Research Journal of Chemical and Environmental Sciences
Res J. Chem. Environ. Sci. Vol 4 [4] August 2016: 40-45
Online ISSN 2321-1040
CODEN: RJCEA2 [USA]
©Academy for Environment and Life Sciences, INDIA
Website: www.aelsindia.com/rjces.htm

RJCES

ORIGINAL ARTICLE

Production of high quality biodiesel from desilked *muga* pupae (*Antheraea assamensis*)

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ABSTRACT
Muga silk is a fine quality silk obtained from the silkworm Antheraea assamensis endemic to Assam, a north eastern state of India. Muga silkworm rearing, reeling and weaving of muga silk play a vital role in the economy of rural areas of this state. The sericulture activities generate huge quantities of waste silkworm pupae after production of silk from the cocoons. The dumping of this huge waste poses a threat to the environment. The present study reports a method to utilize this waste by converting the lipid part of the waste pupae to biodiesel through transesterification. The physicochemical properties of the oil extracted from the waste muga pupae and those of the biodiesel prepared from the same has been reported. The composition of the biodiesel obtained has been determined by GCMS and other techniques. The results obtained clearly indicate that the waste muga silkworm pupae can serve as a good source of high quality biodiesel.
Keywords *Antherea assamensis . Silkworm pupae . Transesterification . Biodiesel . Muga silk . FAME*

Received 07.04.2016 Accepted 15.07.2016
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INTRODUCTION

Biodiesel, a non petroleum fuel consisting of methyl or ethyl esters of fatty acids obtained by transesterification of triglycerides, has been able to draw much attention as a potential substitute for diesel. It has a number of environmental benefits over petroleum diesel. It is biodegradable, non toxic, causes lesser emission of CO and SO_2 and is obtained from renewable sources. It has appropriate viscosity, flash point and cetane number which make it suitable for use in conventional diesel engines without any modification in the design. Biodiesel is mainly prepared by transesterification of vegetable oils such as sunflower oil, soyabean oil, coconut oil, or jatropha oil. The high price of vegetable oil sources is responsible for the fact that in spite of the advantages, the industrial application of biodiesel is still limited in most of the countries excepting a few. Suitable cheap and non edible sources of biodiesel are therefore being explored nowadays to increase the production and use of biodiesel in the developing countries. Though there are reports of new sources from the plant kingdom, animal sources are still underexplored.

Muga silk is known all over the world for its unique golden colour, durability and texture. It is the product of the silkworm *Antheraea assamensis* endemic to Assam a north eastern state of India. *Muga* silk [1, 2] occupies a prominent position in the cultural heritage of the Assamese people. *Muga* silkworm rearing, reeling and weaving of *muga* silk not only represent the tradition of the Assamese people but also play a vital role in the economy of rural areas of the state.

The muga sericulture activities are the main source of income in some districts of Assam where a large number of cocoons are produced. Pupa, which constitutes the major portion of the cocoon weight, is an inevitable byproduct generated in large quantity (75-85%) during the cocoon production. After the reeling is over, the inner pupae are thrown as waste, which putrefy and cause environmental pollution. It has been reported by many workers that these waste muga pupae have tremendous potential for use as poultry feed. The same study [3] also revealed that the dry waste *muga* pupae contain considerable amount of lipid (20 25%). In a recent study the authors reported conversion of the lipid fraction of waste *Attacus ricinii* pupae into biodiesel (4), The present study was therefore designed to convert the oil in the waste muga pupae to methyl ester and to evaluate its potential as biodiesel.

MATERIALS AND METHODS

Extraction of lipid fraction from waste and dried *muga* pupae

The waste pupae locally known as *letuwa* were collected from a muga silk farm of Assam in the North East India. These were dried under sunlight and then further dried in an oven at 100^0C for one hour. The lipid portion was extracted using Soxhlet apparatus with light petrol (boiling range 40 60^0C) as solvent. The temperature was kept around 50^0C. The solvent was removed using rotary vacuum evaporator at 45^0C to get the crude oil which was purified by column chromatography over silica (60-120 mesh) taking a mixture of ethyl acetate and petroleum ether in 1:20 ratio as eluent.

Study of the physicochemical properties of the *Muga* pupae oil and the methyl ester (FAME)

The physicochemical parameters of pupae oil were determined to evaluate its potential for use as a feedstock in biodiesel production. All the parameters iof the oil and the FAME involved, like iodine value, saponification value etc. were determined by the standard AOCS (American Oil Chemists Society) methods [5, 6,7].

Preparation of the immobilized enzyme: Lipase (*Pseudomonas cepasia*) powder (1 g) was dissolved in 10 mL of 20 mM sodium phosphate buffer (pH 7.0) and mixed with 2.0 g of celite and was immediately frozen to lyophilize for 48 hours.

Transesterification of *muga* pupae (*Antheraea assama*) oil

Production of biodiesel from waste muga pupae (*Antheraea assama)* oil was carried out using KOH as catalyst and also with immobilized lipase at milder reaction conditions. Though the alkali catalysed method [8] is cheaper, it does not work well and leads to formation of soaps particularly when there are free fatty acids in the oil. Formation of soap lowers the yield as it makes separation of biodiesel difficult. Enzymatic transesterification [9, 10,11] of oil is therefore carried out with free or immobilized enzymes to obtain good yield. The transesterification of oil was also carried out using Lipase as catalyst and the two methods were compared and reported.

Transesterification using alkali catalyst

The reaction was carried out in a round bottom flask with methanol and oil in 6:1 ratio using KOH as catalyst [8] .The reaction was run approximately at 60^0C and completion of the reaction was monitored by TLC**.** By the end of the experiment the reaction mixture was transferred into a separating funnel, allowing glycerol to separate by gravity separation. The product mixture washed with spraying hot (50^0C) distilled water and crude FAME was partitioned between water and petroleum ether. This process of addition and collection of petroleum ether were repeated for at least three times .Upper layer of light petrol containing crude biodiesel collected in a special container before evaporating the solvent and dried over anhydrous Na_2SO_4. The solvent recovered under vacuum and the product was purified [12, 13] by column chromatography over silica gel (60 – 120 mesh) using a mixture of petroleum ether and ethyl acetate (25: 1) as the eluent.

Transesterification of muga pupae *(Antheraea assama)* oil using lipase as catalyst:

Muga pupae oil and methanol were taken in the ratio 1:4 (mol mol^{-1}) in a screw capped vial. To this mixture, 50 mg of enzyme preparation was added and incubated at 40^0 C with constant shaking at 200 rpm. The reaction was allowed to continue for 12 hours.

Analysis of the biodiesel

The ^{1}H and ^{13}C NMR spectra were recorded $CDCl_3$ at 300 and 75 MHz respectively using Bruker Avance III 300 MHz/54 mm NMR spectrometer. IR spectrum was recorded with a Perkin Elmer RX I FT-IR spectrometer as a thin film on KBr plate. Fatty acid composition of the FAME obtained from muga pupae oil (*Antheraea assama**)*** was analyzed by using Perkin Elmer Clarus 600 GC-MS. The individual peaks of the gas chromatogram (Fig 6) were analyzed and fatty acids were identified using MS database. Relative percentage of fatty acid esters was calculated from total ion chromatography by computerized integrator.

RESULTS AND DISCUSSION

The physicochemical properties of the oil extracted from waste desilked muga pupae are listed in Table 1. The yield of the biodiesel was found to vary with the amount of KOH used in the alkali transesterification process (Table 2). The variation in the yield of biodiesel formed after three hours of transesterification is graphically represented in (Fig 1). Interestingly, the yield shows a decreasing trend with excess of catalyst used.

Transesterification of muga pupae oil with lipase was found to be slower and hence the yield was determined after twelve hours of the reaction. Variation in the yield of biodiesel with the amount of the enzyme catalyst is shown in Table 3 and in Fig 2. Maximum yield of the biodiesel was observed with 30% (w/w) enzyme preparation.

A number of technical standards for biodiesel fuel have been set in order to maintain its quality. This includes the European standard EN 14214. The biodiesel fuel technical standards make sure that the

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products conform to the international standards for biodiesel fuel. The physicochemical parameters such as flash point , cetane number, etc are listed in Table 4 alongwith the desired values as per EN 14214 standard which point out the fact that the methyl ester fulfils the requirements of biodiesel standard. The results indicate that the FAME obtained by transesterification meets the requirements for a good quality biodiesel.

Spectral characterization of the biodiesel ^{1}H NMR($CDCl_3$, 300 MHz): The ^{1}H NMR($CDCl_3$,300MHz) spectra of the FAME (Fig 3) displayed the following signals- δ5.32 5.34(m) for olefinic protons; 3.64 (s) for methoxy protons; 2.73 2.77 (t) bis-allylic protons; 2.26 2.31 (t) α-methylene to ester; 2.00-2.04(m) α-methylene to double bond; 1.58-1.62(m) β-methylene to ester; 1.23 - 1.28 (m) backbone methylenes; 0.86 -0.88 (m) terminal methyl protons.

^{13}C NMR of *Antheraea assama* FAME: The ^{13}C NMR spectra of *Antheraea assama* FAME (Fig 4) displayed singlet (**a)** for carbonyl carbon, doublet (**b**) for olefinic carbons, singlet (**c**) due to methoxy carbon and the multiplet (**d**) indicating methylene and methyl carbons.

IR of *Antheraea assama* FAME**:** Infra-red spectrum (Fig 5) shows the characteristic absorption at 738.8 due to ν = C – H def; the absorption at 1172.39 due to ν C – O str ; 1654 .07 for ν C = C str of unsaturated fatty acids; 1741.65 due to ν C = O str of ester and the band at 2927.96 indicates ν C – H str of – CH_2 – .

The FAME composition (shown in Table 5) as calculated from total ion chromatography reveals that it is composed of 38 % saturated and 62% unsaturated fatty acid which gives it excellent flow properties. It has a high percentage of methyl oleate which is highly desirable for good quality biodiesel [14, 15].

Table 1 Physicochemical properties of waste *muga pupae* oil

Properties	Muga pupae oil
Avg. oil content	22.5 %
pH	6.1
Sp. gravity (35^0C)	0 .966
Moisture content (mg/g)	0.21
Viscosity (35^0C) $mm^2 s^{-1}$	38.3
Acid value (KOH, mg/g)	2.58
Saponification value (mg /g)	187
Iodine Value (mg/g)	112

Table 2 Variation of biodiesel yield obtained from muga pupae oil with varied amount of catalyst (KOH)

Amount of catalyst (KOH) %w/w) of oil	0.5	0.75	1	1.5	1.75	2
Biodiesel yield % (w/w)	68	76.6	81	90	84	80

Table 3 Biodiesel yield from muga pupae oil using varied amount of lipase

Amount of lipase used (% w/w oil)	0.51	1.1	5	10	15	20
Biodiesel yield %	43.7	49	55	66	82	81

Table 4 Physicochemical properties of methyl ester of *muga pupae* oil (MPME)

Properties studied	Results	EN 14214 limits
Biodiesel yield %	88.9	----
Sp. gravity (35^0C)	0.850	0.860-0.900
Kinetic viscosity (35^0C) $mm^2 s^{-1}$ or (cSt)	5.82	3.5-5.0
pH	7.1	6.8-7.2
Acid value (KOH, mg/gm)	0.38	0.5 max
Saponification value	183	-----
Iodine Value (mg/g)	112	120 max
Cetane Number	50.9	51 min
Flash point (^0C)	158	120 min

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Table 5 Composition of muga pupae (*Antheraea assama*) FAME

Retention time (min)	FAME	Ratio of carbon to double bond	%Wt
18.16	Methyl palmitate	(16:0)	20.17
22.09	Methyl linoleate	(18:2)	14.08
22.31	Methyl oleate	(18:1)	47.80
22.88	Methyl stearate	(18:0)	17.95

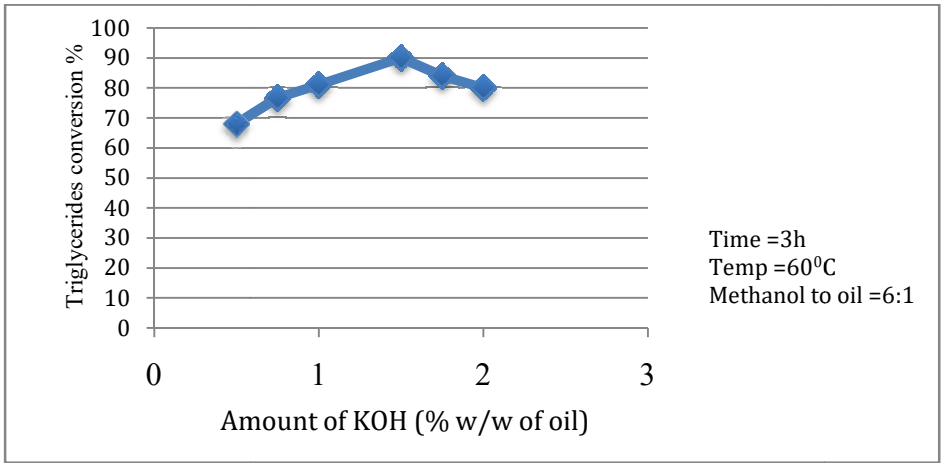

Fig. 1 Percentage yield of Biodiesel with different amounts of catalyst (KOH) used.

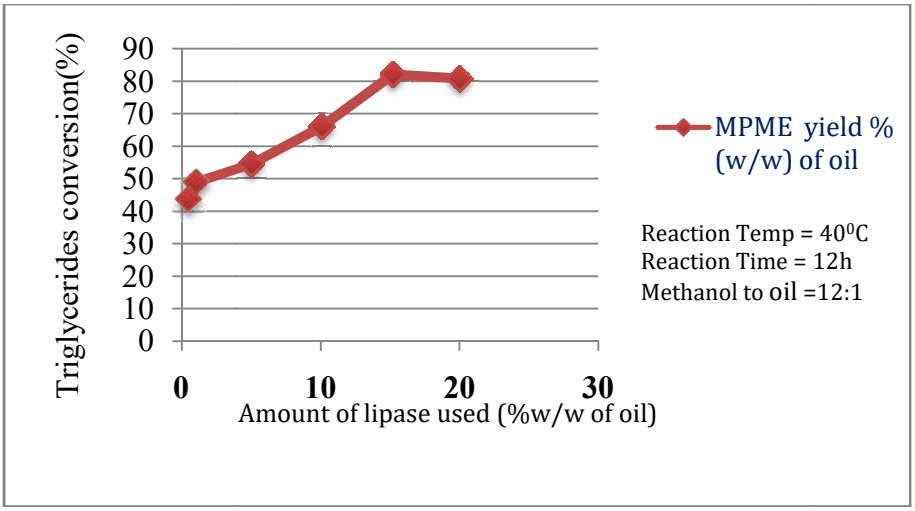

Fig. 2 Percentage yield of biodiesel obtained from muga pupae oil using varied amounts of lipase.

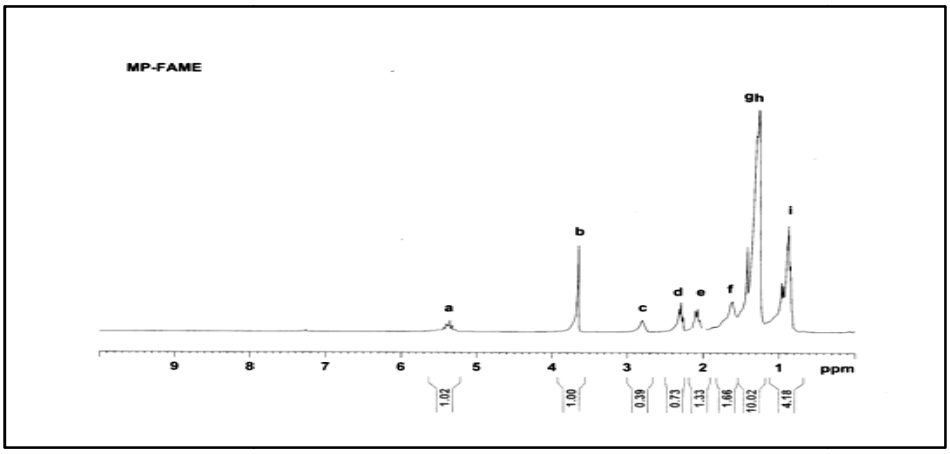

Fig. 3 ^{1}H NMR spectra ($CDCl_3$, 300MHz) of FAME (MPME)

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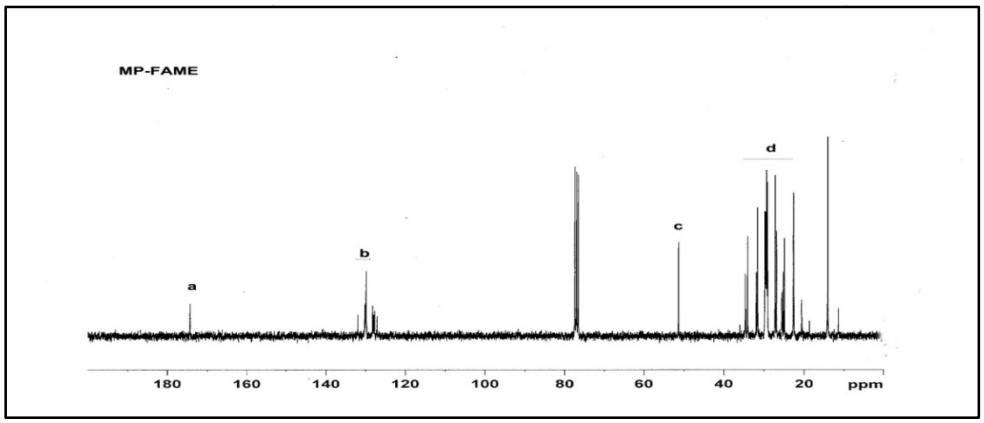

Fig. 4 ^{13}C NMR spectra of *Antheraea assama* FAME

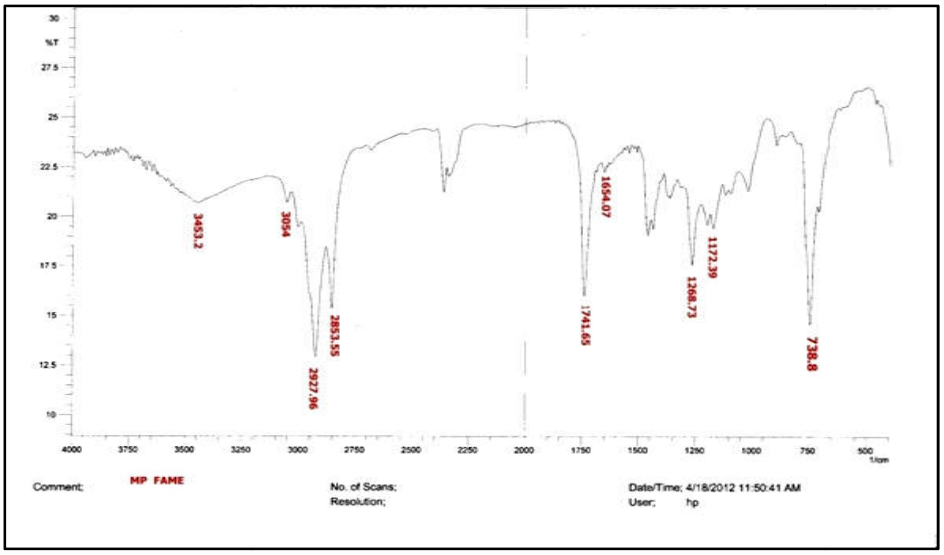

Fig. 5 IR (KBr) spectra of *Antheraea assama* FAME.

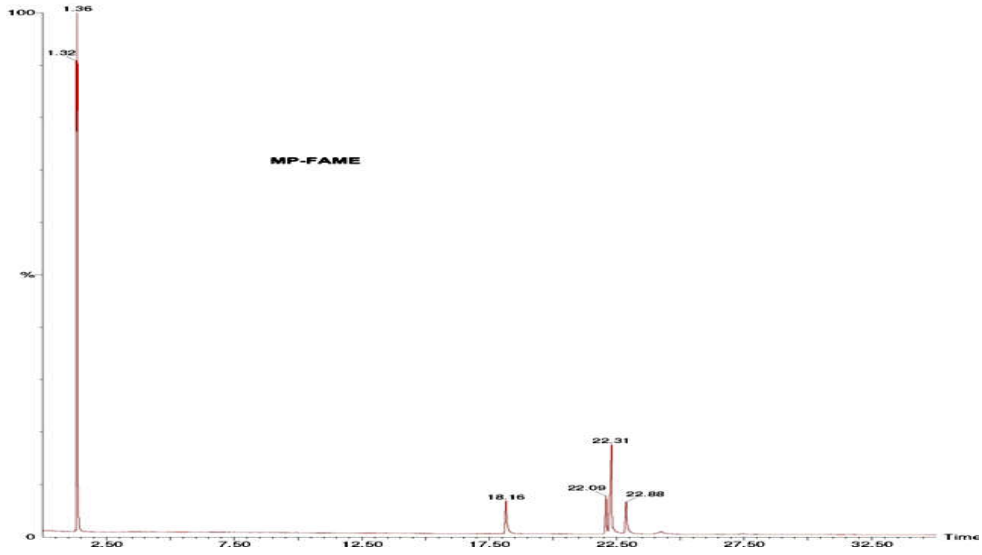

Fig. 6 GC MS of *Antheraea assama* FAME.

CONCLUSIONS

The waste muga pupae, an important by product of the muga silk industries of Assam, can be an excellent raw material for biodiesel production. Both the transesterification methods attempted were found to be successful in producing good yield of excellent quality biodiesel. The oil obtained from waste muga pupae

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contains unsaturated fatty acids as evident from the GCMS studies of the FAME. The FAME obtained from the pupa oil meet the requirements of biodiesel. The parameters such as iodine value, specific gravity, pH and flash point of the FAME are well within the limits. Though the kinematic viscosity is slightly higher, it can be improved by blending the FAME with other fuels. Thus the present work suggests a useful utilization of the waste materials of silk industries by converting them to a high value biodiesel.

ACKNOWLEDGEMENT

One of the authors (M.Sarma) is thankful to UGC for financial support to carry out the work.

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M Sarma, M Ganguly. Production of high quality biodiesel from desilked *muga* pupae (*Antheraea assamensis*). Res. J. Chem. Env. Sci. Vol 4 [4] August 2016. 40-45