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Evaluating the Composition of Biodiesel Synthesized from Black Soldier Fly (*Hermetia illucens*) Larvae

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ABSTRACT

Biodiesel is considered a viable alternative to conventional diesel, particularly for the ground transportation industry. While different plant seeds oils have been the dominant feedstocks for biodiesel synthesis to date. However, they are often expensive due to their limited supply and low reproductive rate. **Objective:** To present a sustainable approach by using the black soldier fly (*Hermetia illucens*) larvae as an alternative feedstock. **Methods:** The larvae were fed with waste chicken rice, fish, soft vegetables and fruits. These wastes provide food and shelter for disease-causing insect larvae and contributes to land pollution. Using petroleum ether as a solvent, 12.2 g of crude grease was extracted from ~ 500 larvae, resulting in about 11.8 g of biodiesel through a two-step acid-base catalyzed transesterification process. **Results:** The resultant biodiesel was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS), revealing a Fatty Acid Methyl Ester (FAME) profile predominantly comprising dodecanoic acid, 9-Hexadecenoic acid, 9- octadecenoic acid and 11- octadecenoic acid etc, emphasizing its potential as a high-quality alternative to conventional diesel fuel. **Conclusions:** This study contributed to develop biodiesel as an eco-friendly renewable energy technology by using BSFL.

INTRODUCTION

In recent decades, the global ground transportation sector has seen a significant rise in the use of non-renewable fossil fuels, propelled by rapid population growth. These fuels are finite, deplete quickly, and come with environmental risks, raising concerns about their sustainability [1, 2]. Therefore, the search for new renewable energy resources is becoming increasingly critical [3, 4]. Biodiesel is emerging as a compelling alternative to petroleum-based diesel for heavy vehicle fueling especially for trains, trucks, buses and tractors [5]. Many researchers encourage the utilization of biodiesel than conventional diesel fuel as it is less explosive, forms low carbon deposits in engine on combustion and hence causes less wear and tear of engine [6]. Biodiesel is not only renewable but also non-toxic, biodegradable, highly combustible and economically competitive [7]. It contains Fatty Acid Methyl Esters (FAME) produced through a transesterification reaction involving lipids, alcohol, and a catalyst [8]. For the synthesis of Black Soldier Fly (BSF) based biodiesel through transesterification reaction, the larval grease is allowed to react with alcoholic solvents (petroleum ether, n-hexane, ethanol) in the presence of acid (sulphuric acid), base (sodium hydroxide) or enzyme (lipase) catalyst [9, 10]. Currently, the production of biodiesel primarily relies on vegetable oils like palm, soybean, sunflower oil, and others [11]. Their limited supply and ethical concerns surrounding the use of such plant-based feedstocks have led to the exploration of alternative

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sources. Moreover, the cost of biodiesel using these feedstocks is significantly higher (1.5 times) than that of traditional petroleum diesel. This expense hampers its large-scale adoption, creating a need for more cost-effective raw materials [12]. Black Soldier Fly (BSF) (Hermetia illucens) is widely distributed non-pest insect, present most commonly in temperate and tropical areas [13]. Black Soldier Fly Larvae (BSFL) have recently gained scientific attention as a potential and more sustainable biodiesel feedstock [14]. They offer advantages such as a rapid reproductive rate, short life cycle, ease of cultivation, high lipid contents and ultimately a higher biodiesel yield [15]. Meanwhile, food waste can be a potential source of the development of many pathogenic insects as well as land pollution. BSFL can feed on a variety of these waste food materials and incorporate them into their bodies as lipids through metabolic processes [16, 17]. The residual material left after lipid extraction can also serve as animal feed [18]. This study was specifically aimed at producing biodiesel from BSFL, fed on food waste, using acid-based catalyzed transesterification. We assessed the composition of the resulting biodiesel to evaluate its suitability as a potential fuel for transportation.

METHODS

BSFL Rearing

The study involved the sustenance of BSFL colony over two generations from November 2023 to February 2024 in the General Zoology Lab at the University of Okara and was originated by the courtesy of Dr. Hafiz Kamran Yousaf from Thal university Bhakkar, Punjab, Pakistan. A feed comprising waste chicken rice, fish, partially rotten vegetables and fruits was offered to provide all essential nutrients for their optimal development. Cultivation conditions were maintained at a temperature of 27 ± 2°C and a humidity range of 60-75%. For this experiment, fully grown, fifth-instar larvae were selected as older larvae yield higher lipid content.

Crude Lipid Extraction from BSFL

In March 2024, approximately 500 BSFL were first cleaned with water and then inactivated by boiling at 80°C for five min. The inactivated larvae were subsequently oven-dried at 70°C for four hours and stored at 4°C. These prepared larvae were then ground into a fine powder using a micro-mill grinder. The resulting powder was immersed in 100 ml of petroleum ether, and allowed to dissolve for 48 hours. To purify the crude lipid extracted, 1 ml of 0.5% sulphuric acid was added to remove impurities like phospholipids, pectin and other solid matter. The purified crude lipid was then evaporated using a rotary evaporator to eliminate any remaining solvent, followed by drying the sample overnight at room temperature. A centrifuge was used at 1000 rpm for two min to separate any residual undesired molecules from the larval lipid. Finally, the acidity level of the prepared sample was measured using a pH meter to ensure that the crude lipid was not overly acidic.

Production of Biodiesel from Crude Larval Lipids

For the synthesis of biodiesel from free fatty acids present in the grease obtained from BSFL, a two-step acid-base catalyzed transesterification was employed to normalize the acidity of the extracted grease [19]. The reaction took place in a sealed system, equipped with a thermometer, reflux condenser, 100 ml reactor vessel, a sample outlet for periodic testing and an electromagnetic stirrer for thorough mixing as mentioned by Jain *et al.*, in 2011.

Acid-Catalyzed Transesterification

A mixture of methanol and grease in an 8:1 molar ratio, along with 1 ml of 0.5% sulphuric acid as a catalyst, was maintained at a temperature of 75°C for 60 min. This was done in a rotatory evaporator operating at a rotational speed of 75 rpm. The outcome of this reaction was a mixture comprising unreacted biodiesel crude grease and some residual solvent. To separate the resultant layers, the mixture was centrifuged at 400 rpm for 10 min. The upper layer, which was the desired product, was then dried in an oven set at 60°C for 20 min. This procedure was specifically designed to reduce the acidity of the crude larval grease.

Alkaline-Catalyzed Transesterification

After acid-transesterification, the resulting mixture was kept in a new reactor for carrying out alkaline-catalyzed transesterification. During this process, the temperature was kept at 65°C for 30 min and methanol: lipid (6:1) was mixed with the catalyst 1 ml of 0.8% sodium hydroxide by using a magnetic stirrer at 500 rpm.

Separation and Purification of Synthesized Biodiesel

After the acid-base transesterification process, the mixture contained two distinct layers: the upper layer comprised biodiesel, and the lower layer contained impurities. These layers were isolated using a centrifuge operating at 400 rpm for 10 min. The biodiesel was then distilled at 80°C to remove any remaining traces of methanol.

Analysis of Biodiesel Composition

The composition of the biodiesel, specifically the FAME profile, was analyzed using gas chromatography-Mass Spectroscopy (GC-MS). In the case of gas chromatography, nitrogen gas was used as the carrier at a flow rate of 29 ml/min. While a capillary column with a flame ionization detector was used during mass spectroscopy. Before analysis, the biodiesel sample was diluted with dichloromethane. The detector and injector temperatures were set at 250°C and 220°C, respectively. The column temperature was initially held at 140°C for 5 min and then raised to 240°C for an additional 15 min to analyze FAME profile as mentioned by Pauline, Sivaramakrishnan, Pugazhendhi, Anbarasan and Achary [19].

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RESULTS

Crude Lipid Extraction

Approximately 500 larvae were used as the larval biomass for petroleum ether extraction, yielding 12.2 g of grease. Additionally, Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed the composition of the extracted grease as 40.8% unsaturated fatty acids and 57.2% saturated fatty acids. The biodiesel yield was calculated to be 96.7% as outlined in table 1.

Table 1: The Quantities of Larval, Grease and Biodiesel Mass and Biodiesel Yield

Number of BSF	BSFL biomass in Powdered Form (g)	Crude BSFL Grease Biomass (g)	Biodiesel Biomass (g)	Biodiesel Yield (%)
~500	32.5	12.2	11.8	96.7%

Biodiesel Production

Following grease extraction, the mixture was allowed to settle for three hours, resulting in the formation of two separate layers. The upper layer, less dense and consisting mainly of fatty acids and triglycerides, was isolated for further processing. This layer was subjected to a two-step acid-base transesterification process. Initially, 0.5% sulphuric acid (1 ml) was used as a catalyst for acid-catalyzed transesterification. This was followed by alkaline-catalyzed transesterification using 0.8% sodium hydroxide (1 ml). After these steps, the resulting biodiesel weighed 11.8 g formed again the upper less dense layer settling below the impurities. Residual solvents were removed using a rotary evaporator at 75°C and 80 rpm for 20 min.

Chemical Composition

The chemical composition of the produced biodiesel was analyzed using GC-MS, identifying 15 FAME. Among these, the most abundant were 9-octadecenoic acid (22.6%), dodecanoic acid (22.3%), 9-hexadecenoic acid (15%), 11-octadecenoic acid (9%), and myristic acid (5.5%). These results, presented in table 2 , suggest that the composition may vary based on the diet provided to the BSFL Retention time is the time period that molecules stay in the column of gas chromatography

Table 2: Composition of biodiesel derived from BSFL through acid-base catalyzed transesterification, (values have been written as mean ± standard deviation)

S. No.	Biodiesel Composition (FAME)	Number of Carbon Atoms	Concentration (%) ± SD	Retention Time (min)
1	Nonanoic Acid	9	1.0 ± 0.02	4.4
2	Dodecanoic Acid	10	22.3 ± 0.8	6.3
3	Undecanoic Acid	11	2.6 ± 0.1	10.2
4	Myristic Acid	14	5.5 ± 0.5	7.8
5	Pentadecanoic Acid	15	1.0 ± 1.0	8.7
6	Hexadecanoic Acid	16	8.0 ± 0.1	18.3
7	9-Hexadecenoic Acid	16	15.0 ± 0.3	9.1
8	7-Hexadecenoic Acid	16	5.2 ± 0.0	4.2
9	Heptadecanoic Acid	17	12.7 ± 0.3	7.9
10	Linoleic Acid	18	2.3 ± 0.5	14.8
11	11- Octadecenoic Acid	18	9.0 ± 1.0	5.6
12	9- Octadecenoic Acid	18	22.6 ± 1.2	8.2
13	Noadecanic Acid	19	1.4 ± 0.8	16.9
14	Nonadecanoic Acid	19	0.9 ± 1.1	11.2
15	Docosanic Acid	22	0.5 ± 1.2	19.9

DISCUSSION

Petroleum diesel, currently the predominant fossil fuel for ground transportation, will no longer be available in the near future [21]. It was now imperative to shift from non-renewable diesel fuel to renewable biodiesel to avoid any future energy crisis [22]. The present study was conducted to investigate the potential of BSFL as a feedstock for biodiesel synthesis. Grease was extracted from these oleaginous larvae using petroleum ether solvent through the chemical extraction method, which was then transformed into fatty acid methyl esters containing biodiesel through two-step transesterification. In terms of biodiesel production, the study was successfully carried out by the acid-base transesterification process, which yielded 11.8 ± 0.4 g of biodiesel from 12.2 ± 3.5 g crude grease, with 96.7 ± 0.76% biodiesel yield. A similar result was reported by in which 23.6 ± 0.5 g of biodiesel was extracted from 25.4 ± 3.5 g larval grease with a 93 ± 0.78% yield of biodiesel [23]. The current study also revealed that the chemical composition of biodiesel further reinforces its viability as a renewable energy source. The significant presence of dodecanoic acid, 9-Hexadecenoic acid, 9-octadecenoic acid, 11- octadecenoic acid, and among other FAME indicate a rich and balanced fatty acid profile, and these results are in line with previous studies [24]. Furthermore, the feeding substrate has an impact on the nutritional profile of the larvae, which may have an impact on the quantity and quality of biodiesel [25]. The life cycle of BSFL was heavily dependent on optimal temperature of 25 to 35°C, any slight change in maintenance of this temperature would negative affect their growth [26]. The constraints pertaining to process scalability, feedstock's impact on biodiesel quality, and the improvement of lipid extraction techniques require more investigation. Further research was required to produce healthier BSFL and ultimately quality biodiesel. In this regard, BSF can be genetically modified to easily ingest or digest the food to accumulate more fat reserves during their larval stage, produce more eggs, and extend the life of their adults through emerging CRISPR Cas9 technology. Future studies would explore how controlled feeding experiments could establish the optimal diet for maximizing biodiesel yield and quality. Additionally, the stability of the produced biodiesel should be investigated further, particularly

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focusing on its long-term storage and how the higher acidic value could be managed effectively. This could include exploring additives or refining processes that could enhance its stability. Furthermore, an economic analysis could be conducted to evaluate the cost-effectiveness of the entire biodiesel production cycle, from larval rearing to grease extraction to transesterification. This could help in formulating business models and policy guidelines for promoting BSFL-based biodiesel as a sustainable alternative. By pursuing these future research directions, a more comprehensive, practical, and economically viable framework for biodiesel production from BSFL can be developed. Moreover, additional studies are needed to investigate how to make BSF grow fast with maximum biomass, the scalability of this process, and to further fine-tune the biodiesel properties to meet varying energy requirements.

CONCLUSIONS

The current study highlighted that the BSFL, a non-pest oleaginous insect larva, carries the capability to consume waste materials, has a higher reproductive rate and a short life cycle, can potentially be used as a biodiesel feedstock. Through experimentation, it was demonstrated that the 12.2 g grease extracted from ~500 BSFL can be effectively converted into 11.8 g of biodiesel that contains the desirable profile of fatty acid methyl esters. Moreover, it elaborated the importance of BSFL-based biodiesel as a biodegradable, harmless, cheap and sustainable energy source that produces the least number of pollutants upon combustion. Hence, it can potentially meet future fuel demands in the transportation sector and can replace the use of fossil fuels. Overall, the study sets the stage for more in-depth investigations that could pave the way for more sustainable energy solutions.

Authors Contribution

Conceptualization: MSS, HKY
Methodology: MSS, FK
Formal analysis: FK, HKY
Writing, review and editing: FK, SH

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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