

Theoretical Assessment of the Potential of Desilked Silkworm Pupae as Feedstock for Biodiesel Production in India

Dhivya Priya N.^{#1}, Thirumarimurugan M.^{*2}

^{#1} Research Scholar, Department of Chemical engineering, Coimbatore Institute of Technology, Tamil Nadu, India

^{*2} Professor, Department of Chemical engineering, Coimbatore Institute of Technology, Tamil Nadu, India

¹dhivya priya.n@cit.edu.in

Abstract - Energy sources have become critical to the growth and development of any nation. In recent times, first-world countries have actively engaged themselves in pursuit of clean and reliable energy sources. Waste accumulation in the biosphere has also become a menace. Waste management practices that double as energy harnessing techniques are on the lookout these days. One such practice is the production of biodiesel from organic and putrescible waste. Biodiesel is produced by the conversion of triglycerides to esters of fatty acids. This paper studies the potential of desilked dried silkworm pupae, waste produced from silk reeling units as feedstock for biodiesel production. These pupae have around 40% lipid, which can be tapped for the production of biodiesel. The characteristics of this lipid and its biodiesel produced have been critically evaluated. This pupae biodiesel meets the standards established for B100. India has a capacity of producing 19 million liters of biodiesel from this waste per year. By 2030, around 25 million liters of biodiesel can be produced from dried, desilked silkworm pupae. This study has reinforced that the production of biodiesel from waste pupae is a potential source for energy production and an effective waste management strategy for the industry.

Keywords — Biodiesel, Sericulture, Waste-management, silkworm pupae, pupal oil

I. INTRODUCTION

Energy in any form is significant and critical for the growth and development of any nation in all respects. It is safe to say that a nation that has access to unhindered access to sources of energy has a large potential for development in every aspect, including industrial and agricultural. In this sight, every first-world country has involved itself in an endless pursuit to find different energy sources. Statistical review of world energy in 2020 indicates that the world is over-dependent on fossil fuels to meet its energy demands.[1] Fossil fuels are petroleum, natural gas, and coal, which are formed by the physiochemical and biological conversion of organic matter under pressure over a prolonged period.[2] According to Key World Energy Statistics – 2020 released by IEA, fossil fuels account for 67% of the total energy consumed around the world.[3]

Fossil fuels, though as efficient they are, leave a rather large carbon footprint.[4] The process involved in the extraction and purification of fossil fuels is energy-intensive. Combustion of these fossil fuels leads to the emission of greenhouse gases, climate change, anthropological changes, biodiversity changes, and also environmental degradation in the areas around the extraction site. [5], [6] The characteristics of emission from the combustion of fossil fuel have been studied by various researchers under different operating conditions. Emission from operating diesel-fuelled compression engines was found to have around 1500ppm NO_x and 25ppm Hydrocarbons. [7] The opacity and temperature of emission also exceeds the allowable limits. Inhaling emissions with high NO_x and hydrocarbon content can result in permanent damage to respiratory system resulting in severe asthma and bronchitis.[8] NO_x emission also turns water surfaces acidic, affecting the inland water bodies and their niche.[9] Elevated emission of hydrocarbons contributes to the greenhouse effect, affecting the atmosphere.[10] Adversities of using fossil fuels are damaging to the biosphere as a whole.

In light of this, various sources of energy have been experimented with, commercialized, and out for public use. Out of these sources, natural gas, coal, and electricity are the most used. On carefully analyzing the use of energy consumption patterns, it can be noticed that the world relies more on non-renewable sources of energy than renewable sources. This is mainly because of the high initial cost incurred in harnessing renewable energy and the lack of public awareness. Various renewable energy sources are being experimented with the recent times.[11] One such source is energy tapped from the oily and greasy matter that is available in non-usable organic wastes. This includes used cooking oil, lipids from plants and animals, and other sources of triglycerides. This lipid of triglyceride can be transesterified to form fatty acid methyl ester (FAME) or Biodiesel (BD).

Biodiesel is an alkyl ester of long-chain fatty acids. These are formed by the reaction of monohydric alcohols on long-chain fatty acids under temperature and pressure in the presence of a catalyst.[12], [13] Biodiesel is a hopeful alternative source of energy, as it is similar to the conventional diesel fuel (DF) that is used by the



public.[14]–[16] Biodiesel can be produced from the diverse feedstock. The various feedstocks that have been explored for biodiesel production have been elaborated in Table 1. Common feedstocks include waste cooking oil, oilseeds such as *Jatropha curcas* and palm kernel, and animal fats such as slaughterhouse waste and meat processing factory waste.[14]–[20] The key factor

affecting the commercialization of biodiesel is the ambiguity in the cost involved in biodiesel production.[21], [22] This is mainly dependent on the cost incurred by the feedstock used. Therefore, to supplement this, several cheaper feedstocks and wastes that double as a feedstock due to their lipid content are being explored.

TABLE I FEEDSTOCKS IDENTIFIED AND USED FOR BIODIESEL PRODUCTION

S.No	Feedstock used	Advantages	Disadvantages
1	Waste cooking oil [23]–[26]	Improved mass transfer in employing heterogeneous catalysis, performance, and emission characteristics similar to DF	Interference due to other macromolecules dissipated in oil during use, High FFA content
2	<i>Jatropha curcas</i> [27]–[29]	Higher yield compared to other oilseeds, better fuel properties, BD produced meets standards in most aspects	Can cause plugging of injection valves or carbon deposit on the engine – maintenance issues
3	Karanja seeds [30]–[32]	A yield of 97% reported BD produced meets standards in most aspects	Not economical due to limited production of Karanja seeds
4	Milkweed seed oil [33], [34]	An untapped source of highly unsaturated fatty acid	Present commercial attention is restricted to the fiber attached to the seed
5	Non – edible oils [35]–[38]	Different varieties of non – edible oil seeds can be used, varied options available for feedstock	The suitable fatty acid composition may not be present, poses competition for water and soil with food culture
6	Microalgae [39]–[42]	High yield, good quality BD produced, suitable fatty acid composition available, can be genetically altered to increase lipid content	Cultivating and harvesting lipid from microalgae at a large scale - not economical.
7	Oleaginous yeast [12], [43]–[45]	Faster growth rate when compared to other single cellular lipid sources	Cultivating and harvesting lipid from yeast at a large scale - not economical.
8	Insect oil [46]–[48]	Insects can be bred using organic waste produced from households and industry	Selection of insect species with appropriate fatty acid composition - difficult
9	Animal fat[49]–[52]	A larger concentration of unsaturated fatty acids, improved oxidation stability, and calorific value	High FFA content, BD produced has high viscosity and poor cold flow temperature properties
10	Soapstock [53]–[56]	Doubles as an efficient waste management method and energy recovery method	High water content and FFA present in soapstock

One such waste that can be used as a feedstock for biodiesel production is the desilked dried silkworm pupae (DDSP), which are produced as waste in the silk reeling process. Silkworm pupae are known as Brown gold due to their rich nutrient value. These waste pupae are mostly discarded or sold as poultry feed. This waste poses a serious menace to the environment at its point of disposal, as in Fig 1. Various measures have been taken to manage this waste, and recently they are being used as poultry feed. Though this method was better than earlier techniques, the nutrient value waste pupae hold has been overlooked. This waste pupae contains almost 35-40% lipid, which is mostly unsaturated fatty acids.[57] In light of this, DDSP can be used as feedstock for BD production.



Fig. 1 Desilked dried pupae at an Automatic silk reeling unit at Udumalpet, Tamil Nadu, India

This paper deals with analyzing the potential of DDSP as feedstock for biodiesel production. A sincere effort has been taken to study the production pattern of pupae, its characteristics, and its suitability as feedstock for biodiesel production. The following sections discuss in detail the process of silkworm rearing, silk reeling, the waste produced (DDSP) and its characterization, biodiesel

production from this waste and its characterization, comparison of pupal oil and its FAME with other common and successful feedstock such as waste cooking oil and *Jatropha curcas* and their FAME respectively. The paper also mentions future research aspects to improve the production of biodiesel from DDSP.

II. SERICULTURE

Sericulture is derivative of the Greek word 'sericos', which means silk.[58] It is the conscious mass rearing of silk-producing organisms to obtain silk from them at a profitable rate.[59] Sericulture involves moriculture, rearing of larvae, mounting of the ripe larvae, silk reeling, and weaving of reeled silk into yarn.[58] There are different species of silkworms. These are classified based on the type of silk fiber spun, volutinism, and the type of feed they consume. Out of all the varieties of silk, mulberry silk is the most significant silk in India.[60] It contributes to over 95% of the total silk production. The different types of silk and silk-producing organisms, along with the steps involved in the silk reeling process, are to be discussed in the following sessions.

A. Types of Silk

- 1) **Mulberry Silk:** This is produced by *Bombyx mori*. These are unique silk fibers of high quality that are produced in long thin strands by *B. mori* silkworms feeding on mulberry leaves alone. This silk is white in color and is composed of single long strands of silk. Sericin, a compound that holds the fibers together, also acts as an anti-allergen making it suitable for use by a diverse population.[61], [62] Mulberry silk is produced in five states in India, namely, Tamil Nadu, Karnataka, Andhra Pradesh, West Bengal, and Jammu and Kashmir.[63]
- 2) **Tasar Silk:** Tasar silk is the most important non-mulberry silk, contributing to over 95% of total non-mulberry silk in the world.[64] Tasar silk is spun by the species *Antheraea*. The tasar cocoons are large, thick, and pedunculate. They feed on asan, arjun, and oak trees according to their subspecies.[65]
- 3) **Eri Silk:** These cocoons are white or brick red in color and smaller than tasar.[58], [66] Unlike other cocoons, they are open at one end. Therefore no single long silk strand can be got from eri cocoons. These silkworms feed on castor and tapioca.
- 4) **Muga Silk:** This type of silk is endemic to Brahmaputra valley and Assam in India,[67] hence the name *A. assamensis*. These cocoons are weakly pedunculate, smaller than tasar, and strong. They feed on som and soalu tree.

B. Rearing and Reeling operations

The rearing of the silkworm is one of the most sensitive animal cultures. Silkworm eggs are stored at lower temperatures of around 5°C before hatching, in sterile conditions.[68] These storage conditions differ according to the stage of diapause of eggs. These eggs may be acid-treated depending upon their stage of diapause. They are then hatched under constant, elevated temperatures (23°C -

27°C) with or without the need of stimulating hormones depending on their storage conditions.[69] The rearing house should be completely disinfected before the commencement of rearing. This can be done by physical, chemical or radiation methods. Once the rearing house has been fully sterilized, the newly hatched larvae have to be transferred to a rearing bed. This process is called brushing.[70] These new larvae are small, black, and are called ants. The quality and size of cocoons are mainly dependent on the quality and quantity of mulberry leaves fed to these larvae during their development stages. In India, 4 feed per day is given.[70] Young larvae are fed with cut, tender leaves, while mature worms are fed with full leaves and young branches. As the silkworm feeds on these leaves, they grow in size over 10,000 times their initial size. Therefore they have to be spaced out frequently to avoid crowding in the rearing beds. Spacing and bed cleaning should be carried out at least once every day. After consuming sufficient feed, silkworms enter the moulting stage, i.e., they shed their skin and grow new soft skin. Silkworms are normally allowed through four moulting stages.[71] After the moulting is complete, silkworms are mature and ripe, and ready for cocoon development. Ripe silkworm is transferred to mountages to enable them to spin cocoons around themselves.[72] The silkworm enters the pupal stage and remains dormant inside the cocoon till it matures and erupts from the cocoon as a moth.

The mounted cocoons are removed and are sent to reeling units. These cocoons are dried or stifled by direct or indirect methods to kill the pupae inside them.[73] Generally, they are dried at 120°C for 7 hours to ensure that moisture in pupae is completely removed. These dried pupae can be stored up to 6 months before reeling. Care should be taken to avoid damage of pupae or cocoons by insects or fungi. Before reeling, dried and stored cocoons are sorted and graded according to their color, texture, diameter of the filament, size and weight of the cocoon, and weight. Each grade of cocoons is reeled separately. The sorted cocoons are defloxed to remove the unreelable tangle silk fibers on the outside. Sericin, a protein secreted by the silkworm, holds the fibers in position and hardens them into a cocoon.[62] Defloxed cocoons are then cooked in soft hot water to loosen the fibers by dissolving the sericin. Cooked cocoons are then lightly brushed to find the reelable end. Reeling or desilking is the process of continuous extraction of silk fibers without any breakage. Multiple cocoons are reeled together according to the size of silk fiber required. Usually, 8 cocoons are reeled together in the case of mulberry silk.[74] Exhausted cocoons are automatically replaced with fresh cocoons such that the fiber quality is maintained throughout. After reeling, the dried pupae inside the exhausted cocoon are collected separately and dried. This dried desilked pupa is the proposed feedstock for biodiesel production in this paper.

III. CHARACTERIZATION OF DRIED DESILKED SILKWORM PUPAE AND PUPAL OIL

The process of silk reeling generates enormous quantities of highly putrescible biomass.[75] This waste mostly comprises dried desilked silkworm pupae (DDSP), its metabolites, and some rudiment silk fibers. These rudiment silk fibers are recovered and are used in silk sheets and carpets. Earlier, after the recovery of these silk fibers, DDSP was disposed of by dumping in landfills. Proximate analysis of this waste has shown that it is made up of protein and lipid, as in Fig 2. [76], [77]. Various attempts have been made by different industries to harvest these macromolecules effectively. The protein content in this waste makes it a suitable ingredient in making animal feed for poultry and fish.[78] Extract of DDSP containing its lipids and proteins can be used in pharmaceuticals, the food industry, and cosmetics.

This review is focused on the lipid content in this waste. It is the proposed feedstock for biodiesel production in this study. Many studies have reported an efficient extraction of this lipid or oil by chemical methods. Soxhlet extraction with solvents such as n-hexane, petroleum ether, and chloroform have been carried out with an efficiency of over 90%.[79]–[87] Microwave-assisted extraction of DDSP oil was studied by Hu et al. [87]. They have reported a similar yield as Soxhlet extraction. Microwave-assisted extraction has also produced a similar yield as Soxhlet extraction in terms of their fatty acid composition and yield percentage. [87]

Fig 3 gives a pictographic representation of the relative percentage of different fatty acids present in silkworm pupal oil according to different studies. The fatty acid composition of two strains of the silkworm, namely, mulberry or *Bombyx mori* and Muga or *Antheraea assamensis*, have been analyzed. Properties of these two species will be discussed in detail in the subsequent sections. On comparing these studies, DDSP oil holds around 30% SFA and 70% UFA. Of the 70% UFA present, 26% is MUFA, and 44% is PUFA. The relative percentage of UFA is the most critical factor in determining the suitability of any lipid as BD feedstock. This is because SFA can hydrolyze on reacting with methanol in the presence of a catalyst causing saponification instead of esterification. DDSP containing 70% UFA is a likely feedstock for biodiesel production. The difference in relative percentage of each fatty acid in the pupal oil is due to the difference in the climatic conditions and nature of feed given to silkworm in their larval stage. This explains the variance in fatty acid composition between DDSP oil of different strains.

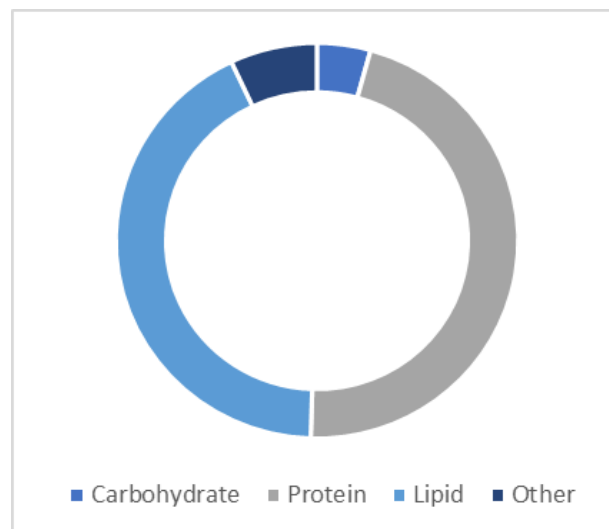


Fig. 2 Composition of DDSP

The most common feedstocks that are used for biodiesel production are waste cooking oil and *Jatropha* oil extracted from the seeds of *Jatropha curcas*. The fatty acid composition of these two feedstocks was compared with the fatty acid composition of DDSP oil to understand its appropriateness for biodiesel production. The relative percentage of UFA present in Waste cooking oil and *Jatropha* is around 85% and 78%. [88], [89] This value is close to that of the UFA of silkworm pupal oil. The content of MUFA and PUFA for these three feedstocks are also comparable. As waste cooking oil and *Jatropha* oil are considered to be efficient feedstocks for biodiesel production, DDSP oil with similar properties can also be used in the production of biodiesel.

Few studies have reported the results of transesterification of DDSP oil. Different catalysts have been used in these studies, and their efficiencies have been compared. Though the application of base-catalyzed transesterification reaction to DDSP oil has resulted in the formation of wax,[81] a two-step transesterification reaction catalyzed by acid followed with base has given the maximum yield. [80] Studies have been carried out on the transesterification of DDSP oil with lipase enzyme as a catalyst. Lipase catalyzed transesterification of muga DDSP oil resulted in about 80% conversion of triglycerides to FAME. Single-step acid catalyzed transesterification of silkworm has also shown a conversion rate of almost 80%. From all these

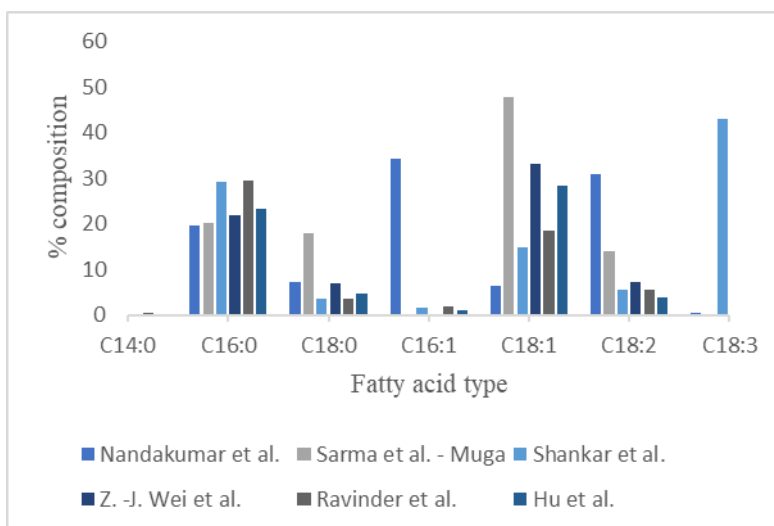


Fig 3. Fatty acid composition of silkworm pupal oil as reported in various studies [80], [81], [84], [87][83], [85]

Studies reported it is clear that there is a minimum of 80% conversion of DDSP oil to BD or FAME. The obtained FAME was allowed to settle, and excess glycerol was removed. Washing biodiesel with hot water to remove any methanol or other impurities has been suggested in many studies as an efficient method for biodiesel purification.

IV. CHARACTERIZATION OF BIODIESEL PRODUCED FROM SILKWORM PUPAL OIL

A. Spectral Characterisation of Biodiesel

Spectroscopy is the study of the interaction between a matter in its physical state and some radiation energy.[90] When a substance is irradiated with radiation of a particular energy or wavelength, the amount of radiation absorbed, scattered, or emitted by the substance is measured to give a knowledge of the type of ions, bonds, or molecules that are present in it. Spectroscopy is generally classified based on the region of the spectral band the irradiating radiation falls in. They can be Ultraviolet (UV), Infrared (IR), Visible, Microwave, and Radiofrequency (RF). Spectroscopic techniques can be used as a confirmatory test and qualitative and quantitative measurements in quality control. [90]

BD bonds are strongly responsive to mid-IR spectrum wavelength. They show the characteristic absorbance of methyl ester at 1700cm^{-1} . According to the studies reported, FTIR analysis of BD produced from DDSP pupal oil has shown characteristic C=O bonds or ester bonds at 1709.6 cm^{-1} . [79] There were no indicative peaks for the presence of sulfur and phosphorous. This proves that this BD would have less SO_x emission than conventional diesel. The absence of broader peaks across the range of 3300cm^{-1} to 2500cm^{-1} shows that there is no alcohol or moisture present in BD extracted. Fig 4 enlists the characteristic peaks that are observed in conventional diesel (DF), biodiesel from *Jatropha curcas*(JCB), sunflower oil

biodiesel (SFOB), and biodiesel from two strains of DDSP, namely Muga pupae biodiesel (MPB) and *B.mori* pupae (BPB). From the data given in Fig 4, it can be confirmed that the biodiesel produced from desilked silkworm is comparable to that of biodiesel from *Jatropha curcas* and sunflower oil diesel. All these exhibit sharp peaks with good absorbance around 2800cm^{-1} and the characteristic ester bond at 1700cm^{-1} .

Spectral characterization of biodiesel can also be done using NMR spectroscopy and UV Visible spectroscopy. Researchers have carried out imaging of biodiesel from DDSP oil using Tetramethylsilane and Chloroform as internal standard and solvent, respectively, under an H^1 NMR spectrum. [80] This was carried out to screen the advancement of the reaction using the following equation

$$C = \frac{2A_{ME}}{3A_{CH_2}} \times 100$$

Where C is the percentage conversion of triglyceride to biodiesel, A_{ME} is the integration of the methoxy protons of methyl ester, while A_{CH_2} is the integration of methylene protons. This equation is in accordance with the study reported by Gelbardet et al., 1995 on spectroscopic application to determine the yield of transesterification reaction. [90], [91] The conversion of triglyceride to Biodiesel was determined to be 98.73%, according to equation 1 mentioned above. [80] The strong signals received for BPB, as reported by Nadankumar et al., 2016 was found to be similar to those values reported by Gelbardet et al., 1995, on analyzing waste cooking oil biodiesel (WBD). [80], [91] Sarma et al., 2016 have studied the characteristics of biodiesel produced from Muga silkworm species (*Antheraea assama*). H^1NMR of this MPB has shown characteristic signals of methoxy protons at 3.64ppm and methylene group at 2.31 ppm. [81] This is

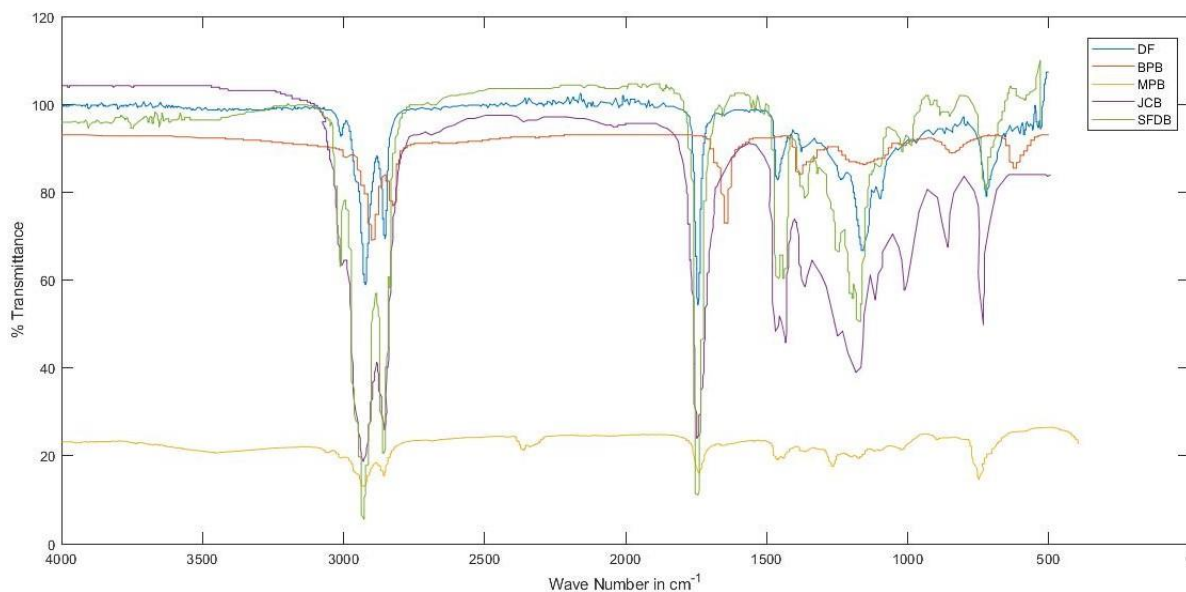


Fig. 4 Comparison of characteristic peak obtained under IR spectrum for biodiesel from different sources and diesel fuel[79], [81], [90], [92], [93]

similar to the spectra received on studying the BPB, which showed signals at 3.66ppm and 2.3ppm, respectively.[80] Analysis of BD under UV Visible spectrum can help understand the degradation properties of BD. [90] No study has reported spectroscopic analysis of silkworm pupae biodiesel under the UV-Visible spectrum. Degradation studies of MPB and BPB have been carried out in other methods and not using UV Visible spectroscopy.

B. Physical Properties of silkworm biodiesel

Table 2 gives an extensive comparison of the physical properties of BPD, MPD, and WBD. These properties have been compared against the standards for biodiesel and conventional diesel to get a clear picture of BD from DDSP. The physical properties tabulated above are mainly dependent on the composition of biodiesel, namely the relative percentage of saturated and unsaturated fatty acid esters that are present in it. These properties are significantly affected by the relative percentage of fatty acids present, the number and position of double bonds in them, and the length of the aliphatic carbon chain and any sidechain if present.

Density and kinematic viscosity are the most basic properties of any diesel fuel. If the fuel fails to fall within these limits, it is deemed unfit to be used as fuels in compression engines. Viscosity and density are dependent on molecular structure and size. The larger the molecule, the greater will be the density and viscosity. The position of the ester bond is very critical in determining the viscosity of biodiesel. If the ester bond is present at the end, then its effect on viscosity is almost negligible. [94] Viscosities, higher or lower than the established limits, can cause difficulties in running a compression engine. While higher viscosities can cause poor combustion and raised exhaust smoke emissions, [29] lower viscosities cause wear

and tear between engine and injection pumps and leakage in some cases. As tabulated above, the density and kinematic viscosity of BPB falls within the desirable range, whereas the density of MPB is lower than the least expected value. [5] This is due to the absence of esters of linolenic acid in MPB. [80], [81] Upper limit of viscosity for biodiesel is greater than that for diesel. Care should be taken while mixing biodiesel with diesel, such that the value does not exceed that of conventional diesel.[29]

An important factor of fuels is the cetane number, which indicates a fuels' ignition quality and its performance in engine and emissions. [95], [96] This property is dependent on the type of hydrocarbons present, the technology used to process the oil, and the growth conditions of the feedstock. [96] It is also determined by the molecular geometry of the comprising fatty acid. Liquid fuel to be used in compression engines is expected to have a minimum cetane value of 51. This value ensures that there are shorter delays in the ignition and a smooth and efficient combustion process. BPD and MPD are reported to have sufficient cetane values. A slight reduction in the MPD cetane value might be due to the rearing conditions of Muga pupae, which are usually under 15°C. Knothe et al. have reported that using alcohols of a longer aliphatic chain would result in producing biodiesel of larger chain length, thereby improving its cetane number. [28] There are no reported studies that have been carried out on BPD or MPD regarding this claim.

The cold flow property (CFP) of any diesel fuel indicates the ability of a fuel to operate in cold weather. This is assessed by measuring the cloud point (CP), pour point (PP), and cold filter plugging point (CFPP) of the fuel. Poor CFPs can cause instability in biodiesel over storage and can affect

TABLE II COMPARISON OF PHYSICAL PROPERTIES OF BIODIESEL PRODUCED FROM BPD, MPD, WBD AGAINST STANDARDS FOR BIODIESEL AND DIESEL

S. No.	Property	Desilked silkworm pupae biodiesel				WCO biodiesel	Standard range as laid by the government	
		BPD [80]	MPD [81]	BPD [79]	BPD [82]	WBD [97] & *[98]	DF [99]	BD [100]
1	Density	870 kg/m ³	850	870	867.6	878.9	815 - 845	860 – 900
2	Kinematic viscosity	4.3 mm ² /s	5.82	3.1	4.79	4.83	2 – 4.5	2.5 – 6.0
3	Cetane number	56	50.9	56.045	57.85	59.7	>51	>51
4	Flash point	160 °C	158 °C	93 °C	113 °C	150 °C*	35 °C	120 °C
5	Cloud point	2 °C	-	12 °C	-	0 °C*	-	-
6	Pour point	-3 °C	-	6 °C	-	-3 °C*	3°C in winter and 15°C in summer	-
7	Total glycerin content	<0.05 w/w	0.02	0.08	0.1	0.2	-	0.25
8	Water content	0.005 v/v	0.01	0.005	0.01	0.0012*	0.02	0.05
9	Sulfated ash	0.002 w/w	-	-	-	0.0005	10	0.02
10	Carbon residue	0.01 w/w	0.097	0.01	0.02	0.19	0.3	0.05
11	Acid value	0.4 mg KOH/g	0.38	0.30	-	0.94*	0.2	0.5
12	Copper strip corrosion	No. 1	-	-	-	-	Not worse than No. 1	Not worse than No. 1
13	Iodine value	-	112 (mg/g)	-	-	88*	-	120
14	Saponification value	-	183	191.48	-	194*	-	-
15	Fire point	-	-	102 °C	135 °C	-	-	-

Engine performance. The presence of high UFA in biodiesel improves the cold flow property and controls the oxidation of biodiesel. CFPs depend on climatic conditions and variations in the seasons. [101] The CP and PP of both MPD and BPD are suitable for operating in cold weather.

Glycerol is formed as a by-product during transesterification and is generally separated by its higher molecular weight. Impure glycerol that is present in biodiesel can cause them to degrade, forming diols and acids. These, in turn, enhance the process of polymerization of the UFA, leading to the degradation of biodiesel. [102] The separation method employed by Nandakumar et al. has proven to be effective as the total glycerin value is within acceptable limits. [80]

Biodiesel, in general is more hygroscopic or absorbs more water than conventional diesel. Water in any form if present, can cause turbidity and can also pave way for microbiological growth. [103] These in turn produce slime which can cause damage to fuel injectors and lines. Water present free form can cause condensation during storage and transportation.

Sulphated ash is an estimation of ash produced by the combustion of inorganic metallic oxides present in biodiesel. These oxides turn into their respective sulphates when thermally treated with sulphuric acid. This metallic ash can be abrasive and cause wear and tear between the piston rings and cylinder walls. [38] Minimum the sulphated ash present, the better it would be for the engine to operate. Sulphated ash on combustion of BPD is found to be within normal limits, as the metallic oxides that are present in pupae are generally eliminated during oil extraction.

The propensity of fuel to form thermal coke on pyrolysis is identified as carbon content and is gravimetrically measured. The carbon residue [CR] of fuel increases with the increase in viscosity. CR can also increase with prolonged storage and type of storage. [104] With storage, the degree of unsaturation of FAME plays an important role in altering the CR of fuel. The CR value of fresh BPD was very minimal. An important reason for lower CR for biodiesel is the absence of cyclic compounds in it. Aromatic esters tend to leave more coke on pyrolysis when compared to aliphatic esters. [104]

The quantity of standard KOH required to neutralise all fatty acid in a known quantity of biodiesel by classical titration is taken as its acid value. [105] The acid value of biodiesel tends to increase with the storage of oil or biodiesel, as free fatty acids are produced by the degradation of triglycerides during storage. [102] The higher the acid value, the higher is the rate of deterioration of BD. [105] The acid value of BPB and MPB lie within the acceptable limits. This indicates stable biodiesel with less corrosive properties. Corrosive property of BPB and MPB are also analysed by a copper strip test. This test is a quantification of sulphur or reactive sulphur species or a mixture of both in BD. BPB has shown corrosion that lies within No.1 grade. There are no studies made on the corrosive properties of MPB.

Iodine value determines the tendency of biodiesel to form deposits on the engine and its rate of degradation by polymerisation and oxidation of TG. It quantifies the amount of iodine that can be incorporated or absorbed into a known quantity of sample. It is an indirect measurement of the percentage of UFA. Iodine value depends on the relative percentage composition of SFA and UFA and their molecular structure and geometry. The maximum acceptable Iodine value for biodiesel is 120 g of iodine per 100 g of biodiesel. [106] The iodine value of BPB is within satisfactory limits.

C. Performance and emission characteristics of silkworm biodiesel

In a report published by Karnataka State Biofuel Development Board, Karnataka State Council for Science and Technology, Bangalore, Karnataka, India, on the production and characterisation of biodiesel from DDSP, the performance and emission characteristics of BPB have been investigated. This was studied on operating a CRDI four-stroke four-cylinder diesel engine run on BPB-Diesel blends. These results were compared with DF analysed in the same conditions. [107]

The performance characteristics of biodiesel, namely, brake specific fuel consumption (BSFC) and brake thermal efficiency (BTE) were determined for DF, BPB20 and BPB40 blends. BSFC was found to increase with the increase in the proportion of blend of BPB. Maximum BSFC was reported for BPB40. BDB20 blend showed a better BTE than DF. This was due to the reduction in density and viscosity on the addition of biodiesel.

Carbon monoxide, Hydrocarbons, Oxides of nitrogen, Smoke capacity and exhaust temperature were measured to analyse the emission properties of BD. Smoke opacity and exhaust temperature increased with the increase in the proportion of BPB blend. The rate of carbon monoxide and hydrocarbon emitted was measured against different brake powers. It was found that with the increase in brake power, the emission of CO and HC reduced. As BPB has a higher cetane number, its combustion in the engine is more complete when compared to DF. Also, as BD has better oxidative properties than DF, any CO produced during the combustion are oxidised to carbon dioxide. The rate of emission of oxides of nitrogen was found to

increase with the increase in BPB in the mixture. The production of NO_x is dependent on the temperature of the engine cylinder, residence time of fuel in the combustion chamber and oxygen concentration. The elevated exhaust temperature of BDD – Diesel blend explains the increase in NO_x production.

V. BIODIESEL PRODUCTION STATISTICS FROM SILKWORM PUPAE

The total production of silkworm pupae and silk in the country, and its imports and exports are being meticulously monitored by Central Silk Board (CSB), Bangalore, Karnataka, India. According to the statistics published by CSB, India has produced 35280 Metric tonnes of silk per year. [108], [109] Considering that the pupae inside the cocoon contribute to at least 60% of the weight of the cocoon, and the silk fibres attribute to the remaining 40%, the total quantity of silkworm pupae produced would be over 52000 Metric tonnes per year. Previous studies have indicated a 40% lipid content in the pupae, and studies have shown a minimum 80% conversion of these triglycerides to FAME. On calculating based on the known factors, DDSP as a feedstock is solely capable of producing over 19 Million litres of biodiesel. According to the GAIN report numbered IN9069, dated 08/02/2019, the total biodiesel used for blending for on-road use was 83 Million litres. [110] Biodiesel from DDSP has shown a potential for production of over 20% of the on-road use blend. Non-edible industrial fats, used cooking oil and animal fats and tallows were the feedstocks accounted for in the GAIN report. The quantity of animal fat and tallow used for BD production is 10,000 Metric tonne in 2019.[110] Fat content in dried pupae sums to almost twice the amount of animal fat that has been used. Therefore, it can be understood that DDSP is a promising feedstock for BD production.

VI. CONCLUSION

On critically reviewing the nature of solid waste produced during the process of silk reeling, the potential of this waste as a resource in biotechnological industries has been overlooked. The quantity of waste produced, quality of lipid in it and the applicability of this lipid as feedstock for biodiesel production has been veraciously studied. Based on this study, it is found that the lipid from desilked dried silkworm pupae has 70% UFA which makes it a viable feedstock for biodiesel. A minimum of 80% conversion of these fatty acids to FAME has been reported. Silkworm pupae alone can be responsible for the production of over 20% of on-road use blend for diesel. The production of silkworm pupae is estimated to over 1 lakh metric tonne by the end of 2030. This would produce approximately 75000 metric tonnes of dried pupae, and total biodiesel of a minimum of 25 million litres. This can further be improved by optimising the transesterification reaction. Studies on employing process intensification techniques for enhancing the yield of biodiesel and the application of novel catalysts such as metallocene compounds can be researched to further improve the prospects of biodiesel production from silkworm pupae.

The organic content after extraction of lipid is rich in mineral nutrients and can be doubled as fertiliser for mulberry plants for the same industry. The use of desilked dried silkworm pupae as feedstock for biodiesel production and the subsequent use of the residue on lipid extraction as fertiliser would not only provide a promising energy source but would lead to a zero solid waste process for biodiesel production.

REFERENCES

[1] B. Looney., Statistical Review of World Energy,69th edition, Bp, (2020) 1–68.

[2] P. Breeze., Chapter 2 - Electricity Generation and the Environment, in Power Generation Technologies (Second Edition), Second Edi., P. Breeze, Ed. Boston: Newnes, (2014) 15–27.

[3] I. E. A. IEA., Key World Energy Statistics, International Energy Agency publication, (2020).

[4] C. Footprint., Carbon footprint, 2008-05-10)[2012-10-01]. <http://www.carbonfoot-print.com/carbonfootprint.html>. (2008).

[5] F. Barbir, T. N. Veziroğlu, and H. J. Plass Jr., Environmental damage due to fossil fuels use, *Int. J. Hydrogen Energy*, 15(10) (1990) 739–749.

[6] J. Liiv, I. Zekker, K. Tamm, and E. Rikmann., Greenhouse gases emission and climate change-beyond mainstream, *MOJ Biorg Org Chem*, 4(1) (2020) 11–16.

[7] P. Janakiraman, M. Gajendiran, and N. Nallusamy., Performance and emission characteristics of diesel engine fueled with diesel, bio diesel and additives, in *AIP Conference Proceedings*, 2161(1) (2019) 020023.

[8] United States Environmental Protection Agency, Overview of the Human Health and Environmental Effects of Power Generation : Focus on Sulfur Dioxide (SO 2), Nitrogen Oxides (NO X) and Mercury (Hg), US EPA, Clear Skies Act, no. X. .

[9] J. E. Jonson, J. Borken-Kleefeld, D. Simpson, A. Ny`iri, M. Posch, and C. Heyes., Impact of excess NOx emissions from diesel cars on air quality, public health and eutrophication in Europe, *Environ. Res. Lett.*, 12(9) (2017) 94017.

[10] B. K. Bose., Global Warming: Energy, Environmental Pollution, and the Impact of Power Electronics, *IEEE Ind. Electron. Mag.*, 4(1)(2010) 6–17. doi: 10.1109/MIE.2010.935860.

[11] W. Stafford, A. Lotter, A. Brent, and G. von Maltitz., *Biofuels technology: A look forward*, (2017).

[12] N. D. Priya et al., A Study on Optimization of Pretreatment for Lipid Extraction from Rice Husk Using Oleaginous Yeast, in *Waste Valorisation and Recycling*, Springer, (2019) 263–272.

[13] F. Ma and M. A. Hanna., Biodiesel production : a review 1, *Bioresour. Technol.*, 70 (1999) 1–15.

[14] C. V. Sagar and N. A. Kumari., Sustainable biofuel production from water Hyacinth (Eicchornia crassipes), *Int J Eng Trends Technol*, 4(10) (2013) 4454–4458.

[15] N. B. Ahmed, B. K. Abdalla, I. H. M. Elamin, and Y. Ibrahim., Biodiesel Production from Roselle Oil Seeds and Determination the Optimum Reaction Conditions for the Transesterification Process, *Int. J. Eng. Trends & Tech*, 39 (2016) 105–111.

[16] I. Nuhu, F. M. Sani, and I. A. Rufai., Investigation of corrosion effects of jatropha biodiesel on the injector of an engine fuel system, *Int. J. Eng. Trends Technol.*, 8(1) (2014) 9–13.

[17] F. Aydin, H. Oguz, and H. Ogut., Fuel property investigation of diesel and mustard oil biodiesel mixtures at different ratios, *Int. J. Eng. Trends Technol.*, 18(2) (2014) 99–102.

[18] A. A. Mamun, S. Siddiqua, and S. M. E. Babar., Selection of an efficient method of biodiesel production from vegetable oil based on fuel properties, *Int J Eng Trends Technol*, 4(8) (2013) 3289–3293.

[19] R. K. Abdulrahman., Effect of Reaction Temperature on the Biodiesel Yield from Waste cooking Oil and chicken fat, *Int. J. Eng. Trends Technol.*, 44 (2017).

[20] V. Manieniyam and S. Sivaprakasam., Vibration analysis in DI diesel engine using diesel and biodiesel, *Int. J. Eng. Trends Technol.*, 1(4) (2013) 3586–3589.

[21] J. Duncan., COSTS OF BIODIESEL PRODUCTION, (2003).

[22] N. D. Priya and M. Thirumarimurugan., Biodiesel—A Review on Recent Advancements in Production, *Bioresour. Util. Bioprocess*,(2020) 117–129.

[23] T. W. Charpe and V. K. Rathod., Biodiesel production using waste frying oil, *Waste Manag.*, 31(1) (2011) 85–90

[24] T. Sabudak and M. Yildiz., Biodiesel production from waste frying oils and its quality control, *Waste Manag.*, 30(5) (2010) 799–803.,

[25] Y. Zhang, M. A. Dube, D. D. Mclean, and M. Kates, Biodiesel production from waste cooking oil: 1 . Process design and technological assessment, *Bioresour. Technology*, 89(1) (2003) 1–16. doi: 10.1016/S0960-8524(03)00040-3.

[26] Z. Yaakob, M. Mohammad, M. Alherbawi, Z. Alam, and K. Sopian., Overview of the production of biodiesel from waste cooking oil, *Renew. Sustain. energy Rev.*, 18 (2013)184–193.

[27] W. Parawira., Biodiesel production from *Jatropha curcas*: A review, *Sci. Res. Essays*, 5(14) (2010) 1796–1808.

[28] M. Y. Koh and T. I. M. Ghazi., A review of biodiesel production from *Jatropha curcas* L. oil, *Renew. Sustain. energy Rev.*, 15(5) (2011) 2240–2251.

[29] S. Gmünder, R. Singh, S. Pfister, A. Adheloia, and R. Zah, Environmental impacts of *Jatropha curcas* biodiesel in India, *J. Biomed. Biotechnol.*, 2012 (2012)

[30] G. A. K. Vivek and A. K. Gupta., Biodiesel production from Karanja oil, *J. Sci. Ind. Res. (India)*, 63(1) (2004) 39–47.

[31] Y. C. Sharma and B. Singh., Development of biodiesel from karanja, a tree found in rural India, *Fuel*, 87(8-9) (2008) 1740–1742.

[32] R. L. Patel and C. D. Sankhavara., Biodiesel production from Karanja oil and its use in diesel engine: A review, *Renew. Sustain. Energy Rev.*, 71 (2017) 464–474.

[33] R. A. Holser and R. Harry-O’Kuru., Transesterified milkweed (Asclepias) seed oil as a biodiesel fuel, *Fuel*, vol. 85(14-15) (2006) 2106–2110.

[34] Z. W. M. M. Phoo et al., Evaluation of Indian milkweed (Calotropis gigantea) seed oil as alternative feedstock for biodiesel, *Ind. Crops Prod.*, 54 (2014) 226–232.

[35] S. Pinzi, I. L. Garcia, F. J. Lopez-Gimenez, M. D. de Castro, G. Dorado, and M. P. Dorado., The ideal vegetable oil-based biodiesel composition: a review of social, economical and technical implications, *Energy & Fuels*, 23(5) (2009) 2325–2341.

[36] A. E. Atabani et al., Non-edible vegetable oils: a critical evaluation of oil extraction, fatty acid compositions, biodiesel production, characteristics, engine performance and emissions production, *Renew. Sustain. energy Rev.*, 18 (2013) 211–245.

[37] I. B. Banković-Ilić, O. S. Stamenković, and V. B. Veljković., Biodiesel production from non-edible plant oils, *Renew. Sustain. Energy Rev.*, 16(6) (2012) 3621–3647.

[38] A. Kumar and S. Sharma., Potential non-edible oil resources as biodiesel feedstock: an Indian perspective, *Renew. Sustain. Energy Rev.*, 15(4) (2011) 1791–1800.

[39] M. K. Lam and K. T. Lee., Microalgae biofuels: a critical review of issues, problems and the way forward, *Biotechnol. Adv.*, 30(3) (2012) 673–690.

[40] Y. Chisti, Biodiesel from microalgae, *Biotechnol. Adv.*, 25(3) (2007) 294–306.

[41] Y. Li, M. Horsman, N. Wu, C. Q. Lan, and N. Dubois-Calero., Biofuels from microalgae, *Biotechnol. Prog.*, vol. 24(4) (2008) 815–820.

[42] T. M. Mata, A. A. Martins, and N. S. Caetano., Microalgae for biodiesel production and other applications: a review, *Renew. Sustain. energy Rev.*, 14(1) (2010) 217–232.

[43] D. E. Leiva-Candia, S. Pinzi, M. D. Redel-Mac`ias, A. Koutinas, C. Webb, and M. P. Dorado., The potential for agro-industrial waste utilization using oleaginous yeast for the production of biodiesel, *Fuel*, 123 (2014) 33–42.

[44] A. Patel, N. Arora, J. Mehtani, V. Pruthi, and P. A. Pruthi., Assessment of fuel properties on the basis of fatty acid profiles of oleaginous yeast for potential biodiesel production, *Renew. Sustain. Energy Rev.*, 77 (2017) 604–616.

[45] M. Spagnuolo, A. Yaguchi, and M. Blenner., Oleaginous yeast for biofuel and oleochemical production, *Curr. Opin. Biotechnol.*, 57 (2019) 73–81.

[46] F. Manzano-Agugliaro, M. J. Sanchez-Muros, F. G. Barroso, A. Mart`inez-Sánchez, S. Rojo, and C. Pérez-Bañón, Insects for biodiesel production, *Renew. Sustain. Energy Rev.*, 16(6) (2012) 3744–3753.

- [47] C.-H. Su, H. C. Nguyen, T. L. Bui, and D.-L. Huang., Enzyme-assisted extraction of insect fat for biodiesel production, *J. Clean. Prod.*, 223 (2019) 436–444.
- [48] H. C. Nguyen, S.-H. Liang, S.-S. Chen, C.-H. Su, J.-H. Lin, and C.-C. Chien, Enzymatic production of biodiesel from insect fat using methyl acetate as an acyl acceptor: optimization by using response surface methodology, *Energy Convers. Manag.*, 158 (2018) 168–175.
- [49] M. Gürü, B. D. Artuko\uglu, A. Keskin, and A. Koca., Biodiesel production from waste animal fat and improvement of its characteristics by synthesized nickel and magnesium additive, *Energy Convers. Manag.*, 50(3) (2009) 498–502.
- [50] J. M. Dias, M. C. M. Alvim-Ferraz, and M. F. Almeida., Production of biodiesel from acid waste lard, *Bioresour. Technol.*, 100(24) (2009) 6355–6361.
- [51] G. M. Tashtoush, M. I. Al-Widyan, and M. M. Al-Jarrah., Experimental study on evaluation and optimization of conversion of waste animal fat into biodiesel, *Energy Convers. Manag.*, 45(17) (2004) 2697–2711.
- [52] P. Adewale, M.-J. Dumont, and M. Ngadi., Recent trends of biodiesel production from animal fat wastes and associated production techniques, *Renew. Sustain. Energy Rev.*, 45 (2015) 574–588.
- [53] A. Keskin, M. Gürü, D. Altiparmak, and K. Aydin., Using of cotton oil soapstock biodiesel–diesel fuel blends as an alternative diesel fuel, *Renew. Energy*, 33(4) (2008) 553–557.
- [54] M. J. Haas., Improving the economics of biodiesel production through the use of low value lipids as feedstocks: vegetable oil soapstock, *Fuel Process. Technol.*, 8(10) (2005) 1087–1096.
- [55] E. Öztürk., Performance, emissions, combustion and injection characteristics of a diesel engine fuelled with canola oil–hazelnut soapstock biodiesel mixture, *Fuel Process. Technol.*, 129 (2015) 183–191.
- [56] C.-Y. Lin and R.-J. Li., Fuel properties of biodiesel produced from the crude fish oil from the soapstock of marine fish, *Fuel Process. Technol.*, 90(1)(2009) 130–136.
- [57] S. Sridhara and J. V Bhat., Lipid composition of the silkworm *Bombyx mori* L., *J. Insect Physiol.*, 11(4)(1965) 449–462, doi: [https://doi.org/10.1016/0022-1910\(65\)90051-X](https://doi.org/10.1016/0022-1910(65)90051-X).
- [58] G. Ganga and S. C. J., *An Introduction to Sericulture*, 2nd ed. Oxford & IBH Publishing Co. Pvt. Ltd., 2018.
- [59] T. A. Bhat and T. Choure., Study of growth and instability in raw silk production and marketing in India, *Eur. J. Bus. Manag.*, 6(14)(2014) 108–111.
- [60] D. Gangopadhyay., *Sericulture Industry in India – A Review*, India, *Sci. Technol. Rural India Incl. Growth*, 47(5)(2008) 1–25 [Online]. Available: <http://www.nistads.res.in/indiants2008/t6rural/t6rur16.htm>.
- [61] M. N. Padamwar and A. P. Pawar., *Silk sericin and its applications: A review*, (2004).
- [62] Y.-Q. Zhang., Applications of natural silk protein sericin in biomaterials, *Biotechnol. Adv.*, 20(2)(2002) 91–100.
- [63] M. Manjunath, K. C., Narayanaswamy, Savithamma, S. H. Babu, and H. V Harishkumar, Scenario of mulberry and cocoon production in major silk producing States of India-Application of exponential growth function, *Indian J. Econ. Dev.*, 3(8)(2015).
- [64] R. Bukhari, K. P. Singh, and R. H. Shah., Non: Mulberry Sericulture, *J. Pharmacogn. Phytochem.*, 8(4)(2019) 311–323.
- [65] S. K. Dewangan, K. R. Sahu, K. V Achari, and others., Sericulture-A tool of eco-system checking through tribal, *J. Environ. Res. Dev.*, vol. 6, no. 1, pp. 165–173, 2011.
- [66] E. O. Oduor, L. Ciera, V. Adolkar, and O. Pido., Physical characterization of eri silk fibers produced in Kenya, *J. Nat. Fibers*, 18(1)(2021) 59–70.
- [67] C. Kalita., Study of Physical Properties of Muga and Eri with the Help of Xrd (Undegummed), *Confluence*, 1(1) (2014) 78–85.
- [68] K. V Benchamin, V. Rao, and P. J. Raju., Effect of cold storage of newly hatched larvae on survival rate, growth and egg production in silkworm *Bombyx mori* L., *Proc. Anim. Sci.*, 98(1)(1989) 27–33.
- [69] T. Yokoyama., *Sericulture*, *Annu. Rev. Entomol.*, 8(1) (1963) 287–306.
- [70] N. MN and others., *Manual on sericulture, silkworm rearing*, 2(1973).
- [71] T. Karthik and R. Rathinamoorthy, Sustainable silk production. Elsevier Ltd, (2017).
- [72] K. S. Shinde, S. B. Avhad, S. V Jamdar, and C. J. Hiware., Comparative studies on the performance of mountages on cocoon quality of *Bombyx mori* L, *Trends life Sci.*, 1(2012) 8–11.
- [73] S. D. Aznar-Cervantes, A. Pagan, B. M. Santesteban, and J. L. Cenis., Effect of different cocoon stifling methods on the properties of silk fibroin biomaterials, *Sci. Rep.*, 9(1) (2019) 1–11.
- [74] M. R. A. O. NR, S. TS, and others., *Manual on sericulture*. 3(1972) silk reeling.
- [75] M. Łochyńska and J. Frankowski., The biogas production potential from silkworm waste, *Waste Manag.*, 79(2018) 564–570, doi: <https://doi.org/10.1016/j.wasman.2018.08.019>.
- [76] S. R. Patil, S. Amena, A. Vikas, P. Rahul, K. Jagadeesh, and K. Praveen., Utilization of silkworm litter and pupal waste—an eco-friendly approach for mass production of *Bacillus thuringiensis*, *Bioresour. Technol.*, 131(2013) 545–547.
- [77] A. Karthikeyan and N. Sivakumar., Sericulture pupal waste—A new production medium for mass cultivation of *Bacillus thuringiensis*, *Indian J. Biotechnol.*, 6(2007) 57–559.
- [78] D. S. Mahesh, B. S. Vidhathi, T. K. Narayanaswamy, C. T. Subbarayappa, R. Muthuraju, and P. Shruthi., A Review – Bionutritional Science of Silkworm Pupal residue to Mine New ways for utilization, *Int. J. Adv. Res. Biol. Sci.*, 2(9) (2015) 135–140.
- [79] N. R. Pradeep, S. Kumarappa, and B. M. Kulkarni., Characterization and Evaluation of Fuel Properties of Pupae Biodiesel-Diesel Blends, *Int. J. Latest Technol. Eng. Manag. Appl. Sci.*, 6(8)(2017) 85–90.
- [80] V. Nadanakumar, A. A. Arivalagar, and N. Alagumurthi., Studies on Production and Optimization of silkworm biodiesel, *J. Chem. Pharm. Sci.*, 9(4)(2016) 3063–3069.
- [81] M. G. M. Sarma., Production of high quality biodiesel from desilked muga pupae (*Antheraea assamensis*), *Res. J. Chem. Environ. Sci.*, 4(4)(2016) 40–45.
- [82] R. Ravikumar, H. Kumar, K. Kiran, and G. S. Hebbar, ., Extraction and Characterization of Biofuel from Industrial Waste organic Pupae-Silkworm, *Int. J. Recent Technol. Eng.*, 8(3)(2019) 1603–1607.
- [83] K. S. Shanker et al., Isolation and characterization of neutral lipids of desilked eri silkworm pupae grown on castor and tapioca leaves, *J. Agric. Food Chem.*, 54(9)(2006) 3305–3309.
- [84] Z.-J. Wei, A.-M. Liao, H.-X. Zhang, S. K. Liu, and S.-T. Jiang., Optimization of supercritical carbon dioxide extraction of silkworm pupal oil applying the response surface methodology, *Bioresour. Technol.*, 100(18)(2009) 4214–4219.
- [85] T. Ravinder, S. S. Kaki, S. Kanjilal, B. Rao, S. K. Swain, and R. B. N. Prasad., Refining of castor and tapioca leaf fed eri silkworm oils, *Int. J. Chem. Sci. Technol.*, 5(2)(2015) 32–37.
- [86] B. S. Vidhathi et al., Isolation and detection of alpha linolenic acid from silkworm pupal residue oil (*Bombyx mori* L.) using HPLC, *Int. J. Curr. Microbiol. Appl. Sci.*, 6(7)(2017) 2202–2206.
- [87] B. Hu et al., Microwave-assisted extraction of silkworm pupal oil and evaluation of its fatty acid composition, physicochemical properties and antioxidant activities, *Food Chem.*, 231(2017) 348–355.
- [88] L. F. Bautista, G. Vicente, R. Rodriguez, and M. Pacheco., Optimisation of FAME production from waste cooking oil for biodiesel use, *Biomass and Bioenergy*, 33(5)(2009) 862–872.
- [89] E. F. Aransiola, M. O. Daramola, T. V. Ojumu, M. O. Aremu, S. K. Layokun, and B. O. Solomon., Nigerian *Jatropha curcas* oil seeds: prospect for biodiesel production in Nigeria., *Int. J. Renew. ENERGY Res.*, 2(2)(2012)317–325.
- [90] S. O'Donnell, I. Demshemino, M. Yahaya, I. Nwadike, and L. Okoro., A review on the spectroscopic analyses of biodiesel, *Eur. Int. J. Sci. Technol.*, 2(7)(2013) 137–146.
- [91] G. Gelbard, O. Bres, R. M. Vargas, F. Vielfaure, and U. F. Schuchardt., ¹H nuclear magnetic resonance determination of the yield of the transesterification of rapeseed oil with methanol, *J. Am. Oil Chem. Soc.*, 72(10)(1995) 1239–1241.
- [92] J. Nisar et al., Enhanced biodiesel production from *Jatropha* oil using calcined waste animal bones as catalyst, *Renew. Energy*, 101(2017) 111–119.
- [93] M. Qasim, T. M. Ansari, and M. Hussain., Combustion, performance, and emission evaluation of a diesel engine with biodiesel like fuel blends derived from a mixture of Pakistani

- waste canola and waste transformer oils, *Energies*, 10(7)(2017) 1023.
- [94] N. Isioma, Y. Muhammad, O. Sylvester, D. Innocent, and O. Linus., Cold flow properties and kinematic viscosity of biodiesel, *Univers. J. Chem.*, 1(4)(2013) 135–141.
- [95] E. Lois, E. L. Keating, and A. K. Gupta., Fuels, in *Encyclopedia of Physical Science and Technology (Third Edition)*, Third Edit., R. A. Meyers, Ed. New York: Academic Press, (2003) 275–314.
- [96] K. Wadumesthrige, J. C. Smith, J. R. Wilson, S. O. Salley, and K. Y. S. Ng., Investigation of the parameters affecting the cetane number of biodiesel, *J. Am. Oil Chem. Soc.*, 85(11)(2008) 1073–1081.
- [97] Ö. Can., Combustion characteristics, performance and exhaust emissions of a diesel engine fueled with a waste cooking oil biodiesel mixture, *Energy Convers. Manag.*, 87(2014) 676–686.
- [98] M. R. Uddin, K. Ferdous, M. R. Uddin, M. R. Khan, and M. A. Islam., Synthesis of biodiesel from waste cooking oil., *Chem. Eng. Sci.*, 1(2)(2013) 22–26.
- [99] Bureau of Indian Standards, IS 1460:2017, Automotive diesel - Specification (Bharat Stage IV). (2017).
- [100] Bureau of Indian Standards, IS 15607:2005, Bio-diesel (B100) Blend Stock for Diesel Fuel - Specification. (2005).
- [101] L. Gouveia et al., Biodiesel from microalgae, in *Microalgae-Based Biofuels and Bioproducts: From Feedstock Cultivation to End-Products*, (2017) 235–258.
- [102] S. Banga and P. K. Varshney., Effect of impurities on performance of biodiesel: A review, *J. Sci. Ind. Res.*, vol. 69(2010) 575–579.
- [103] P. B. L. Fregolente, W. M. Wolf Maciel, and L. S. Oliveira., Removal of water content from biodiesel and diesel fuel using hydrogel adsorbents, *Brazilian J. Chem. Eng.*, 32(4)(2015) 895–901.
- [104] V. Thangarasu and R. Anand., 11 - Physicochemical fuel properties and tribological behavior of aegle marmelos correa biodiesel, in *Advances in Eco-Fuels for a Sustainable Environment*, K. Azad, Ed. Woodhead Publishing, (2019) 309–336.
- [105] J. N. Conceição et al., Evaluation of molecular spectroscopy for predicting oxidative degradation of biodiesel and vegetable oil: Correlation analysis between acid value and UV-Vis absorbance and fluorescence, *Fuel Process. Technol.*, 183(2019) 1–7.
- [106] R. A. de Mattos, F. A. Bastos, and M. Tubino., Correlation between the composition and flash point of diesel-biodiesel blends, *J. Braz. Chem. Soc.*, 26(2)(2015) 393–395.
- [107] M. N. . (KARNATAKA S. B. D. B. S.Kumarappa, PRODUCTION, CHARACTERIZATION OF BIODIESEL FROM PUPAE A BYPRODUCT OF SILK REELING INDUSTRY AND ENGINE PERFORMANCE ANALYSIS AND EMISSION CHARACTERISTICS OF CRDI DIESEL ENGINE, (2012).
- [108] K. Central Silk Board, Bengaluru., RAW SILK PRODUCTION STATISTICS, (2020).
- [109] K. Central Silk Board, Bengaluru., SERICULTURAL STATISTICS IN INDIA - A GLANCE, (2020).
- [110] U. F. A. Service., India biofuels annual, IN9080, 2009, [Online]. Available: [http://gain.fas.usda.gov/Recent GAIN Publications/General Report_New Delhi_India_6-12-2009.pdf](http://gain.fas.usda.gov/Recent%20Publications/General%20Report_New%20Delhi_India_6-12-2009.pdf).