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Resource-Efficient Technologies 2 (2016) 126–132
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Ultrasonication: An effective pre-treatment method for extracting lipid from *Salvinia molesta* for biodiesel production

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Received 10 March 2016; received in revised form 26 July 2016; accepted 28 July 2016
Available online 25 August 2016

Abstract

Biodiesel is considered as one of the promising alternative fuels for diesel engines due its renewability and environment friendly nature. As the process of lipid extraction from the biomass consumes about 90% of the total energy spent for biodiesel production, an efficient and economic method is very important. The amount of lipid extracted from the biomass could be increased if it is pre-treated before the extraction process. This work was an attempt to compare the various pre-treatment methods before extracting lipids from dried *Salvinia molesta* (aquatic weed), such as autoclaving, microwaving, ultrasonication, sand, and glass grinding. After each pre-treatment method, Bligh and Dyer's method was used to measure the total lipid content in percentage dry weight (% dwt), which was then compared with the untreated *S. molesta*. It was found experimentally that the lipid yield was 19.97% dwt for ultrasonication > 16.60% dwt for microwaving > 16.46% dwt for glass grinding >16.26% dwt for sand grindin, > 15.72% dwt for autoclaving > 15.36% dwt for untreated. The one-way ANOVA with Tukey's test was then used to validate the experimental results and showed that ultrasonication method of pre-treatment was the most efficient and had resulted in the highest lipid yield among all the methods used which was followed by the microwaving method. The Taguchi method with L9 orthogonal array was then used for the optimization of ultrasonic assisted pre-treatment method before extracting lipid from *S. molesta* and showed a maximum lipid of 20.86% using 100% amplitude and sonication time of 15 min. The fatty acid methyl ester (FAME) of *S. molesta* lipid was analyzed using gas chromatography mass spectroscopy (GCMS) with flame ionization detector. It showed fatty acids such as C14:0, C14:1, C16:0, C16:1, C18:0, C18:1, C20:1, C20:4, C22:0 which contributed 97.38% weight of the total fatty acids. FAME consisted of 63.59% monounsaturated, 33.18% saturated and 0.73% polyunsaturated fatty acids in % weight. The physical properties such as specific gravity, kinematic viscosity, cetane number, flash point, cloud point, pour point, saponification value and iodine value of *S. molesta* biodiesel, which were estimated based on fatty acid profiles are comparable with ASTM 6751-08 biodiesel standard.

Keywords: Biodiesel; *Salvinia molesta*; Autoclaving; Microwave; Ultrasonication; Bligh and Dyer method; Taguchi method

1. Introduction

The escalating prices with environmental pollution concerns of using fossil fuels has explored the interest in biodiesel as one of the promising fuels for diesel engines. The reasons for considering biodiesel as a promising alternative fuel are properties like renewability, high biodegradability and non-toxicity [1]. Biodiesel can be produced from edible, non-edible vegetable oils, macro and microalgae and aquatic weeds. The increased demand for edible crops as a food source with the finite availability of arable land makes the use of edible crops such as corn, sugar cane and rapeseed and non-edible crops such as jatropha, miscanthus and switch grass for biodiesel production unsustainable [2,3]. To avoid foresaid problems associated with edible and non-edible crops, macro and microalgae and aquatic weeds are tried for the biodiesel production. Also, the excessive growth of aquatic weeds restricts fishing, swimming and makes water unsuitable for drinking [4] and is a nuisance to the environment. The increased productivity and lipid yield of aquatic weed, *Salvinia molesta*, make them a good choice for biodiesel production and help to clean the clogged water bodies [5].

The processes such as cultivation, harvesting, biomass processing, extraction of lipid involved in the production of biodiesel from microalgae and aquatic weed are similar in nature.

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http://dx.doi.org/10.1016/j.reffit.2016.07.005

Among these processes, the lipid extraction consumes about 90% of the process energy for biodiesel production [3]. The extraction of lipids at a reduced cost and its eco-friendly nature are the most important advantages for the commercial production of biodiesel from aquatic weed [6]. The aquatic weed cells normally possess robust cell wall which prevents the release of intracellular products and thus breaking them can be energy intensive [7]. Hence, it becomes necessary to disrupt these cell walls using pre-treatment methods, for liberating the intracellular products for extracting lipid [8].

The disruption or pre-treatment methods can be categorized as mechanical and non-mechanical. The mechanical methods are further categorized as liquid and solid shear methods while non-mechanical methods as lysis and desiccation [9]. The liquid shear methods are subdivided into ultrasonication, microwaving, high pressure homogenization while solid shear methods are divided as bead mill, sand grinding, glass grinding and freeze press. The non-mechanical methods of cell disruption can be done using enzymes, acids, alkalis and autoclaving [9]. The pre-treatment methods such as ultrasonication, bead beating, microwaving, osmotic shock, autoclaving, manual grinding with liquid nitrogen, electro floatation by alternating current, laser treatment are already reported in literature for different biomasses like mixture of microalgae, *Nanochloropsis oculata*, *Botryococcus braunii* UTEX 572, *Chlorella vulgaris* [7,10–12]. According to Suganya et al. [13], ultrasonication requires minimum time and solvents and results in improved release of lipids from marine macroalgae, *Ulva lactuca*. This was followed by microwaving method which also results in higher lipid yield in reduced time as compared to other methods [14]. To find an effective method of pre-treatment at optimized conditions is important for efficient and economic method of lipid extraction from biomass for biodiesel production. The classical method of optimization uses one variable at a time, keeping other variables constant, and is time consuming [15]. Hence, Taguchi method could be used which is a simple, efficient, and systematic approach to optimize the design of experiments and reduce the number of experiments [16]. This method uses fractional factorial matrix named as orthogonal array (OA) for the experimental design. The signal to noise (S/N) ratio proposed by Taguchi was useful for finding the favorable levels of the control factors named as input parameters. Depending on the nature of experiments, S/N analysis can be either, lower-the-better, nominal-the-better or larger-the-better [17].

Fatty acid methyl ester or biodiesel of an oil can be produced using transesterification reaction as follows: one mole of oil reacts with three moles of alcohol in the presence of catalyst such as acid, base, or enzyme to form one mole of glycerol and three moles of fatty acid esters [18]. The fatty acid composition of biodiesel can then be measured using GCMS with flame ionization detector. A few studies have been reported based on the prediction of biodiesel properties such as specific gravity, kinematic viscosity, cetane number, calorific value, flash point, cloud point, pour point, saponification value and iodine value from its fatty acid composition using empirical formulae [19,20].

Since *S. molesta* is a novel feedstock with rigid cell wall, extraction of lipid requires to use an efficient pretreatment method. This study explores the effectiveness of various pre-treatment methods such as ultrasonication, microwaving, autoclaving, sand grinding and glass grinding were analyzed experimentally and compared with the untreated *S. molesta*. Taguchi method was used for optimization of most efficient pretreatment followed by FAME analysis using GCMS. Then, the properties of biodiesel produced from *S. molesta* were estimated using its FAME profiles with empirical formulae.

2. Methodology and materials

2.1. Collection and stock preparation

About 4 kg of wet *S. molesta* was collected from a nearby inland water body in Calicut, Kerala, India during the monsoon season. The collected *S. molesta* was washed thoroughly in water with care to remove mud and all other impurities, sundried for 3 weeks and then powdered to finer particle size of less than 1 mm using a mechanical pulverizer.

2.2. Pre-treatment methods used before extracting lipid from S. molesta

Pre-treatment methods like ultrasonication, microwaving, sand grinding, glass grinding and autoclaving were done as shown in Fig. 1. Each method of cell disruption was done using 0.5 g dwt of *S. molesta* in triplicates followed by Bligh and Dyer method [21] for extracting total lipid content in % dwt.

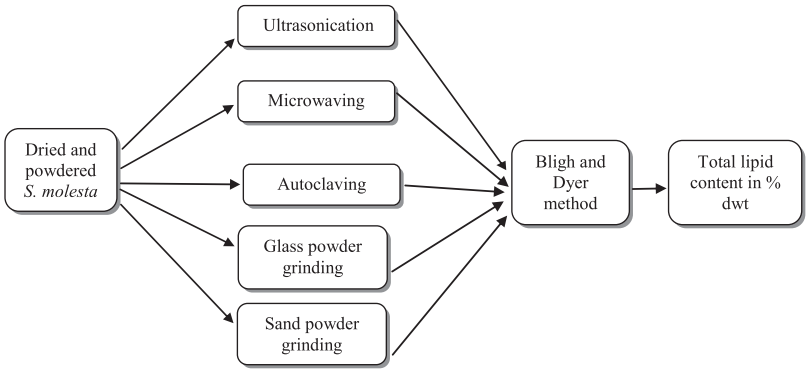

Fig. 1. Flowchart for different pre-treatment methods used for extracting lipid from *S. molest.*

Specifically, (1) ultrasonication: 0.5 g dwt of *S. molesta* was mixed with 1 ml methanol and 0.5 ml chloroform in a glass beaker and sonicated at 20 kHz for 5 min in ice bath using Sonics Vibra-Cell ultrasonic liquid processor Model VC-750, USA; (2) microwaving: 0.5 g dwt of *S. molesta* was mixed with 1 ml methanol and 0.5 ml chloroform in a glass centrifuge tube and microwaved at 500 W with a frequency of 2450 MHz for 5 min; (3) autoclaving: 0.5 g dwt of *S. molesta* was added to 1 ml distilled water in a glass centrifuge tube and autoclaved at 121 °C and 15 kPa for 15 min; (4) sand grinding: 0.5 g dwt of *S. molesta* was manually ground by adding 0.1 g of neutralized sand in mortar and pestle for 5 min; (5) glass powder grinding: 0.5 g dwt of *S. molesta* was manually ground in mortar and pestle by adding 0.1 g of glass powder for 5 min.

2.3. Extraction and estimation of lipid

Bligh and Dyer's method [21] was then used for extracting lipid from each sample of pre-treated *S. molesta*. A mixture of 1 ml methanol and 0.5 ml chloroform was added to the samples of *S. molesta* pre-treated with autoclaving, sand grinding and glass grinding. The samples pre-treated with ultrasonication and microwaving were used directly because they were already pre-treated with methanol and chloroform mixture. After keeping all the pre-treated samples in methanol and chloroform mixture at room temperature for 18 h, each mixture was then vortexed for 2 min. Half a milliliter of chloroform was again added and mixed vigorously for 1 more min. After that, 0.5 ml of distilled water was added and vortexed again for 2 min. The layers were separated by centrifugation for 10 min at 2000 rpm. The lower layer was separated and the procedure was repeated with the pellet. The two supernatants collected were allowed to settle for 2 h and the lower organic layer with the lipids was transferred to a clean pre-weighed vial (W_1) and allowed to evaporate in hot air oven at 80 °C for 50 min. The weight of the vial was again recorded (W_2) and the lipid content expressed as % dry weight (% dwt) was calculated by taking the ratio of final to the initial weight of *S. molesta* used for each pre-treatment [22].

2.4. Statistical analysis for finding effective pre-treatment method

The lipid content extracted from untreated and five pre-treated *S. molesta* samples were compared using one-way ANOVA followed by Tukey's test. The level of significant difference was checked at p-value <0.01. Tukey's test was used to validate significant pre-treatment method by comparing critical mean difference (CMD) which is the minimum of difference between any two group means and it is calculated using Eq. (1) [23] with difference in means of each pre-treatment method.

$$\mathrm{CMD} = Q_{(k,df,MSE)}\sqrt{\frac{MSE}{2}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)} \quad (1)$$

where Q is the Studentized range static obtained from standard table corresponding to the degree of freedom value and number of groups obtained from one way ANOVA table; MSE is the mean square error value within groups; n_1 and n_2 are sample sizes of individual pre-treatment method. Since all the pre-treatment was performed in triplicate, therefore sample size is 3.

2.5. Taguchi design of experiments and statistical analysis

Taguchi design of experiments with L9 orthogonal array was used for the optimization of the ultrasonication method of pre-treatment for extracting lipid from *S. molesta*. Two factors involved in the ultrasonication process such as amplitude (%) and time (min) were used in three levels *i.e.*, 50, 75, 100 and 5, 10 and 15, respectively. Totally, 9 experiments were performed in triplicates with 9 combinations of these factors in Taguchi method using Minitab 14 software. 0.5 g dwt of *S. molesta* was used for each of these experiments and the total lipid content was measured using Bligh and Dyer's method in % dwt.

The experiments were performed in triplicates and S/N (signal to noise) ratio was calculated using Eq. (2).

$$\frac{S}{N} = -10\log\left(\frac{1}{n}\right)\sum_{i=1}^{n}\frac{1}{y_i^2} \quad (2)$$

where y is the response factor and n is the number of observations. The S/N ratio for "larger-the-better" case was selected to maximize the lipid yield of *S. molesta* in % dwt. The design of experiments based on orthogonal array was applied and the highest S/N ratio gave the optimum values of amplitude and time of ultrasonication for lipid yield in % dwt.

2.6. Fatty acid methyl ester (FAME) analysis of S. molesta lipid

The fatty acid composition of *S. molesta* lipid, extracted after ultrasonically pre-treated using 100% amplitude for 15 min was analyzed by gas chromatography mass spectroscopy (GC–MS) using JEOL GCMATE II GC–MS. The acid value of *S. molesta* lipid was 34 with a free fatty acid value of 17 which is greater than 2.5%. Thus, FAME of *S. molesta* lipid was prepared using acid esterification followed by base catalyzed transesterification reactions. The esterification was done by treating 5 ml of *S. molesta* lipid with 1 ml methanol containing 0.5% sulphuric acid with stirring at 50 °C for 45 min and kept overnight at room temperature for the separation of methanol layer. The bottom layer containing esterified *S. molesta* lipid was then used for transesterification in which 0.5 ml of methanolic NaOH was added and kept overnight at room temperature. The mixture was placed inside an ultrasonicator bath of 30 kHz frequency for 25 min and centrifuged at 3000 rpm for 10 min for separating the top layer of FAME. The excess methanol and impurities are removed after washing with 10% by volume of hot distilled water. The components of FAME were identified by comparing the retention times of eluted fatty acids which showed different peaks, obtained from GC–MS analysis with the standard retention time of individual fatty acids. The percentage weight of each fatty acid was then estimated by using the ratio of the peak area of individual fatty acid to the total area of all peaks.

2.7. Estimation of properties of S. molesta biodiesel based on FAME profiles

The quality of biodiesel produced from *S. molesta* lipid was estimated through the following properties such as specific gravity (SG), kinematic viscosity (KV), cetane number (CN), flash point (T_f), cloud point (CP), pour point (PP), saponification value (SV), iodine value (IV), calorific value (CV), degree of unsaturation (DU) based on the following empirical equations [20,24]:

$$SG = 0.8463 + \left(\frac{4.9}{MW_i}\right) + 0.0118\ N_D \quad (3)$$

$$KV = 0.235\ W_C - 0.468\ W_{db} \quad (4)$$

$$CN = 3.930\ W_C - 15.936\ W_{db} \quad (5)$$

$$T_f = 23.362\ W_C + 4.854\ W_{db} \quad (6)$$

$$CP = 18.134\ W_C - 0.790\ W_{US} \quad (7)$$

$$PP = 18.880\ W_C - W_{US} \quad (8)$$

$$SV = \frac{\sum(560 P_{FA})}{MW_i} \quad (9)$$

$$IV = \frac{\sum(254 P_{FA} N_D)}{MW_i} \quad (10)$$

$$CV = 46.19 - \left(\frac{1794}{MW_i}\right) - 0.21\ N_D \quad (11)$$

$$DU = W_{MUFA} + (2W_{PUFA}) \quad (12)$$

where W_c, W_{db}, N_D, W_{MUFA}, W_{PUFA}, MW_i, W_{US}, P_{FA} are the weighted average number of carbon atoms in the fatty acids, weighted average number of double bonds, number of double bonds, monounsaturated fatty acids in weight %, polyunsaturated fatty acids in weight % and the molecular weight of the ith FAME component, unsaturated fatty acids in weight %, % weight of each fatty acid, respectively. The % weight of each fatty acid obtained from FAME analysis of *S. molesta* lipid was used for the estimation of properties. The molecular weight of each fatty acid and number of double bonds were obtained from its chemical structure.

3. Results and discussion

Various pre-treatment methods before the extraction of lipid from *S. molesta* using one way ANOVA followed by Tukey's test showed that the ultrasonication method was the most efficient method. The optimization of ultrasonication method was then performed using Taguchi method with L9 orthogonal array. FAME analysis was done using GC–MS from the lipid extracted after ultrasonication was done and its properties are estimated using empirical formulae.

3.1. Comparison of pre-treatment methods

The extent of cell disruption was indicated by the amount of lipid content in % dwt of *S. molesta*, *i.e.*, higher amount of lipid released indicates higher degree of cell disruption. In this study, a comparative analysis of the lipid extracted from *S. molesta* using different pre-treatment methods and from the untreated *S. molesta* was done as shown in Fig. 2. It was found that degree of lipid extracted from *S. molesta* was 19.97% dwt for ultrasonication > 16.79% dwt for microwaving > 16.60% dwt for glass grinding > 16.36% dwt for sand grinding > 15.82% dwt for autoclaving > 15.36% dwt for untreated. The % hike in lipid extracted was 29.9, 9.20, 2.90, 7.13 and 6.22 for ultrasonication, microwaving, autoclaving, glass grinding and sand grinding, respectively as compared to untreated *S.*

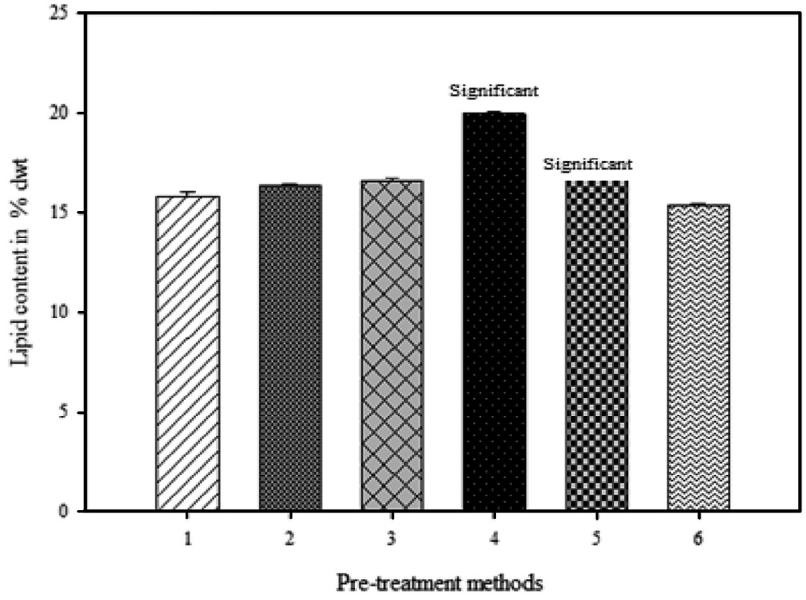

Fig. 2. Lipid extracted (% dwt) from *S. molesta* after various pre-treatment methods. 1. Autoclaving; 2. Sand grinding; 3. Glass grinding; 4. Ultrasonication; 5. Microwaving; 6. Untreated *S. molesta.*

molesta. Hence, ultrasonication was found to be the most efficient pre-treatment method and could be used for large scale extraction of lipid from *S. molesta*.

Only a few studies are reported in the literature on the various pre-treatment methods of different species of microalgae such as *Chlorella sp.*, *C. vulgaris* [24,25]. Prabakaran and Ravindran [24] used pre-treatment methods such as ultrasonication, microwaving, autoclaving for extracting lipid from *Chlorella sp* and achieved a lipid yield of 40, 38 and 24% dwt, respectively. As *S. molesta* (fresh water weed) is totally an unexplored source of biomass, this study is the first attempt to use various pre-treatment methods before extracting lipid from *S. molesta* and therefore, could not be compared with any previous work.

3.2. Statistical analysis for finding effective pretreatment method

As shown in one-way ANOVA Table S1 (Supplementary material), p-value of 2.856×10^{-7} which is less than 0.01 and the higher F-value of 43.339 illustrate that each pre-treatment method was significant for improving the lipid yield of *S. molesta*. The mean square error and degree of freedom values from the ANOVA table for within groups were used for Tukey's test for identifying the most significant pre-treatment method with increased lipid yield.

The CMD value of 0.0119 was calculated using Eq. (2) and compared with the difference in means of all the pre-treatment methods as shown in Table S2 (Supplementary material). The difference between the means of lipid extracted after ultrasonication and those of other pre-treatment methods showed greater values than the calculated CMD value. This indicates that ultrasonication method of pre-treatment has significant difference between other pretreatment methods for extracting lipid from *S. molesta*. The difference in mean values of microwaving and untreated *S. molesta* was greater than the CMD value, whereas other pre-treatment methods showed lesser value of mean. Hence, microwaving can be suggested as the second most effective method after ultrasonication.

3.3. Taguchi method of optimization

The results of 9 experiments which were performed with 9 combinations of amplitude and time in triplicates for Taguchi method using Minitab 14 software are shown in Table 1, where 19.34, 20.2 and 20.86 of lipid was extracted in % dwt using ultrasonically pre-treated *S. molesta* with 100% amplitude and sonication time of 5, 10 and 15 min, respectively. It was quite evident from these results that 100% amplitude of ultrasonication wave showed improved values of lipid extracted (%dwt) with increase in time used for sonication. This could be due to the higher energy waves created at 100% amplitude generating a series of microbubble cavitations on the surface of *S. molesta* cells which imparts kinetic energy causing its disruption.

Table 1
L9 array of Taguchi experimental design with measured lipid extracted from *S. molesta.*

Exp no	Time (min)	Amplitude (%)	Lipid extracted % dwt
1	5	50	16.38
2	5	75	17.98
3	**5**	**100**	**19.34**
4	10	50	18.05
5	10	75	18.79
6	**10**	**100**	**20.20**
7	15	50	18.53
8	15	75	19.02
9	**15**	**100**	**20.86**

Bolded values are best three values of lipid extracted % dwt.

As shown in Table S3 (Supplementary material), the ANOVA of S/N ratio supports the significance of amplitude of ultrasonic wave used for pre-treating *S. molesta* with ultrasonication. The higher F value of 11.67 with a lower p-value of 0.021 ($p < 0.05$) indicates that the amplitudes of ultrasonic waves are more significant than the sonication time for improving the lipid extraction from *S. molesta*. Also the % contribution of 75.78 for amplitude additionally supports its significance. The correlation coefficient, R^2 value should be at least 80% for statistical validity [26] and in the present study, a value of 88.5% supports the statistical validity of the experiments conducted.

The results of analysis of variance of S/N ratios are shown in Fig. 3, where also the lipid extracted was found to increase normally with the time (min) and increase steeply with the amplitude of ultrasonication waves. At higher amplitudes and time, the intense sonication of *S. molesta* containing methanol and chloroform generates sound waves that propagate and forms high and low pressure cycles. The small vacuum bubbles created during low-pressure cycle collapse violently during high-pressure cycle causing a phenomenon called cavitation. The high-pressure liquid jets created break the cell walls of *S. molesta* and release lipid. Hence, sonicating at higher amplitudes and time results in the improved release of lipid.

3.4. FAME analysis of S. molesta lipid

As shown in Table 2, the fatty acid profile of *S. molesta* biodiesel consists of: C14:0, C14:1, C16:0, C16:1, C18:1, C18:0, C20:1, C20:4, C22:0 with a total content of 97.38% weight fatty acids. It can be seen that 63.49% weight contains monounsaturated fatty acids (with one double bond) followed by 33.16% weight of saturated fatty acids (with no double bond) and only 0.73% contains polyunsaturated fatty acids (with more than two double bond). Also as shown in Table 2, the fatty acid profiles reported by Rozentsvet et al. [27] for leaves and sorus of *S. natans* that consists of C14:0, C14:1, C16:0, C16:1, C18:1, C18:0, C20:1, C20:4, C22:0 are comparable with those of *S. molesta*. In another study, Brouwer et al. [28] reported the fatty acids profiles of *Azolla filiculoides* showed dominations of C16:0, C18:2 and C18:3 whereas in *S. molesta* with C16:0, C16:1 and C18:1. It is clear that domination of C16:0 is common for both biodiesels and C18:2 and C18:3 present in *Azolla filiculoides*, more amount of polyunsaturated fatty acids (with two or more double bonds). The more amount of polyunsaturated fatty acids in biodiesel reduces oxidation stability [29] indicates that *S. molesta* biodiesel has good oxidation stability.

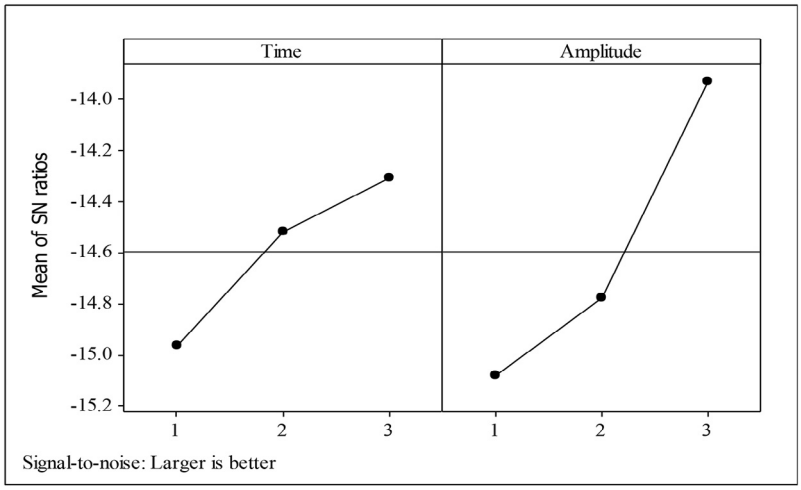

Fig. 3. Main effect plots for lipid extracted from *S. molesta* (% dwt) with time and amplitude.

Table 2
Comparison of fatty acid profiles of *S. molesta* and *S. natans* biodiesel [27].

Compound	Fatty acid	*S .molesta** weight %	*S. natans* [27] weight %		Type of fatty acid
			Leaves	Sorus	
Methyl myristate	C14:0	4.08	2.84	3.66	Saturated
Methyl myristoleate	C14:1	1.54	1.25	0.42	Monounsaturated
Methyl palmitate	C16:0	25.96	6.9	11.14	Saturated
Methyl palmitoleate	C16:1	30.29	6.78	1.46	Monounsaturated
Methyl oleate	C18:1	29.88	5.5	15.9	Monounsaturated
Methyl stearate	C18:0	1.49	1.33	2.02	Saturated
Methyl eicosenoate	C20:1	1.78	0.17	0.55	Monounsaturated
Methyl arachidonate	C20:4	0.73	3.64	–	Polyunsaturated
Methyl behenate	C22:0	1.63	0.29	4.66	Saturated

* % weight of total fatty acids: 97.38%.

The quality of *S. molesta* biodiesel produced from ultrasonically pre-treated *S. molesta* was checked by comparing its estimated physical properties with ASTM 6751-08 biodiesel standard. As shown in Table 3, the properties of biodiesel produced from *S. molesta* are comparable with ASTM 6751-08 biodiesel standard. The specific gravity of *S. molesta* biodiesel was 0.875 which is well within the range of ASTM biodiesel standard. The kinematic viscosity of *S. molesta* biodiesel was found to be 3.657 mm^2/s, which is the range of 1.9–6 mm^2/s. Higher viscosity of biodiesel creates the problem of poor flow properties and atomization inside the combustion chamber. The cetane number of *S. molesta* biodiesel was found to be 55, which is above 47 as per ASTM 6751-08 standard as it influences the good cold start behavior and smooth running of the engine. The calorific value of *S. molesta* biodiesel was 39.73 MJ/kg which is close to all other biodiesels [30]. The degree of unsaturation (DU) is directly proportional to the iodine value of *S. molesta* biodiesel which is the measure of total unsaturation within a mixture of fatty acid.

Table 3
Properties of biodiesel produced from *S. molesta* lipid obtained from ultrasonication pre-treatment.

Biodiesel properties	Units	*S. molesta* biodiesel	ASTM D6751-08 biodiesel standard
Specific gravity		0.875	0.890
Kinematic viscosity	$mm^2\ s^{-1}$	3.657	1.9–6
Cetane number		55	47 min
Degree of unsaturation	%	64.95	ND
Calorific value	MJ/kg	39.73	ND
Flash point	°C	139	93
Cloud point	°C	1.5	ND
Pour point	°C	1.4	−15–16
Saponification value	mg KOH/g oil	196.12	ND
Iodine value	gI_2/100 g oil	98.98	ND
Ester content	weight %	97.38	ND

4. Conclusions

The following conclusions could be drawn from this study:

- It was found that the degree of lipid extracted from *S. molesta* was 19.97% dwt for ultrasonication > 16.79% dwt for microwaving >16.60% dwt for glass grinding >1 6.36% dwt for sand grinding >15.82% dwt for autoclaving >15.36% dwt for untreated.
- The Taguchi method of optimization using ultrasonication method of pre-treatment gave a maximum

lipid extraction of 20.86% dwt using 100% amplitude and 15 min sonication time and amplitude has 75.78% contribution for enhancing lipid extraction of *S. molesta*.

- The FAME analysis of *S. molesta* lipid showed fatty acids such as C14:0, C14:1, C16:0, C16:1, C18:0, C18:1, C20:1, C20:4 and C22:0 which consists of monounsaturated, saturated and polyunsaturated fatty acids in %weight as 63.59, 33.16 and 0.73, respectively.
- The physical properties estimated based on fatty acid profiles of *S. molesta* biodiesel are comparable with ASTM 6751-08 biodiesel standard.

Acknowledgements

The authors are thankful to Kerala State Council for Science, Technology and Environment (KSCSTE), Kerala, India for financially supporting (Order No.1237/2015/KSCSTE) this investigation.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.reffit.2016.07.005.

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