily separated from the reaction medium and can be moved as desired by applying a magnetic field and are stabilized in the fluidized bed reactors. In addition, the use of magnetic particles causes little pollution and reduces the process costs.

One of the important criteria in biodiesel production is the methanol/oil molar ratio. Therefore, in the next stage, the effect of the methanol/oil molar ratio on biodiesel production was investigated and the methanol/oil molar ratio was varied as 3:1; 5:1; 7:1, and 10:1. The experiments were carried out at a constant reaction temperature of 55 °C, with the lipase concentration maintained at 4000 U, a parameter previously determined to yield optimal results. The graph indicated that the highest biodiesel production efficiency (97.45%) was achieved under conditions employing a methanol/oil molar ratio of 5:1 in conjunction with the use of immobilized lipase (Fig. 4). It was found that the 7:1 methanol/oil molar ratio reached rates close to 5:1. However, at the 10:1 methanol/oil molar ratio, the yield decreased to 80%. Therefore, as the methanol ratio increased, the biodiesel yield decreased. The probable reason for this is that the excess methanol in the medium started to inhibit the lipase enzyme. On the other hand, immobilized lipases were found to be more efficient than free lipase at all methanol/oil molar ratios. The impact of the methanol:oil ratio was highly significant for ratios of 5:1 and above ($p < 0.001$). However, at a 3:1 ratio, the difference between the two systems (free and immobilized lipase) was not statistically significant ($p = 0.1401$), suggesting similar performance at lower methanol concentrations. 3 moles of methanol, which is a highly reactive alcohol, is used for biodiesel production by a transesterification reaction with 1 mole of triacylglycerol, and it is generally recommended to use excess methanol [69]. However, it has been observed that the use of excess methanol increases saponification in other non-enzymatic biodiesel production methods, but this problem can be solved by enzymatic biodiesel production [70, 71].

After determining the optimum lipase amount and methanol/oil molar ratio, the temperature was changed to 45, 50, 55, 60, and 65 °C to determine the ideal reaction temperature. As depicted in Fig. 5, the experiments were conducted under the optimal conditions identified in the earlier stages, with the lipase concentration maintained at 4000 U and the methanol/oil molar ratio kept constant at 5:1. Biodiesel production was not achieved at 45 °C. Therefore, these temperature data are not included in Fig. 5. Analysis of the graphical data revealed that the highest biodiesel production yield of 97.45% was attained at a reaction temperature of 55 °C under enzymatic catalysis employing immobilized lipase. At 65 °C, the biodiesel yield (83.44%) decreased due to the possible onset of protein denaturation. The immobilized lipase exhibited superior performance over the free lipase at 50, 55, 60, and 65 °C, confirmed by high statistical significance ($p = 0.0072$, $p = 0.0006$, $p = 0.0056$ and $p = 0.0084$, respectively).

In the literature, it has been reported that the optimum biodiesel production yield with lipase enzyme immobilized on polyhydroxybutyrate was reached at 45 °C [66], 50 °C was used for the optimum biodiesel production yield obtained with lipase immobilized on amino functional silica material [67], and the optimum yield temperature for lipase immobilized on $CaCO_3$ was 55 °C [68]. It is likely and expected that there will be such differences in the optimum temperatures of the lipase enzymes from different sources.

Determination of the reusability performance of the biodiesel production system prepared with immobilized lipase

The most important advantage offered by immobilized enzymes is that they provide the opportunity for reuse. Therefore, in this study, the reuse performance of lipase immobilized on $MnFe_2O_4$-pHEMA magnetic nanogels in biodiesel production was investigated. During the evaluation of the reuse performance, the previously optimized conditions—namely, a lipase concentration of 4000 U, a methanol/oil molar ratio of 5:1, and a reaction temperature of 55 °C—were maintained consistently throughout the experiments. The percentage biodiesel yield was maintained at 100% for the first 6 cycles and at 50% for 13 cycles. After the 20th cycle, the percentage biodiesel yield could not be obtained (Fig. 6). In the context of industrial feasibility, a biocatalyst is typically considered effective if it maintains at least 50% of its initial activity or yield. Based on this criterion, the immobilized lipase developed in this study remained effective for 13 consecutive cycles. Although this reuse number is quite high, it was also desired to determine the cycle in which the efficiency would be completely lost. After the 20th cycle, it is assumed that the failure to obtain the percentage biodiesel yield is due to the inactivation and/or desorption of the immobilized lipase by washing after many cycles, and this is an expected situation. In the literature, the percentage biodiesel yield of lipase immobilized on $CaCO_3$ decreased to 50% after 10 cycles and to 0% after 14 cycles [68]. Control experiments conducted with blank magnetic nanogels resulted in no detectable biodiesel yield, confirming that the support material itself possesses no intrinsic catalytic activity and that the reaction is solely mediated by the immobilized lipase.

Evaluation of C. gibelio as a sustainable feedstock for biofuel industry

Various methods have been developed to manage or eradicate invasive species; while efforts to apply these targets using chemical and physical methods or biomanipulation have sometimes been successful, they have proved difficult to sustain and their harmfulness to the native or endemic populations has been debated. The rapid development of genetically based technologies (e.g., gene-silencing, RNA-guided gene drives, and the use of transgenes) has created excitement regarding the elimination or control of invasive species [72]. However, removing a fish with a specialized reproductive strategy such as gynogenesis and high ecological tolerance, such as *C. gibelio*, from all invaded water bodies using chemicals or genetic techniques would be a very costly and time-consuming practice. Chemical intervention, intensive fishing, or eradication programs are generally recommended to control this invasive fish; if control fails, alternative uses are suggested, such as utilizing them for human consumption, employing their skins medically treat wounds, or applying them as fertilizer in soil or aquaponic systems [73]. Instead, applying intense fishing pressure to this fish and converting it into a value-added product is proposed as a new and promising approach in this study. Considering the need for renewable energy, the production of biodiesel from this fish demonstrates the originality of the research regarding its potential economic benefits. In terms of ethical considerations, using invasive fish biomass rather than merely discarding it after culling presents a more responsible approach. Discarding captured biomass can sometimes raise ethical issues regarding animal waste and environmental impact. Therefore, repurposing this biomass as a renewable energy source offers a more acceptable way to utilize these organisms.

In addition to being a source of protein, fish serve an important energy source due to their valuable lipid content and composition. These characteristics make fish ideal for both human food and animal feed. The lipid ratio in fish varies depending on species, ontogenetic development, sex, and even habitat [74, 75]. Previous studies have reported lipid ratios ranging from as low as 0.78% in *Carasobarbus luteus* in its natural habitat to as high as 8.2% in the cultured species *Hypophthalmichthys nobilis*. Therefore, from a biodiesel production and yield perspective, the lipid ratio of the fish is of critical importance. Compared with many other species mentioned in the previous studies [76-78], the lipid ratio calculated for *C. gibelio* was relatively high (3.61%). Moreover, this species, which can reach a length of approximately 40 cm [79], is characterized by high biomass in both reservoirs and natural lakes across many countries [35, 80-82]. Consequently, *C. gibelio*—as an invasive species—may be considered as a promising candidate for biodiesel production due to its sustainable availability through regular catches and its high oil content. Furthermore, the potential of fish waste for sustainable biofuel production is generally considered to be an efficient and viable process, primarily due to its high lipid content. Numerous studies have demonstrated that the use of fish waste as a substrate in biofuel production results in high efficiency [11, 83-85]. Notably, the present study also represents the first attempt to directly evaluate an invasive fish species for biodiesel production by utilizing the whole body of the fish for its high lipid content.

The United Nations Sustainable Development Goal 7 (energy) outlines key targets such as ensuring access to clean and affordable energy, which is key to development in many sectors. The results of this study are expected to provide the necessary reliable and sustainable energy data required to meet the targets set out in Goal 7, particularly utilizing high lipid invasive fish such as *C. gibelio* as a carbon source. In addition, Goal 14 (life below water) focuses on the conservation and sustainable use of aquatic resources, as they provide essential natural resources such as food, medicines, biofuels and other products. This study, which proposes that freshwater resources can be protected by managing invasive fish species and converting these harmful organisms into value-added products, provides critical data to support the achievement of the Goal 14 targets.

Conclusion

Since *C. gibelio* is generally not preferred as food due to its bony structure, repurposing this invasive species for biodiesel production could offer a practical mitigation strategy. This approach may help integrate a recognized biological threat into a more beneficial ecological cycle. In this study, based on this perspective, this invasive fish was used for the first time as a feedstock to produce high value-added biodiesel. The method used was the enzymatic catalysis method, which offers numerous advantages in biodiesel production; specifically, lipase enzyme was immobilized on $MnFe_2O_4$-pHEMA magnetic nanogels, enabling its of reuse (maintaining at least 50% yield over 13 cycles). In addition, high yields were achieved by optimizing the production conditions. Furthermore, given that the use of biological waste or harmful organisms as raw materials for biofuel production is regarded as an affective waste management practice, it is believed that the approach presented constitutes a sustainable practice and will provide benefiting to both the ecosystem and the economy. This study offers a new approach for managing invasive species, which are the drivers of biological invasions, while also advancing the growing body of knowledge in species-specific biodiesel production.

Furthermore, the economic sustainability of this approach can be significantly enhanced by adopting a biorefinery-based model. The present findings indicate that *C. gibelio* oil contains valuable long-chain polyunsaturated fatty acids, such as DHA (4.56%), which are highly sought after in the pharmaceutical and nutraceutical sectors. Integrating the recovery of these high-value compounds with biodiesel production would provide an additional revenue stream, effectively offsetting the operational costs of enzymatic transesterification. This integrated strategy transforms the management of invasive species from a purely ecological intervention into a circular bioeconomy model that is both environmentally restorative and economically competitive.

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Authors' contributions

GS: Conceptualization, Fish Survey, Investigation, Writing Original Draft, Visualization. GA: Formal Analysis, Data Curation. AÇ: Formal Analysis, Data Curation. SA: Investigation, Review and Editing. MÖ: Investigation, Review and Editing. YİD: Conceptualization, Investigation, Formal Analysis, Data Curation, Visualization, Writing Original Draft, Review & Editing.

Data availability statement

The datasets generated and analyzed during the current study are available from the authors on reasonable request.

Competing interests

The authors declare no conflicts of interest.

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Ethics approval

According to the national guidelines for animal care, this study was conducted with the permission of İstanbul University Local Ethics Committee for Animal Experiments (Date: 04.09.2023, No: 2023/26).

Figure legends

Figure 1. Scheme describing the laboratory processes for biodiesel production from *Carassius gibelio*.

Figure 2. SEM images of $MnFe_2O_4$-pHEMA nanogel (100× and 1000× magnification)

Figure 3. Effect of the amount of lipase on biodiesel production from *Carassius gibelio* [*Data are presented as mean ± SD (n=3). Differences between free and immobilized lipase were found to be statistically significant ($p < 0.05$]).*

Figure 4. Effect of the methanol/oil molar ratio on biodiesel production from *Carassius gibelio* [*Data are presented as mean ± SD (n=3). Differences between free and immobilized lipase were found to be statistically significant for ratios of 5:1 and above ($p < 0.05$)].*

Figure 5. Effect of temperature on the biodiesel production of *Carassius gibelio* [*Data are presented as mean ± SD (n=3). Differences between free and immobilized lipase were found to be statistically significant ($p < 0.05$]).*

Figure 6. Reuse performance of lipase immobilized on $MnFe_2O_4$-pHEMA magnetic nanogels in biodiesel production from *Carassius gibelio* [*Data are presented as mean ± SD (n=3)].*

Table legends

Table 1. Major fatty acid compositions of the *Carassius gibelio* fish oil.

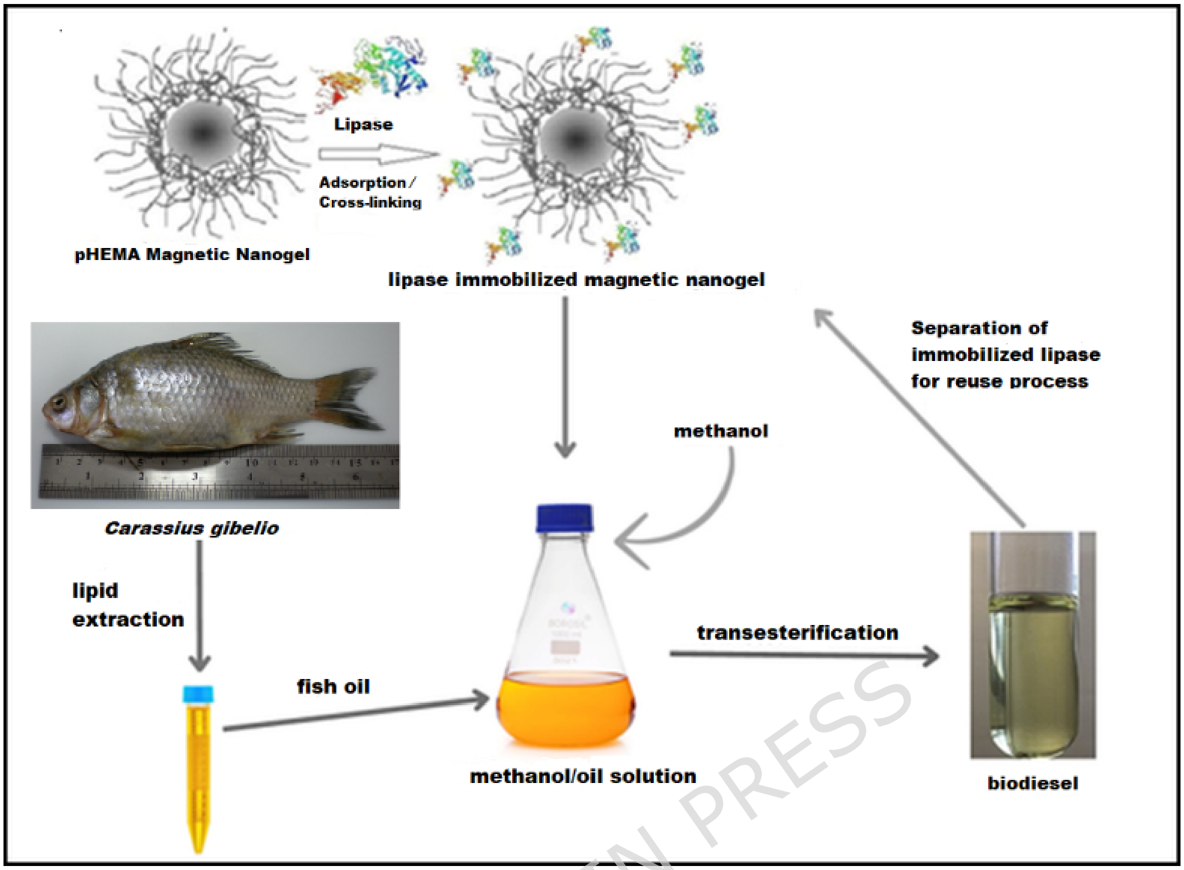
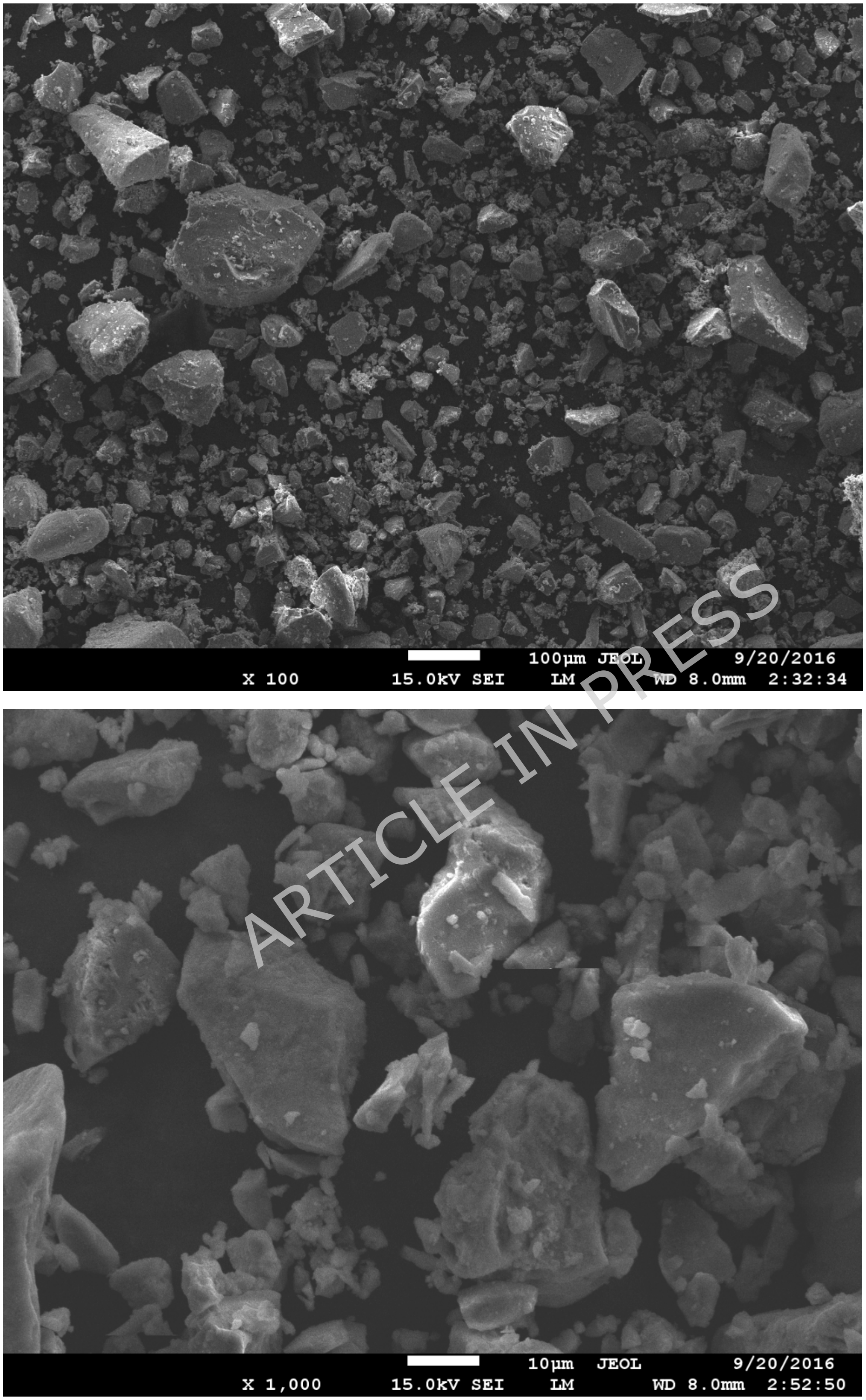

Lipase
Adsorption / Cross-linking
pHEMA Magnetic Nanogel
lipase immobilized magnetic nanogel
Separation of immobilized lipase for reuse process
methanol
Carassius gibelio
lipid extraction
transesterification
fish oil
methanol/oil solution
biodiesel

Figure 1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X 100 15.0kV SEI LM WD 8.0mm 2:32:34
10μm JEOL 9/20/2016
X 1,000 15.0kV SEI LM WD 8.0mm 2:52:50

Figure 2

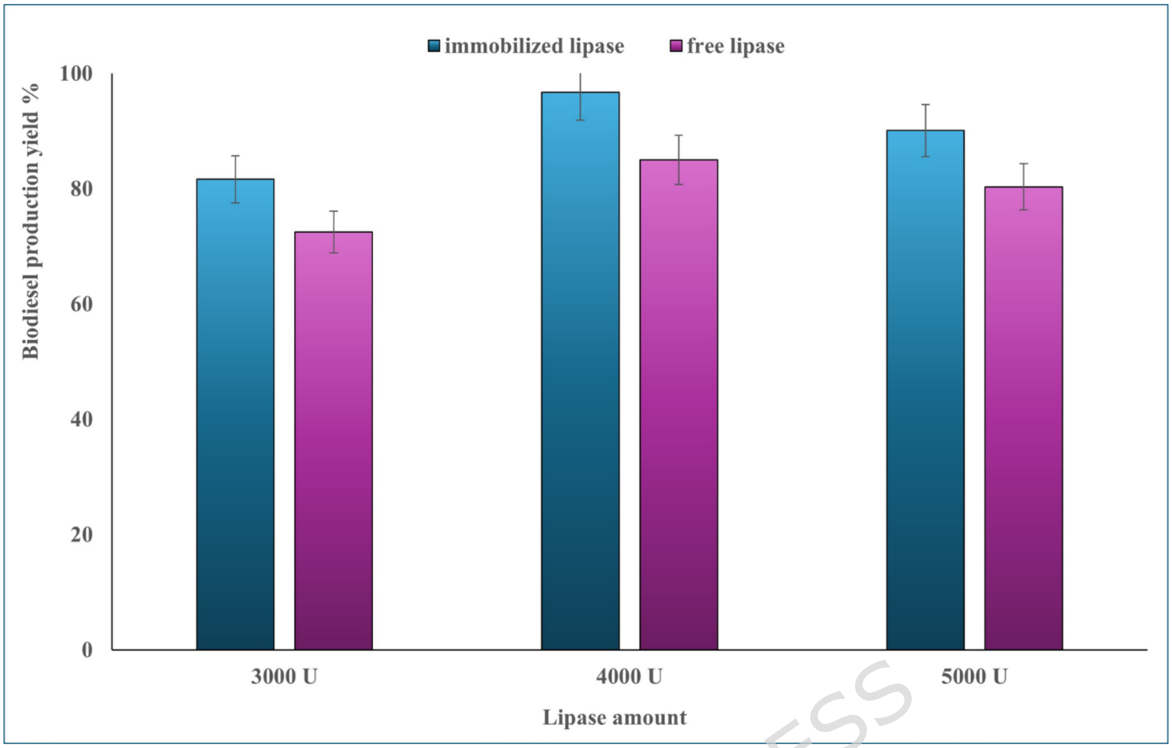

Figure 3

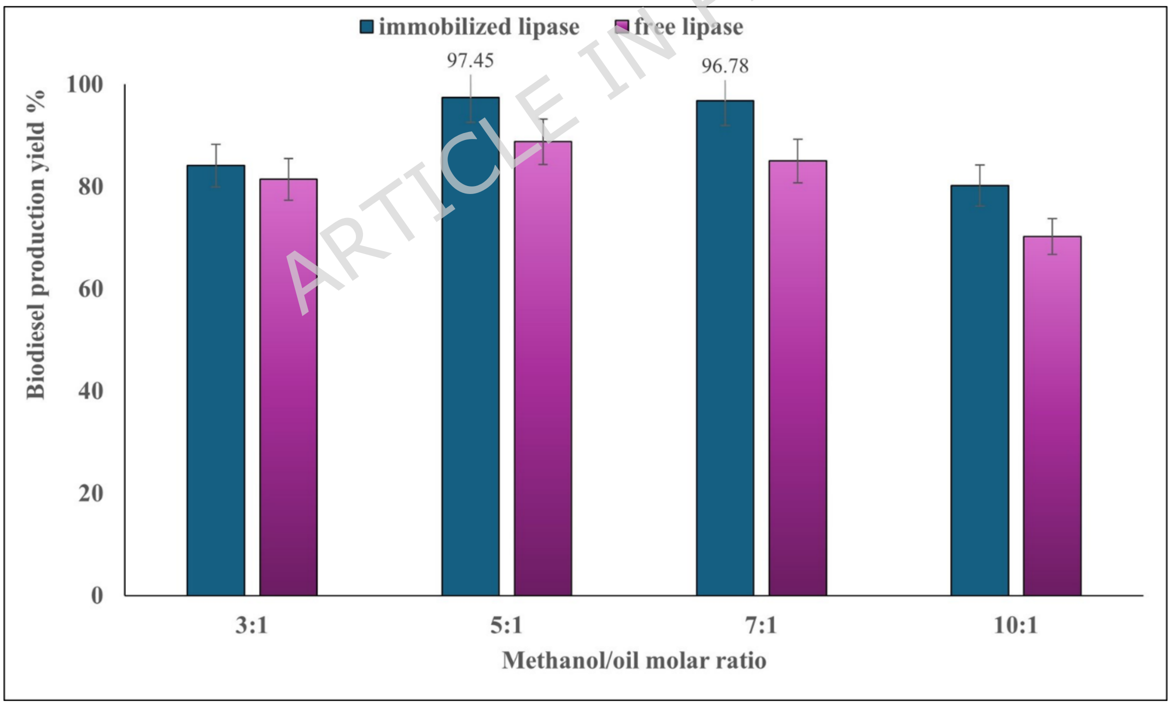

Figure 4

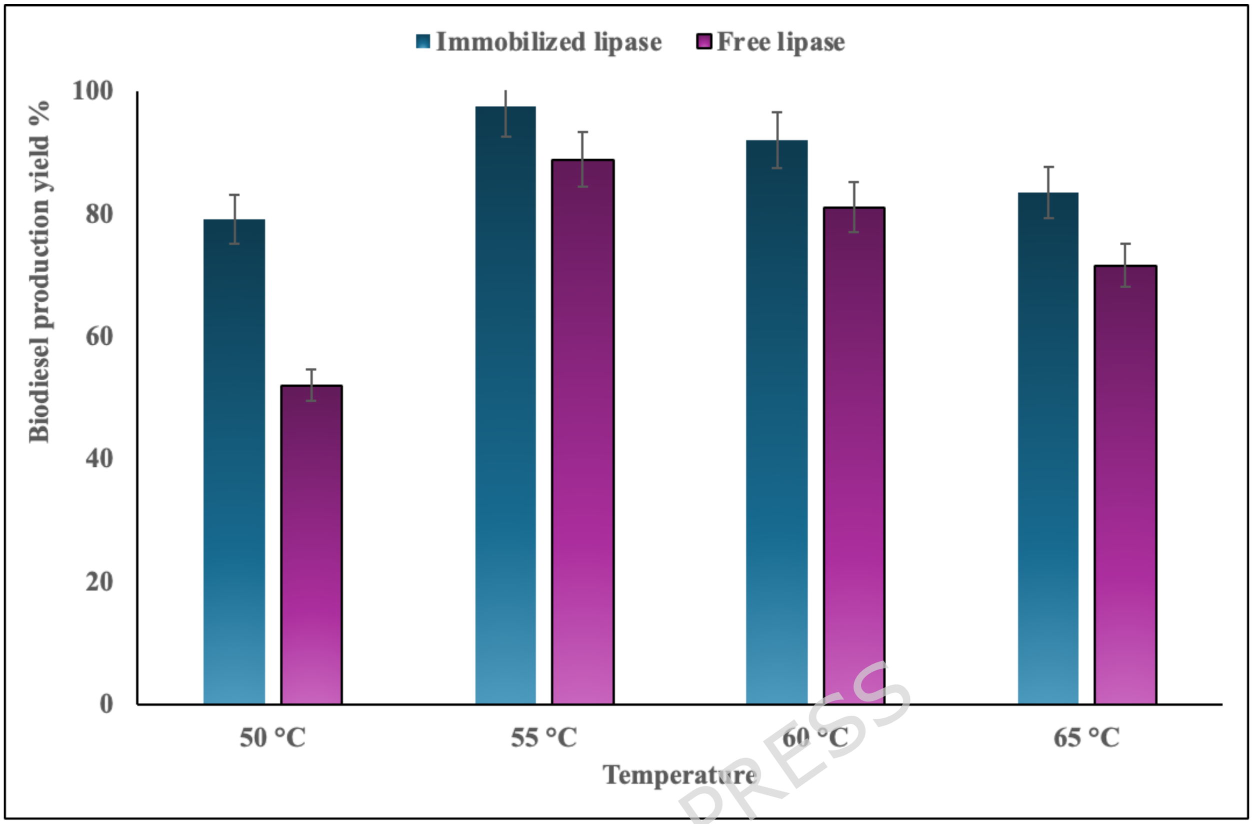

Figure 5

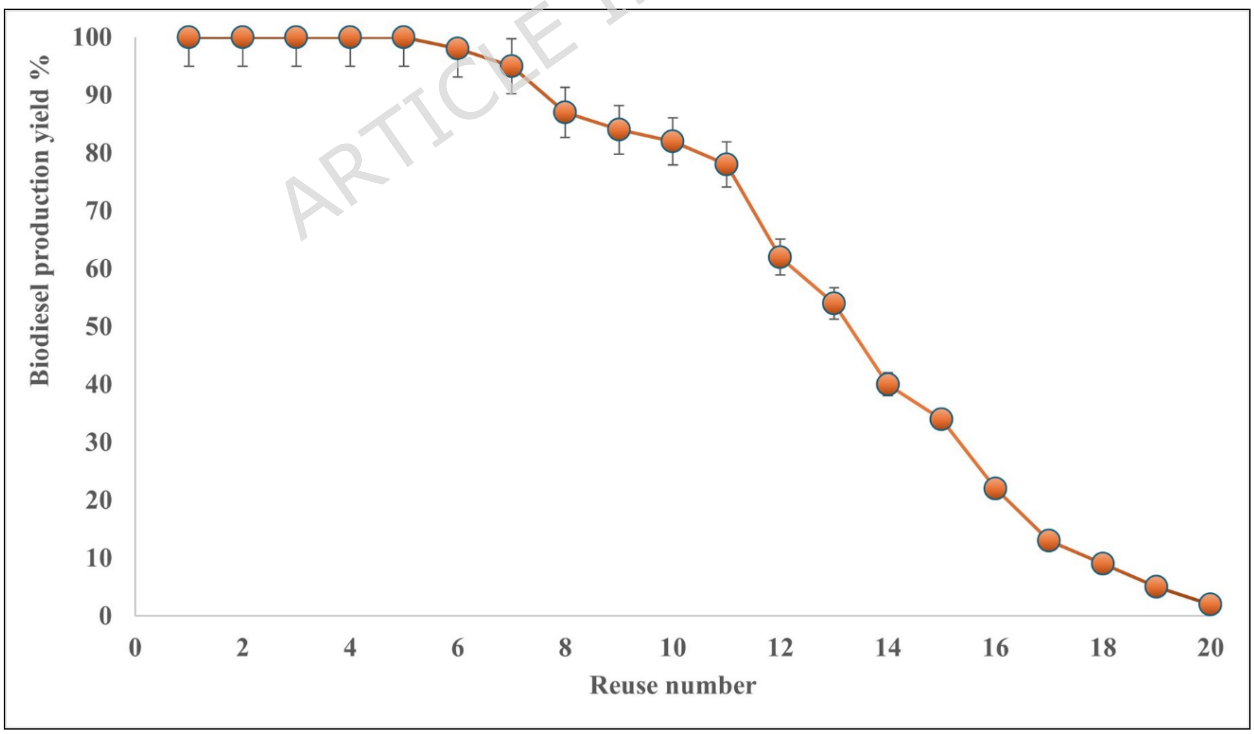

Figure 6

Table 1.

Fatty acids	**Carbon chain length**	**Molecular Weight (g/mol)**	**Retention Time (min)**	**Relative %**
Myristic acid	14:0	228.37	9.652	2.92
Palmitic acid	16:0	256.42	11.245	26.98
Palmitoleic acid	16:1	254.41	11.645	7.75
Stearic acid	18:0	284.48	12.931	6.72
Oleic acid	18:1	282.47	13.585	22.14
Linoleic acid	18:2	280.45	14.218	16.86
□-linoleic acid	18:3	278.43	14.961	5.48
Docosahexaenoic acid	22:6	328.49	19.860	4.56