

## Studies on Production and Optimization of Silkworm Biodiesel

V.Nadanakumar<sup>1\*</sup>, A.A.Arivalagar<sup>2</sup>, N.Alagumurthi<sup>3</sup>

<sup>1</sup> Pondicherry Engineering College, Pudhucherry, India

<sup>2</sup> G.R.T. Institute of Engineering and Technology, Thiruttani, India

<sup>3</sup> Pondicherry Engineering College, Pudhucherry, India.

\*Corresponding author: E-Mail: vin.nadanakumar@gmail.com

### ABSTRACT

This study utilizes the de-silked silkworm *Bombyx mori* .L as a raw source for the production of methyl ester by the way of two step (esterification and transesterification) reaction technique. The feasibility of lipids were collected and characterized by Soxhlet and GC (gas chromatography), respectively. Fatty acid methyl ester production from de-silked worm using H<sub>2</sub>SO<sub>4</sub> homogeneous catalyst were performed and transesterification experimental condition such as reaction temperature, catalyst loading, methanol-oil molar ratio, reaction time and stirring rate were studied. The highest conversion of oil to fatty acid methyl ester was achieved as 98.9 wt% at the favorable conditions of 65 °C, 12:1 molar ratio of methanol-oil, 2 wt% of H<sub>2</sub>SO<sub>4</sub> and 400 rpm stirring rate with 50 min of reaction time. The conversion and methyl ester properties were confirmed by <sup>1</sup>H-NMR (Nuclear magnetic resonance) and ASTM standards, respectively.

**KEY WORD:** *Bombyx mori* .L bio-oil; methyl ester, esterification, transesterification, homogeneous process, H<sub>2</sub>SO<sub>4</sub>.

### 1. INTRODUCTION

Energy is an significant source mainly for transportation, industries and agricultural sectors. Presently, the petroleum energy utilisation has been enhanced continuously, which speed up the diminution of limited petroleum deliver and necessarily increase the petroleum prices (Kasirajan Ramachandran, 2011; Jatropha curcas, 2012). It is very important to find an alternative energy, particularly the fuel for transport diesel engines, in direct to extend the petroleum supply. Nowadays, renewable and sustainable fuel has received great consideration as an alternate for present fossil fuels. Approximately 90% of the biofuel souk is confined by bio-ethanol and biodiesel (Hamidreza Jaliliannosrati, 2013; Jain, 2010).

A prospective diesel oil alternative is fatty acid methyl ester (biodiesel). Biodiesel have been used honestly or mixed with diesel oil at various proportions in many countries (Zhang, 2013; Zeng, 2009; Refaat, 2010). It is derived from a renewable and domestic resource, thereby relieving dependence on petroleum imports. Compared to petroleum fossil diesel oil, biodiesel has a most favourable combustion emission outline such as less emission of CO, SO<sub>2</sub>, particulate matters and unburned hydrocarbons, (Ramachandran, 2013). In addition, biodiesel have the proper viscosity, higher cetane number, higher flash point and can be used in an engine without any engine modification (Pramanik, 2003). The price of feedstock is accounted as a major cost of biodiesel production. The utilization of low prize substrates is a best alternative source for sustainable production of biodiesel (Sen Yang, 2014, Sharma, 2010).

There are several sources available for biodiesel production such as, used restaurant oil residues, waste greases, waste fish oils, waste animal fats, industrial residues and waste marine algae's (Alptekin, 2011; Phan, 2008; Vinothkumar, 2016). But, the silkworm residues were having more than 30 wt % of lipids. The annual dry de-silked *Bombyx mori* .L is approximately above 450 tons. China and India has become the highest silk-reeling countries in the world. However, most of the de-silked *Bombyx mori* .L is used as a constituent of fish and chicken feed and some cases used as a fertilizer, in certain cases they are directly dumped in the land pits and some are even considered as industrial waste. Consequently, highly value added products were not developed from this de-silked *Bombyx mori* .L. Therefore, the oil extracted from the de-silked *silkworm* is considered as a best source for biodiesel production.

There are several homogeneous and heterogeneous catalyst (acid or base) presently available for biodiesel production such NaOH, KOH, silica supported base and acid catalyst, metal oxides, zeolites and enzymes. But, in commercial process homogeneous catalyst only give more conversion then other catalysts. Due to the higher acid value and higher free fatty acids presence in de-silked *Bombyx mori* .L oil, the acid catalyzed transesterification reaction only the possible way to achieve higher conversion of oil to biodiesel. The base catalyst will induce the soap formation during the reaction, when acid value and free fatty acids are more than 2 mg of KOH/gm of oil and 1wt%, respectively (Shuangshuang, 2013).

In this study, we analyzed the capability of the de-silked *Bombyx mori* .L source used for methyl ester production and optimized some of key variables distressing the conversion of acid- base catalyzed production of methyl esters such as the reaction time, temperature, molar ratio of methanol to oil, amount of catalyst and stirring rate. We also analyzed the most important fatty acid components of the *Bombyx mori* .L oil and evaluated the fuel properties of the resultant *Bombyx mori* .L based biodiesel to the ASTM standard.

## 2. MATERIALS AND METHODS

**Materials:** De-silked *Bombyx mori* .Lhas been collected in Tamil Nadu and Andhra Pradesh states in India. The 98 % of sulphuric acid have been purchased from Merck India Ltd, Chennai. Methanol (purity of 99.9 %), Hexane (purity of 99 %) were obtained from SRL Chemical India Ltd, Chennai.

**Extraction procedure:** The de-silked *Bombyx mori* .Lwas utilized as a raw source in this study. Initially, de-silked *Bombyx mori* .Lwasdried in a hot air oven at a constant temperature of 45 °C over night to obtain equilibrium weight loss. Then the dried *Bombyx mori* .L weregrounded to shrinkthe particle size in a blender and sieved to the particle size in the range between 0.25 to 0.5 mm. After size reduction, the fine particle size was separated using avibrator sieve-shaker (mesh type) into a range of 0.25 to 0.5 mm. The maximum amount of oil content of the de-silked *Bombyx mori* .L was determined using the conventional method.25 gm of biomass loaded in to the Soxhlet extraction apparatus with n-hexane was utilized as the solvent for 8 hours at solvent reflux temperature (Xin Deng, 2010). After the completion of extraction process, n-hexane was recovered using batch distillation and the traces amount of solvent has been removed by rotary evaporator and the extracted oil was quantified by weighing method and the yield was measured by the following equation. The oil content of the de-silked *Bombyx mori* .L was obtained as 30.4 wt%.

$$\text{Yield \%} = \frac{[\text{Amount of oil extracted}]}{[\text{Amount of biomass taken}]} \times 100 \quad (1)$$

**Oil characterization:** The properties such as average molecular weight, density, acid value, iodine value, saponification value and FFA (free fatty acid) content of extracted de-silked *Bombyx mori* .L oil were analyzed by using standard procedures (Suganya, 2012). The fatty acid composition present in *Bombyx mori* .L oil was analyzed by gas chromatography (GC) analysis. A sample with one gram of oil was taken and fatty acid composition analysis was carry out by gas chromatograph, which consisted of CHEMIT GC 8610 flame ionization sensor in the column BPX-70. Hydrogen and nitrogen gas were used as carrier gas and oxygen was used for ignition purpose. The data was self-possessed with the use of Winchrom software (AOCS, 1998).

**Two-step reaction procedure:** First step, only H<sub>2</sub>SO<sub>4</sub> catalyst was used for acid esterification pre-treatment in the reactor to convert the FFAs into methyl ester. In this step, required quantity of catalyst (with respect to oil wt %) and required methanol to oil molar ratio were used to study their influence on diminution of the FFAs of *Bombyx mori* L. oil. The three-neck round bottom flask was connected with a water-cooled reflux condenser was used as a reactor and filled with 25 gm of *Bombyx mori* L.oil and appropriate molar ratio of anhydrous methanol and H<sub>2</sub>SO<sub>4</sub>. The reaction mixture was energetically stirred and refluxed up to the required reaction time. After the reaction completion, the unreacted methanol was separated from the liquid phase using rotary evaporation.

At the second step, NaOH catalyst was used for the reason that this catalyst had a superior catalytic activity for the transesterification reactions.The treated *Bombyx mori* L. by acid catalyzed esterification was considered as the pre-treated material for transesterification process. The appropriate process conditions such as molar ratio of methanol to oil, reaction time and Catalyst loading for transesterification reaction were maintained. All experiments were performed in 250 mL of batch reactor with reflux condenser and the reaction carried out at reflux temperature and constant stirring speed of 400 rpm.After the completion of reaction, the mixture was allowed to settling overnight to remove byproduct of glycerol then the traces amount of methanol was removed by open pan evaporation.

**Biodiesel characterization:** Methyl ester produced from *Bombyx mori* L. oil by the two step techniques was characterized by <sup>1</sup>H-NMR. <sup>1</sup>H-NMR spectra were acquired using a Bruker 500 MHz Avance III tool with the Chloroform-d (CDCl<sub>3</sub>) as solvent and tetramethylsilane (TMS) as an internal standard. A <sup>1</sup>H spectrum was recorded with pulse duration of 45 °C and 16 scan. The conversion was calculated using simple formula (Eq. 2).

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

Where C is the percentage conversion of *Bombyx mori* L. oil to methyl ester, A<sub>ME</sub> is an integration value of the methoxy protons of the methyl ester, A<sub>CH<sub>2</sub></sub> is an integration value of the methylene protons. Factors 2 and 3 were predicted from the fact, that the methylene carbon possesses 2 protons, while the methyl alcohol (alcohol derived) carbon has 3 attached protons. The properties such as density, viscosity, flash point, fire point, cetane number, sulphur content, moisture content and etc... of biodiesel were analysed as per the ASTM standard.

## 3. RESULT AND DISCUSSION

**Characterization of *Bombyx mori* L. oil:** Table 1 demonstrates the characteristics of *Bombyx mori* L. that were used in this study. Based on the GC results, the average molecular weight was calculated as 866.9 and the free fatty acid content of the *Bombyx mori* L.was determined as 58.55%. Fatty acids compositions of *Bombyx mori* L. are very important in identify the carbon chains and its properties. Table 2 shows that the fatty acid compositions of *Bombyx mori* Land analysed as per the IS548 standard method. The results showed that the highest fatty acids were oleic, linolenic,palmitic, stearic and linoleic acid. Saturated fatty acids in *Bombyx mori* L. were 27.08 wt% while mono and poly unsaturated fatty acids were 35.04 and 37.83 wt%, respectively.

**Esterification reaction studies:** Esterification technique was used in order to pre-treat the *Bombyx mori L.* oil by converting the high content of free fatty acids into methyl ester using an acid catalyst. The initial content of FFAs of the *Bombyx mori L.* used in this study was 58.55%, which would not be favourable for biodiesel production. Transesterification reaction will not occur if the FFAs content in oil is more than 1.5%. Therefore, the limits of FFAs were set to a maximum of 1% for all esterification experimentation. The major factors affecting the esterification process were reaction time, molar ratio of methanol to oil, reaction temperature, catalyst concentration and stirrer speed.

**Reaction time effect on esterification:** Adequate contact time must be provided to complete the esterification reaction. Fig. 1 shows that effect of reaction time on the reduction of FFAs and conversion of FFAs to methyl ester. It was observed that the conversion of FFAs to methyl ester increased with an increase in reaction time. The FFAs content at 60 min up to 80 min was less than 1% FFAs. Consequently, in sequence to save the energy and to reduce the cost of the pre-treatment process, 70 min of reaction time is adequate for the completion of esterification reaction, which gave 98.5% conversion of FFA's to methyl ester and the FFA content decreased from 58.55% to 0.9%. Veljkovic et al. (2006) also investigated that esterification process reduced the FFAs content from about 35% to less than 2% in within 50 min, with 13:1 molar ration of methanol to oil (Ramachandran Kasirajan, 2014). In the present study, FFAs content were reduced from 58.55% to less than 1% in 60–80 min with 12:1 Molar ratio. Therefore, 70 min was selected as a suitable time for further studies.

**Reaction temperature effect on esterification:** The diminution of acid value and FFAs content was considerably controlled by the temperature of an esterification pre-treatment. The temperature was selected from the range of 35 to 65 °C. Temperature higher than 65 °C was not investigated in the esterification reaction because at higher temperature sulphuric acid could burn some of oil. The experiment was carried out with catalyst concentration of 2% and methanol to oil ratio of 12:1 at 400 rpm of mixing intensity and up to 70 min reaction time. When the reaction temperature increased from 35 to 60 °C, the FFAs content found to be reduced from 40.8 to 0.9 mg KOH/g and the (Fig. 2). It specifies that, the increase in the temperature augment the solubility of the solvent with superior diffusion rate. Continuing rise in the temperature demonstrates the positive effect on the conversion from 30.3% to 98.5%. The optimum temperature of this examination was 65°C for the acid esterification of *Bombyx mori L.* oil. Ghadge and Raheman (2005) investigated that 60 °C is the appropriate temperature for esterification pre-treatment to reduced FFAs from 38 to 4.84 mg of KOH/g of the crude *mahua* oil (Veljkovic , 2006).

**Catalyst concentration effect on esterification:** Catalyst concentration optimization studies was conducted with different concentration ranges from 0 to 3.5 wt.% and other reaction conditions were kept constant as 12:1 molar ratio of methanol to oil, 65 °C of temperature, 70 min of reaction time and 400 rpm of mixing intensity. Initially from 0 to 2.5 wt.% of catalyst loading, the FFAs content was reduced from 58.55 to 99.15 wt%. Further increase in the catalyst concentration from 2.5 to 3.5 wt.% suddenly reduced the conversion of FFAs from 99.15 to 91.1 wt% (Fig. 3). Conversely, the concentration of catalyst increased further 2.5 %, the acid value and FFAs content augmented again. The possible reason that the higher acid concentration was hydrolyzed the triglyceride to form FFAs and low-molecular weight alcohols. The similar trend was observed by Tiwari et al. (2007) in terms of acid value (Ghadge, 2005).

**Methanol to oil molar ratio effect on esterification:** Molar ratio of methanol-oil is also an important key factor distressing the conversion of FFAs to methyl ester, as well as the overall processing cost of biodiesel. The esterification reaction requires more methanol than transesterification. However, in practice the methanol-oil molar ratio should be superior to that of the stoichiometric ratio in order to force the reaction towards complete conversion (Tiwari, 2007). In this investigation, the molar ratio of methanol to *Bombyx mori L.* oil was varied from 3:1 to 18:1. Fig. 4 shows that the consequence of the molar ratio on the reduction of the FFAs content in *Bombyx mori L.* oil and conversion of FFAs to methyl ester. The conversion of treated *Bombyx mori L.* oil slightly increased when the molar ratio of methanol-oil was increased from 3:1 to 12:1 and further increases of molar ratio, no significant changes was observed with a higher molar ratio. Because, an excess of methanol was achieved higher conversion but too large quantity of methanol would not capable to contribute on the esterification reaction due to the mass transfer restriction. Conversely, a minimum of 10:1 molar ratio was required to reduce the FFAs content of *Bombyx mori L.* oil from 45.4% to 0.5%, which is the boundary of FFAs for transesterification reaction in this study. An insufficient quantity of methanol in the esterification reaction tends to be slower, thus decreases the rate of conversion. Therefore, 12:1 molar ratio of methanol-oil was considered for further reaction studies. Di Serio et al. (2008) also used 12:1 molar ratio to decrease the FFAs content presence in the soybean oil from 20.5% to 1.1% catalyzed by PTSA as an acid catalyst (Ramadhas, 2005).

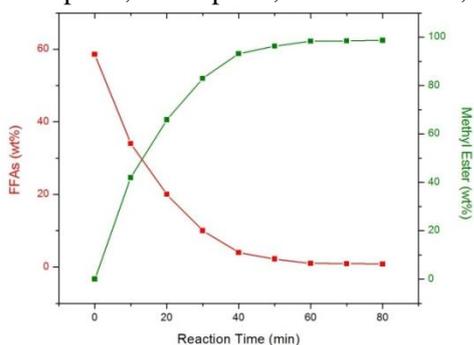
**Mixing intensity effect on esterification:** The intensity of mixing is one of the main factors for the esterification reaction, in order to enhance the diffusion mass transfer between the reactants. It increases the contact area between oil and catalyst in methanol solution to initiate the reaction. The lower conversion is due to inappropriate mixing

because the reaction takes place only at the interface of the two layers. Agitation of oil and methanol-catalyst mixture augments the reaction rate and leads to complete the reaction with short duration.

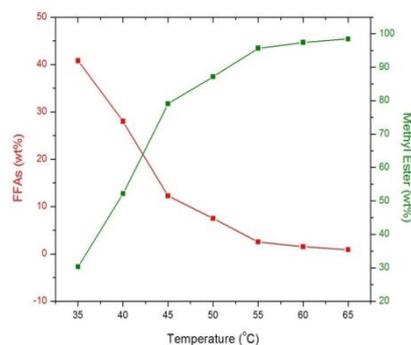
Mixing intensity was studied in the range from 150 to 500 rpm. Fig. 5 shows that an effect of mixing intensity on the reduction of FFAs content and the conversion of FFA to methyl ester. The results exposed that the stirrer rates of 150 and 400 rpm were adequate for the completion of esterification reaction and to diminish FFAs content from 34% to 0.5%. However, the conversion for 400 rpm was slightly higher than that for 150 rpm. Mixing rate significantly affects the reaction rate. Inadequate mixing could lead to lower reaction rate, thus inferior the conversion value. Therefore, 400 rpm stirrer speed was selected as the optimum stirrer speed in the pre-treatment process of *Bombyx mori L.* oil. On the other hand, higher mixing intensity would negatively impact to the reaction as well. The results showed that increasing the stirrer speed more than 400 rpm, not only consumes more energy but also results in a lower conversion than that of 400 rpm. The mixing rate of 500 rpm was not recommended because the FFAs content increased above 3%, and the conversion of FFAs to methyl ester was decreased.

**Alkyl transesterification of *Bombyx mori L.* oil:** In two step processes after esterification, the pre-treated *Bombyx mori L.* oil was converted into methyl ester (biodiesel) using NaOH as a homogenous base catalyst. The pre-treated oil contains only triglycerides that conversion study was examined by varying the catalyst concentration from 0.5 to 3 wt% at constant temperature of 65 °C, Methanol-oil molar ratio of 12:1, 400 rpm and 60 min of reaction time. The results are shown in table 3. It was observed that the conversion of triglycerides to methyl ester found to be increased with increase in catalyst concentration up to 1.5 wt%. Further increases, there is no any significant impact on conversion of triglycerides to methyl ester. The maximum conversion was achieved as 98.3% with the use of 1.5 wt% of catalyst.

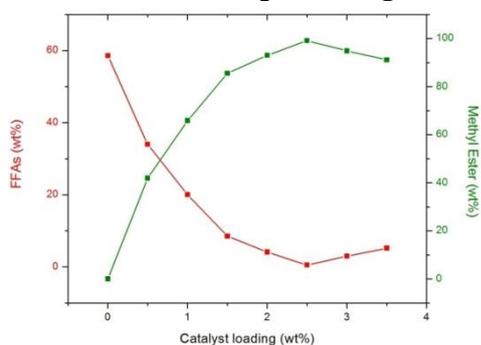
**Characterization of biodiesel:** Figure 6 represents the <sup>1</sup>H-NMR spectra of *Bombyx mori L.* biodiesel. The quality gesture formed from the protons of methylene group adjacent to the ester group of a molecule in TG (a-CH<sub>2</sub>) was eluted at 2.3 ppm and the protons in the methoxy group of the methyl ester were detected at 3.66 ppm. The conversion of biodiesel was accomplished based on these attribute proton signals in the NMR spectra. The conversion of the methyl ester from *Bombyx mori L.* oil was determined as 98.73 wt%. The physical properties of kinematic viscosity, density, flash point, cloud point, cetane number, moisture content and etc... were shown in table 4.



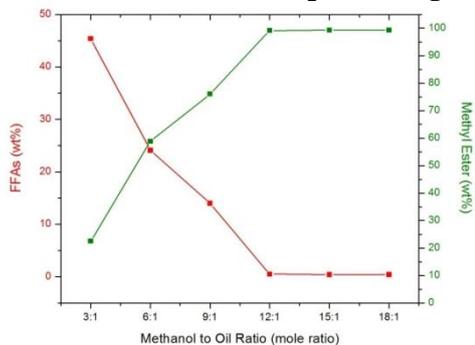
**Figure.1.** Effect of reaction time at 65°C temperature, 2wt% of catalyst, 12:1 molar ratio of methanol-oil and 400 rpm mixing intensity.



**Figure.2.** Effect of reaction temperature with 70 min of reaction time, 2wt% of catalyst, 12:1 molar ratio of methanol-oil and 400 rpm mixing intensity.



**Figure.3.** Effect of Catalyst Concentration at 65°C temperature with 70 min of reaction time, 12:1 molar ratio of methanol-oil and 400 rpm mixing intensity.



**Figure.4.** Effect of Methanol-oil Molar ratio at 65°C temperature with 70 min of reaction time, 2.5 wt% of catalyst and 400 rpm mixing intensity.

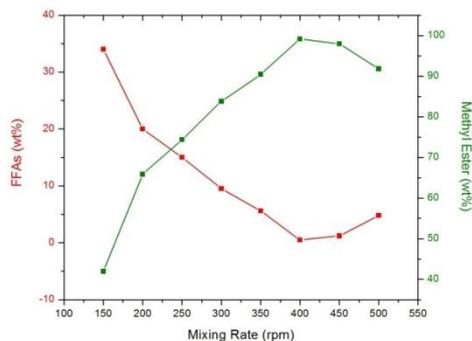


Figure.5. Effect of mixing intensity at 65°C temperature with 70 min of reaction time, 2.5 wt% of catalyst and 12:1 molar ratio of methanol-oil

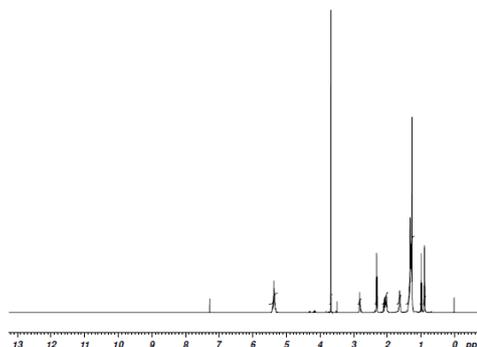


Figure.6. <sup>1</sup>H-NMR Spectra of *Bombyx mori L.* biodiesel

Table.1. Properties of *Bombyx mori L.* oil.

Properties	Values	Units
Average molecular weight of Oil	866.9	g/mol
Density	0.901	g/cc
Acid value	115.92	mg KOH/g
Saponification value	199.64	mg KOH/g
Free fatty acids	129.48	wt%
Saturated fatty acids	27.08	Relative%
Mono unsaturated fatty acids	35.04	Relative%
Poly unsaturated fatty acids	37.83	Relative%
Trans fatty acids	<0.01	Relative%

Table.2. Fatty acid compositions of *Bombyx mori L.* oil.

Fatty acids	Carbon atoms	Relative %
Myristic acid	C14:0	0.17
Palmitic acid	C16:0	19.68
Stearic acid	C18:0	7.23
Palmitoleic acid	C16:1	0.73
Oleic acid	C18:1	34.31
Linoleic acid	C18:2	6.34
Linolenic acid	C18:3n3 b	30.99
Y-linolenic acid	C18:3n6	0.50
Other fatty acids	-	0.05

Table.3. Alkyl transesterification (effect of catalyst concentration) at constant temperature, methanol-oil molar ratio, stirring speed and time.

Catalyst Concentration (wt%)	Triglyceride concentration (wt%)	Methyl ester conversion (wt%)
0	41.44	0
0.5	32	45.34
1	12	79.50
1.5	0.94	98.39
2	0.93	98.41
2.5	0.93	98.41
3	0.93	98.41

Table.4. Comparison of *Bombyx mori L.* biodiesel with ASTM standards.

Content	<i>Bombyx mori L.</i> Biodiesel	ASTM D 6751	Units
Density at 15 °C	0.87	0.82-0.9	g/cc
Kinematic viscosity (40 °C)	4.3	1.9-6.0	mm <sup>2</sup> /s
Cetane number	56	47 min	-
Flash point	160	>130 min.	°C

Cloud point	2	N/A	°C
Pour point	-3	N/A	°C
Total glycerin content	<0.05	0.24% max.	w/w
Water content	0.005	0.05% max.	v/v
Sulfated ash	0.002	0.02% max.	w/w
Carbon residue	0.01	0.05% max.	w/w
Acid value	0.4	0.8	mg KOH/g
Copper strip corrosion	1	No. 3 max.	-

#### 4. CONCLUSION

The lipids (oil) were extracted from *Bombyx mori L.* biomass and utilized for biodiesel production by two step processes. Acid catalysed esterification was performed to diminish the FFAs in *Bombyx mori L.* oil from 58.55% to 0.5% with optimized reaction parameters. After the two step process completion, the maximum conversion of biodiesel was achieved as 98.73% with optimum parameters conditions using H<sub>2</sub>SO<sub>4</sub> and NaOH used as an acid and base catalyst, respectively. The obtained biodiesel was confirmed by <sup>1</sup>H-NMR. The properties of the *Bombyx mori L.* biodiesel were in good agreement with ASTM standards. It has been recommended that *Bombyx mori L.* could be used as a potential resource for biodiesel production.

#### REFERENCE

- Alptekin E, Canakci M, Optimization of transesterification for methyl ester production from chicken fat. *Fuel*, 90, 2011, 2630-2638.
- AOCS (American Oil Chemical Society), Official methods and recommended practices of AOCS, 5th Ed., American Oil Chemical Society, Champaign, IL, USA, 1998.
- Di Serio M, Tesser R, Pengmei L, Santacesaria E, Heterogenous catalyst for biodiesel production, *Energy Fuels*, 22, 2008, 207-217.
- Ghadge SV, Raheman H, Biodiesel production from mahua (*Madhuca indica*) oil having high free fatty acids. *Biomass Bioenergy*, 28, 2005, 601-605.
- Ginting MSA, Azizan MT, Yusup S, Alkaline insitu ethanolsysis of *Jatropha curcas*, *Fuel* 93, 2012, 82-85.
- Hamidreza Jaliliannosrati, Nor Aishah Saidina Amin, Amin Talebian-Kiakalaieh, Iman Noshadi, Microwave assisted biodiesel production from *Jatropha curcas L.*, seed by two-step *in situ* process, Optimization using response surface methodology, *Bioresource Technology*, 136, 2013, 565-573.
- Jain S, Sharma MP, Prospects of biodiesel from *Jatropha* in India. *Renewable Sustainable Energy Rev*, 14, 2010, 763-771.
- Kasirajan Ramachandran, Pandian Sivakumar, Tamilarasan Suganya, Sahadevan Renganathan, Production of biodiesel from mixed waste vegetable oil using an aluminium hydrogen sulphate as a heterogeneous acid catalyst. *Bioresource Technol*, 102, 2011, 7289-7293.
- Manop Charoenchaitrakool and Juthagate Thienmethangkoon. Statistical optimization for biodiesel production from waste frying oil through two-step catalyzed process. *Fuel Processing Technology*, 92, 2011, 112-118.
- Phan AN, Phan TM, Biodiesel production from waste cooking oils, *Fuel* 87, 2008, 3490-3496.
- Pramanik K, Properties and use of *Jatropha curcas* oil and diesel fuel blends in compression ignition engine, *Renewable Energy*, 28, 2003, 239-248.
- Ramachandran K, Suganya T, Nagendra Gandhi N, Renganathan S, Recent developments for biodiesel production by ultrasonic assist transesterification using different heterogeneous catalyst, A review, *Renew. Sust. Energy. Rev.* 22, 2013, 410-418.
- Ramachandran Kasirajan, Sivakumar Pandian, Suganya Tamilarasan and Renganathan Sahadevan. Lipid extraction from natural plant source of *Adenantha pavonina* using mixed solvent by superheated extractor. *Korean J. Chem. Eng.* 31, 2014, 509-513.
- Ramadhas AS, Jayaraj S, Muraleedharan C, Biodiesel production from high FFA rubber seed oil, *Fuel* 84, 2005, 335-340.
- Refaat AA, Different techniques for the production of biodiesel from waste vegetable oil. *Int. J. Environ. Sci. Technol*, 7, 2010, 183-213.

Sen Yang, Qing Li, Yang Gao, Longyu Zheng, Ziduo Liu. Biodiesel production from swine manure via housefly larvae (*Musca domestica* L.), *Renewable Energy*, 66, 2014, 222-227.

Sharma YC, Singh B, An ideal feedstock, kusum (*Schleichera triguga*) for preparation of biodiesel, *Fuel* 89, 2010, 1470-1474.

Shuangshuang Gu, Jun Wang, Na Pang, Fangqin Eang, Cong Li and Fuan Wu, Biodiesel production from silkworm pupae oil using solid base catalyst. *Advanced materials research*, 634-638, 2013, 711-715.

Suganya T and Renganathan S, Optimization and kinetic studies on algal oil extraction from marine macroalgae *Ulva lactuca*, *Bioresour. Technol*, 107, 2012, 319-326.

Tiwari AK, Kumar A, Raheman H, Biodiesel production from *Jatropha* oil (*Jatropha curcas*) with high free fatty acids, an optimized process, *Biomass Bioenergy*, 31, 2007, 569-575.

Veljkovic VB, Lakicevic SH, Stamenkovic OS, Todorovic ZB, Lazic ML, Biodiesel production from tobacco (*Nicotiana tabacum* L.) seed oil with a high content of free fatty acids, *Fuel*, 85, 2006, 2671-2675.

Vinothkumar P, Nadanakumar V, Alagumurthi N, Performance and emission characteristics of DI Diesel engine run by fish oil biodiesel, *Advances in Natural and Applied Sciences*, 10(7), 2016, 342-348.

Xin Deng, Zhen Fang, Yun-hu Liu. Ultrasonic transesterification of *Jatropha curcas* L. oil to biodiesel by a two-step process. *Energy Conversion and Management* 51, 2010, 2802-2807.

Zeng J, Wang X, Zhao B, Sun J, Wang Y, Rapid in situ transesterification of sunflower oil. *Ind. Eng. Chem. Res*, 48, 2009, 850-856.

Zhang Y, Dube MA, McLean DD, Kates M, Biodiesel production from waste cooking oil, *Process design and technological assessment, Bioresource Technol*, 89, 2003, 1-16.