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Insect Biorefinery: A green approach for Biodiesel production through Bioconversion of Waste materials

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Abstract

Recently the world is suffering from twin crises of fossil fuel depletion and environmental degradation. Increasing demand for fuel and uncertainty in their availability are two important triggering factors for searching an alternative energy source that can supplement or replace fossil fuels. Recently bio diesel has become more attractive for its environmental benefits and renewable resources, and it can be produced from insect fat content. Moreover, certain species of saprophagous insects have contributed to recycle the organic matter to fulfil their biological needs. Insects can be used to degrade organic waste materials to obtain economically viable biomass for animal feed. Fat content of insects varies widely between orders, species, stages of development, and dietary habits. Through their life cycle, insects are cultured with agricultural, restaurant wastes or urban by-products to accumulate a large amount of fat for conversion into energy through bio diesel production. This study mainly focuses on black soldier fly larvae as a bio conversion agent of the organic wastes such as crop residues, dairy manure, restaurant wastes etc. All these reasons make the mass rearing of insects a viable future for bio diesel production through bio conversion of waste materials, and it arises as an environmentally sustainable technology.

Recently two major problems are the fossil fuel depletion and environmental degradation[4]. Most of the countries have been convinced to develop clean and renewable energies in order to replace fossil fuels and to help achieve a development that is balanced, harmonious, and respectful to the environment. Gradual depletion of fossil fuel is the main cause for introducing an alternative source of energy that can replace fossil fuels[2]. Renewable energy sources have several advantages, including reducing carbon emissions and the dependency on fossil fuel resources[6]. Recently bio diesel has become more attractive because of its environmental benefits like portability, renew-ability, higher combustion efficiency, ready availability, lower sulphur and higher biodegradability[9]. Ninety-

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five percent of global bio diesel is produced from edible vegetable oils[23]. The cost of these raw materials accounts for 60–75% of the total cost of bio diesel fuel[3]. On the other hand, animal fats contain higher levels of saturated fatty acids and are therefore solid at room temperature. For example, BSF larvae consume organic matter for their biological needs, and thus they can be used to degrade organic waste to obtain economically viable bio fuel.

The Concept of Bio Refinery :

Bio refinery is a technological process that combines biomass conversion, equivalent to crude oil processing plants where substrate is crude oil, natural gas, or other fossil energy sources. These sources are processed to make various products, mainly fuel, heat, chemicals, electricity, *etc*. Substrates in bio refineries are organic waste materials that are processed in bio refinery processes. The term bio refinery can be represented as a tool for the execution of sustainable development. Such type of installation procuring energy is the most optimal solution according to the concept of sustainable development. It simultaneously considers the continuous technological development, production of clean energy, reducing greenhouse gases & harmful compounds[31].

Overview of Bio diesel:

Bio diesel is the mono-alkyl esters of long-chain fatty acids derived from renewable feed stock, such as vegetable oil or animal fats for use in compression–ignition engines[16]. Bio diesel has the potency to provide power similar to conventional diesel and it can be used safely in currently used diesel engines without any modification. The most common process for bio diesel production is trans-esterification, which involves combining natural oil with alcohol and a catalyst. The high energy efficiency makes it favourable in the competitive energy market. In general, bio diesel feed stock can be categorized into four groups: vegetable oils, animal fats, used cooking oil, and oleaginous microorganisms. Bio diesel is mainly produced from edible vegetable oils, but animal fats frequently offer an economic advantage over plant crops for bio conversion because of their favourable price[29]. Advantages of bio diesel are :

- ***Smaller Trade Deficit:*** Producing more bio-diesel can reduce energy expenditures and it will save foreign exchange also.

- ***Cleaner Air:*** Bio diesel burns more cleanly than gasoline & diesel and produces fewer emissions of carbon monoxide, particulates, and various toxic chemicals that cause smog, heart disease, respiratory problems and prevent thousands of premature deaths each year.

- ***Less Global warming:*** Fossil fuels are adding huge amount of stored carbon dioxide to the atmosphere, and it traps the Earth's heat like a thick blanket and causes the world to warm. A number of research works show that bio diesel reduces the carbon dioxide emissions.

Insect Fat: A promising resource for Bio Diesel :

Bio diesel is a promising approach to reduce the consumption of petroleum.

However, bio diesel production has been hampered by the less production of oil-seed plants. So, alternative feed stocks are urgently needed to produce bio diesel from cheaper materials. An insect is the most diverse animal group which are rich and ubiquitous in the world. Insects can convert organic waste into insect fat which is further extracted as bio diesel. Insects often establish metabolic reserves, especially during the immature stages, as they are necessary during specific non-feeding periods of their life cycle. Insects possess fat body that plays a significant role in metabolism. It is the storage system of nutrients and act as energy reservoirs[2]. It is well known that the type of food ingested by insects is one of the most important factors affecting body composition[26]. It has been found that the BSF larvae which are fed with poultry manure, contained 34.8% fat but those are fed with pig manure, contained only 28.0% fat. For example, Yellow Meal Worm (YMW) take decayed vegetables to produce environment friendly bio diesel reported by Zheng *et al*.[35] in the year 2013. Bio diesel production using BSFL fed with rice straw[18], restaurant wastes[32], animal manure[5] and corncob[27] have been reported. BSFL have ability to accumulate a huge amount of saturated fatty acids (C18 & C16) with desirable physical & chemical properties such as kinematic viscosity, oxidation stability and calorific value conducive to further conversion into bio diesel[17].

Main criteria for selecting Insects for Bio Diesel Production :

- ***Fat content (of larvae):*** Fat content is variable throughout the life cycle of an insect. For example, many dipterans are characterized by the existence of a mobile non-feeding pre-pupa stage. Such types of larvae have unique lipid concentrations to produce bio diesel

- ***Speed of completion of the life :*** The life cycles of most insects are very fast, particularly for the species those feed on decaying organic matter. This is due to the ephemeral nature of this type of habitat and the combination of their small size and high metabolic rates.

- ***Requirements of space and reproductive capacity:*** Space requirements of many insect species are also reduced compared to other animal groups. A large number of insects can be gathered in limited spaces for artificial rearing because of their small size.

- ***Less expensive feeding:*** Omnivorous and decomposer species can be fed with a great variety of by-products & organic waste. Certain coprophagous species are capable of feeding on dung. Some necrophagous species could be fed with organic waste derived from restaurants.

Black Soldier Fly Larvae (BSFL) : Potential Bio diesel Source :

We can highlight an insect species with great potential in bio diesel production, the larvae of *Hermetia illucens*, also called Black Soldier Fly. This insect currently presents a worldwide distribution from about 46°N to 42°S latitude[33], including India, Australia, Africa, and Europe[1]. BSF is associated with saprophagous environments such as manure and obtains biomass to produce bio diesel[8]. Its main advantages are the simultaneous degradation of organic waste as animal feed, increasing of the manure micro flora and reducing harmful bacteria[22]. Black Soldier flies have self-harvesting ability due to the migratory nature of their larvae[10], which pupate away from the source of development, allowing effective separation of the decomposed matter. In this stage (pre-pupa) they have their largest size, with a large store of fat. During this period, BSFL has transformed its mouth into a beak-like structure to crawl from feeding substrate and pupae on dry surfaces. BSFL biomass also contains protein (~40%) and lipid (~30%) sources[11], which could be used as aquaculture, poultry and broilers feed (Fig 1).

Resource value of Black Soldier Fly Grease:

Although the grease content in BSF larvae is greatly affected by the rearing substrate[33], crude grease content is much higher in these larvae than in other insects. 1200 BSFL can digest 1248 g of cow dung by bio conversion in 21 days and 15.8g of bio diesel can be extracted from the grease of BSFL. BSF larva convert 1 kg of pig manure, 1 kg of chicken manure and 1 kg of cow dung into 60.4g, 95.5g & 38.2g of crude grease respectively which accounted for 29.1%, 30.1% and 29.9% of the total biomass of the BSF larvae respectively. Seven days after feeding of 1 kg kitchen waste to 1000 BSF larvae, 64.9 g of larvae dry matter was obtained. After subsequent refining, 23.6 g of bio diesel was obtained[20]. The fuel properties of bio diesel largely depend on the fatty acid distribution of the triglycerides i.e., the fatty acid of cow dung is enriched in palmitoleic acid, palmitic acid, myristic acid, linolenic acid, stearic acid *etc*.[7]. According to EU bio diesel standard, the parameters of the bio diesel converted from the crude grease of larvae fed on poultry and dairy manure and restaurant waste met the standard and exhibited higher oxidation resistance compared with bio diesel converted from rapeseed oil[19]. Seven days after feeding of 1 kg kitchen waste by 1000 BSF larvae, 64.9 g of larvae dry matter was obtained. After refining, 23.6 g of bio diesel was obtained that contains with 96.9% ester and the fuel performance of this bio diesel almost reach the EU biodiesel standard[20]. Experimental studies have indicated that *H. illucens*can effectively convert kitchen waste and rice straw into organic matter containing polypeptides byco-biotransformation and that the resulting crude grease can be refined into biodiesel that meets the standard[34]. The larval grease has been used for replacing the other traditional grease sources in feed formulations without any adverse effects on animal performance and product quality[28].

Bio-conversion of Dairy manure into Bio Diesel by Black Soldier Fly :

A considerable amount of dairy manure is produced from modern dairies, which is a potential hazard to the environment. We can also use dairy manure as a principal larval resource for black soldier fly (BSF). Dairy manure, treated by BSF larvae, is considered as new biotechnology to convert it into bio diesel. Grease could be extracted from BSF larva by petroleum ether and then it will be passed through a two-step method to produce bio diesel.

Biomass**:** About 12000 BSFL (10 days old) were inoculated into 1248.6-gram fresh dairy manure (582g dry weight) in an open barrel with cotton gauze. Cultivation conditions were temperature 27°C, humidity 60 - 75%. Pre-pupated larva crawled out from the manure near after 21 days. After being washed with distilled water, pre-pupa were inactivated at 105°C and then dried at 60°C for 2 days.

Crude grease extraction**:** Grease was extracted from dried BSF larva with petroleum ether using three methods. At first dried BSFL were placed into a Soxhlet extractor for 16 hour with petroleum ether. After that, the dried BSFL were immersed into petroleum ether at room temperature for 48 hours, and then the dried BSFL were exposed in petroleum ether under ultrasound for30 min. Extracted samples were dried at the temperature 60°C for overnight.

Bio diesel production from BSFL grease: The extracted grease contained various impurities, including phospholipids, water, pectin etc. It was purified by adding 0.5% H_2SO_4. A two-step method was performed for biodiesel production in which H_2SO_4-catalyzed pretreatment was introduced to reduce the acid value of BSFL grease and NaOH catalyzed transesterification was performed subsequently. After filling the reactor with BSFL grease, it was heated at 73°C in a water bath. Methanol and oil were used in a ratio of 8:1 and 1% w/w H_2SO_4 was added as the catalyst. The mixture was poured into a funnel after 2 hours to separate the bio diesel and neutral oil from water. After transferring the upper layer into the reactor, methanol and oil were used in a ratio of 8:1 and 0.8% w/w NaOH was added as a catalyst for trans esterification. Then the mixture was gently stirred and allowed to react in a water bath for 30 min at 65°C and then the mixture was poured into other funnel for separation. Finally, the bio diesel was washed with distilled water until the washing liquor was neutral.

Bio-conversion of crop waste into bio diesel by black soldier fly :

Crop residues occupy more than half of the total world's agricultural phytomass[25] and it has been identified as a suitable raw material for bio fuel production due to its high cellulose content & its vast availability at low price[12]. Yellow mealworm (*Tenebrio molitor*) & black soldier fly (*Hermetia illucens*) can degrade organic matters and transform wastes into larval biomass[24]. Bio diesel production from YMWL with decaying vegetable matter was first reported[35], and using BSFL with rice straw[34] and corncob[21] have been reported.

First stage of multi-insect bio-refinery process: 1700 Yellow Meal Worm Larva (YMWL) were introduced into 200 g dry mass CS (Coffee Silverskin, a coffee roasting by-products) feeding substrate and reared at the temperature of 28°C and the relative humidity at 70%. 100g CS substrate was provided twice to the YMWL during the development. YMWL were separated from the residues, washed with distilled water and inactivated for 5 min at the temperature of 105°C and then dried the larval biomass at the temperature of 60°C for two days to produce bio diesel.

Second stage of multi-insect bio-refinery process: Remaining CS and residues from the first stage were used for BSF larval development. In this process, 400 BSF larvas were treated in the greenhouse into 750 g

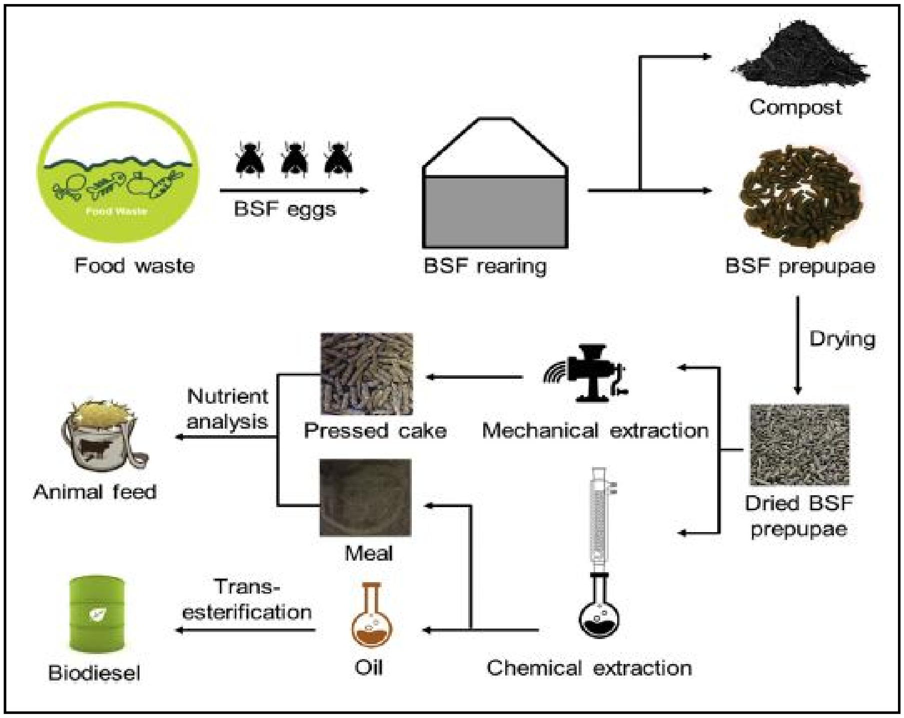

Fig. 1 : Schematic diagram of bio conversion of food waste materials to produce bio diesel
Source : researchgate.net

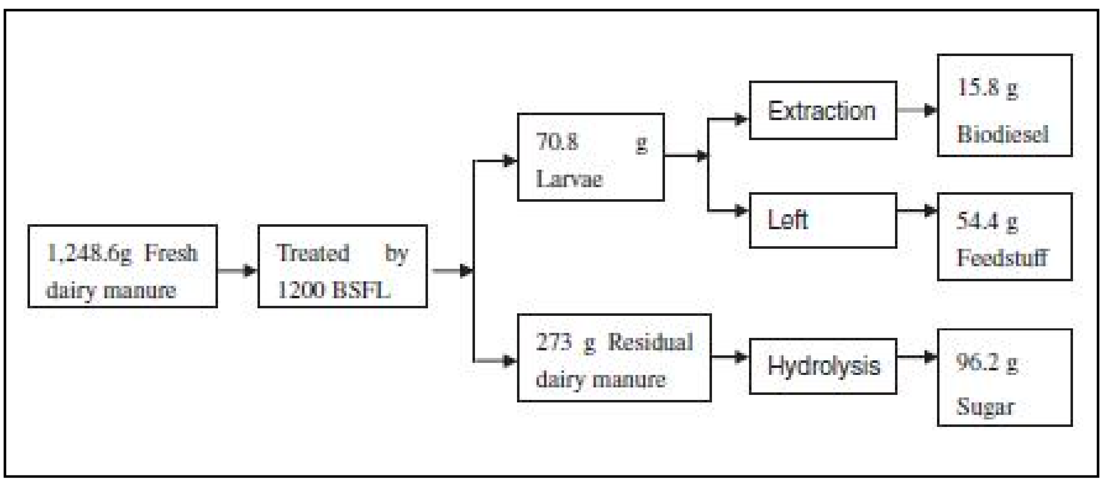

Fig. 2: Schematic diagram bio-conversion of dairy manure into biodiesel
Source :Source : researchgate.net

residues at 27°C and 70% relative humidity. Mature BSF larva was separated from the residues and dried for bio diesel production.

Control experiment using BSF larvae only: 200 g dried CS was used for BSF pre treatment. 400 BSFL were treated into a prepared matrix & incubated in the greenhouse at 27°C and 70% humidity. Mature BSFL were separated from residues & dried for further processing.

Crude grease extraction: Samples were subjected with 200 ml petroleum ether in a Soxhlet extractor and extracted twice for eight hours. After that, the crude grease was obtained with the rotary evaporator by combining the leaching solution and evaporating petroleum ether.

Bio diesel production through multi-insect bio-refinery process: Extracted grease was purified by adding 0.5% H_2SO. A two-step method including acid-catalyzed esterification of free fatty acids to decrease the acidity of crude grease & alkaline-catalyzed transesterification was chosen for bio diesel production. The process was conducted in a reactor equipped with a reflux condenser, thermometer, stirring & sampling outlet. Free fatty acids were transformed into bio diesel after esterification. Then the mixture was poured into a funnel for separating, and the upper layer was transferred to a reactor for alkali catalyzed transesterification. Fat and methanol in a ratio of 6:1, 0.8% NaOH were added and then the mixture was heated in a water bath for 30 min at 65°C after that stirring it with a magnetic stirrer. Then the mixture was separated in a hopper by gravity, and the upper layer was purified at 80°C to remove the residual methanol[35].

Bio-conversion of restaurant waste into bio diesel by Hermetia illucens :

Bio diesel production through insect larvae culture on restaurant waste is believed to be an environment and pocket-friendly approach.

Rearing of BSFL: Rearing of BSFL was performed under an open barrel at 25-32°C and humidity of 60-75%. After 20 days, BSFL was separated from restaurant waste, rinsed with deionized water, and inactivated at 105 °C for 5 min followed by oven-dried at 60 °C for 24 h.

Extraction of crude grease: Extraction of crude grease was performed in a Soxhlet system. About 30 g of dried BSFL with different solvents were placed in holders. Then BSF larval oil was obtained by removing the solvent using the distillation process. The flask was placed in the oven at 103°C and heated to constant weight followed by cooling in the desiccators for 30 min. Larval oil was treated with 1% (v/v) of H_3PO_4 (85%) at 30ºC after extraction and finally it was centrifuged to separate the pectin. Thus high purity of BSF larval oil was collected.

Production of bio diesel: Bio diesel was produced through a two-step transesterification process. Acid-catalysed esterification process was performed to lower the FFA content in the crude oil. Alkaline catalyzed transesterification process converts the esterified oil to bio diesel. For acid catalyzed esterification process, about 10 g of BSF larval oil was transferred into a 250 ml of conical flask and the mixture of methanol and sulfuric acid was added. Then resulted solution was stirred for certain reaction times and centrifuged at 400 rpm for 10 min. After that, the upper layer was transferred to a conical flask & dry in an oven at 105°C for 10 min. The alkaline-catalysed transesterificationwas conducted by placing about 10 g of esterified oil into a conical flask, heat to certain temperature, added with the mixture of methanol and sodium hydroxide & stirred for a certain reaction of times. The solution was then separated using the centrifuge at 400 rpm for 10 minutes and bio diesel was extracted.

Regulators of Transesterification :

Effect of methanol and oil molar ratio: Stoichiometrically, one mole of triglyceride requires 3mol of alcohol in transesterification. Excess alcohol is used to shift the reaction to product side[14]. High bio diesel yield (>90%) was observed when the methanol and oil molar ratio was between 8:1 to 10:1. Further increase of the methanol and oil molar ratio up to 11:1 to 12:1, leads to decrease of bio diesel yield.

Effect of reaction time: High biodiesel yield (>90%) was observed when reaction time was between 40 to 60 min. Increase of reaction time up to 80 min with increasing catalyst amount up to 1.5 weight % would decrease the bio diesel production[15].

Effect of reaction temperature: High bio diesel yield (>90%) can be obtained between 55 to 65 °C. Bio diesel yield was reduced at higher temperature due to vaporization of methanol resulting in a two-phase interface in the reaction mixture[13].

Effect of catalyst amount: Catalyst application will enhance the transesterification process by accelerating the breaking of triglycerides bond[14]. High bio diesel yield (>90%) was observed between catalyst amounts 0.5 - 1.0 weight %. An increase in catalyst amount upto 1.5 weight % has resulted in a gradual decrease in bio diesel yield.

Sustainability and Environmental Benefits of using Bio Diesel :

Many countries are moving from voluntary to obligatory legislation, increasing the market share of bio fuels in the transport sector. A number of African countries, including Ethiopia, South Africa and Egypt are initiating large-scale bio fuels production to capture the benefits of its value chain. Thus, socioeconomic and environmental implications are required for addressing the potential impacts of biomass resource development and to guide appropriate national policies and strategies development.

- ***Socioeconomic implications***: Producing biomass resources locally reduces the country's dependence on foreign energy sources. Biomass resource cultivation and processing could have a direct impact on rural development. Biomass and bio fuels production could improve rural livelihoods by providing new income opportunities to their families. However, biomass production should not be made to affect food security in the country. It positively contribute to increase the productivity of food crops as bio energy sources. Efforts should be made to avoid human health impacts and risks through regular training and awareness.

- ***Environmental implications***: Potential environmental benefits derived from the local production and use of biomass resources and bio fuel production include waste utilization and erosion control. Biomass technology may benefit the environment as well as solve some pressing environmental problems. It is reported that using biomass to produce energy is carbon-neutral. For every MWh of power generated using biomass, approximately 1.6 tonnes of CO_2 are avoided, and thus it helps to tackle global warming. The use of MSW reduced soil fertility, the accumulation of toxic substances, and reduced organic matter.

Due to its biological characteristics, *H. illucens* has been widely used for the pollution free treatment of common organic waste like kitchen waste, poultry manure, domestic waste and agricultural waste. Such treatment converts the organic waste to beneficial forms that is also used as the nutriments to insect biomass. BSF prepupae-derived oil has a good fatty acids profile which could produce a high-quality bio diesel. Bio diesel production from insects depends on organic waste has great potential in the era of bio fuels and it has become popular in developing continents (*i.e.*, Asia, Africa, and Middle-east). It will decrease the fossil fuel consumption, relieve from the negative impact on the environment and reduce the cost of bio diesel. This investigation employed an innovative environment-friendly technology of bio diesel production through bio-conversion.

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