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Optimization and Kinetic studies of biodiesel production from the green alga *Ulva fasciata* Delile

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ABSTRACT

In this investigation, optimization experiments were carried on lipid extraction and biodiesel production parameters from *Ulva fasciata* as a potential feed stock. This study was carried by running one to one optimization model. The results showed that, the highest yield and the best quality of biodiesel was achieved at the optimum condition of <0.16 mm algal particle size, 55^{o}C extraction temperature, 25:1 v/w solvent to solid ratio, 60 min extraction time and 250 rpm shaking speed, using chloroform: methanol: H_2O (2: 2: 1) solvent mixture. The recorded lipid yield was 28.84 mg g^{-1} with total fatty acids ΣTFAs content 1148.94 µg g^{-1}, saturated fatty acids ΣSFAs=979.43 µg g^{-1}, monounsaturated ΣMUFAs=136.98 µg g^{-1} and polyunsaturated ΣPUFAs fatty acids =32.53 µg g^{-1}. The quality of the produced biodiesel at these optimum conditions was determined by its physicochemical properties which showed a very high quality. Cetane number (CN) was 73.21, while kinematic viscosity (υ), density (ρ), higher heating value (HHV) and iodine value (IV) were 4.68 mm^2s^{-1}, 0.87 g cm^{-3}, 39.85 MJ kg^{-1} and 18.48 gI_2100g^{-1}fat, respectively. Other properties such as degree of unsaturation (DU), saponification value (SV) and long chain saturation factor (LCSF) recorded 17.58, 200.66mg $KOHg^{-1}$ and 23.06, respectively. On the other hand, Linolenic acid (C18:3) % and Polyunsaturated fatty acid methyl esters containing ≥ 4 double bonds % recorded 0.68% and 0.98%, respectively. The initial extraction rate (h) was 6.169 ml g^{-1} min^{-1}; the extraction capacity (Cs) was 31.036 g ml^{-1}; the second order extraction constant (k) was 0.0064 ml g^{-1} min^{-1} and the coefficient of determination (R^2) was 0.9994.

INTRODUCTION

At recent years, the increase of global demand for energy by domestic and industrial sectors is being paralleled with population increase and the simultaneous increase in fossil fuel consumption. A large quantity of fossil fuel is still available at considerable cost but gradually inching towards depletion due to overexploitation of fuel reserves. Besides, excessive burning of fossil fuel has resulted in increased

greenhouse gas emissions (GHG) and thereby contributed to global warming (Imasiku *et al*., 2019).

So, the interest in using renewable energy, hydroelectricity, or nuclear energy as alternative sources for petroleum based fuels has remarkably risen (Banerjee and Chakraborty, 2009). Particularly, biodiesel is considered as one of important renewable fuels (Da Siva Filho *et al*., 2018). As fatty acid methyl esters (FAMEs) has many advantages such as biodegradability and nontoxicity (Adipah, 2018). Biodiesel also has a favorable combustion-emission profile, producing much less carbon monoxide, sulphur oxides, nitrogen hydride, particulate matter, and unburned hydrocarbons compared to the petroleum-based diesel (Cheng and Li, 2018). Therefore, using biodiesel is beneficial for the sake of reducing air pollution and minimizing the emission of greenhouse gas (Eshton *et al*., 2013).

Although, biodiesel seems to be an applicable choice, but the most significant drawback is the cost of crop oils, which accounts for 80% of total operating cost, used for the biodiesel production (Demirbas, 2007).Therefore, there is an increase demand to find new suitable substrate for oil supply, which does not compete with the edible crops. One alternative is the algae as they have higher biomass production rate per unit area, do not compete with agricultural plants for land, require no agricultural input such as fertilizer, and pesticides, and easier depolymerization as it does not contain lignin in their cell wall (Raikova *et al*., 2017).

In this context, the green seaweed *Ulva fasciata* Delile was selected as a potential feedstock for biodiesel production. It has high lipid and fatty acid contents compared with other algal species (Shaltout and Shams El-Din, 2015). It grows luxuriantly, widespread along Alexandria shores and has worldwide distribution regardless of geographical barriers (Aleem, 1993; Abdallah, 2010; El-Nemr *et al*., 2012).

Moreover, Shaltout and Shams El-Din (2015) concluded that *Ulva fasciata* collected from Abu Qir Bay can be considered as the most suitable and promising source for biodiesel production.

The kinetic of lipid extraction is the investigation on effect of experimental conditions influencing the speed of extraction mechanism (Meizane and Kadi, 2008).The kinetics of oil extraction from oil substrate depends on a number of factors, among which the most important are temperature and duration of the extraction, as well as the polarity of the solvent used for extraction. The driving force in this mechanism is the difference between the concentration of the lipid being transferred at the solid interface and in the solvent.

Many studies have been conducted to describe the kinetics and mechanism of the extraction process. Solid-liquid extraction usually fitted the second order mechanism model (Meizane and Kadi, 2008) which means that the extraction occurs in two simultaneous processes.

The first part is when the solute gets extracted quickly caused by the driving force of the fresh solvent. Meanwhile, the extraction gets much slower in the second stage as the remainder solute will diffuse into the solution (Uhm and Yoon 2011). Both of these processes have a different kinetic coefficient.

The purpose of this work was to find out the optimum conditions of the different extraction parameters (algal particle size, extraction temperature, extraction time, solvent-to-solid ratio and mixing intensity) which influence the biodiesel production from *Ulva fasciata* for the sake of reducing biodiesel coast, increasing yield and enhancing the quality.

Also, this work aimed to study the kinetics and mechanism of solid-liquid extraction of *Ulva fasciata* based on a second order model.

MATERIALS AND METHODS

Collection of *Ulva fasciata*

The green alga *Ulva fasciata* Delile was collected during May (2014) from the beach of the touristic site "Bardiss" located at the extremely western head of Abu Qir Bay located on the Egyptian Mediterranean Sea at longitudes 30° 04` 18.732``E and latitudes 31° 18` 36.049`` N. The species was identified according to Aleem (1993).

Healthy specimens of the alga were handpicked whole, from their bases, scraping the substrata on which they were adhered, and then kept at 4 °C in icebox. The collected alga was brought to the laboratory and was washed with tap water to separate any potential contaminants such as adhering impurities, sand particles, epiphytes and animal castings. Algal biomass was dried at room temperature (25°C) in shade for about four days, then dried in a drying oven (Model: DX302) at 60°C, to remove the water content from the biomass as it will interfere with lipid extraction (Jegathese and Farid, 2014).Thereafter, it was desiccated at room temperature (25°C). The dried seaweed was hand crushed, grinded as coarse powder with a mixer grinder, and particle size distribution was determined using a sieve shaker (Cisa - BA 200N), following ASTM standards.

Extraction and purification of total lipids

The dried algal biomass (<0.16 mm particle size) was weighted (1 g ± 0.001) into 100 ml screw top bottles. A total of 25 ml solvent was added in a predetermined sequence according to Folch *et al.* (1957) with some modifications set by Shaltout and Shams El-Din (2015), where lipids were extracted from the alga with 15 ml of chloroform/methanol (2/1, v/v) by shaking at 250 rpm in an orbital shaking incubator (model: JSSI-100T) and 55°C for 30 minutes. This was followed by the addition of a mixture of methanol/water (10 ml, 1:1, v/v) to achieve a final solvent mixture ratio of 2:2:1 for chloroform: methanol: water. The bottles were well caped and re-shacked for another 30 minutes. Thereafter, the mixture was filtered by using Whatman filter paper No. 1 (Whatman, USA). The supernatants were collected and the residues were re-extracted with 5 ml chloroform (Afify *et al*., 2010). The extract was shaken vigorously for one minute and allowed to undergo phase separation for 15 min in a separating funnel (Doan *et al*., 2011). The lower organic phases were collected by using the separating funnel in pre-weighted 25 ml dried clean screw top tubes and the chloroform-methanol mixture was evaporated on a water bath until dryness leaving a residue at the bottom of the tube and then was dried in an oven at 60°C to a constant weight. The total extracted lipid yield (%w/w) was then quantified gravimetrically according to Gutierrez *et al*. (2008).

Determination of fatty acids

The extracted total lipid was reacted directly with a freshly prepared mixture of methanol, chloroform and HCl (10:1:1 v/v/v) at 90°C for 120 min for esterification process (Lewis *et al.,* 2000). The fatty acids methyl esters (FAMEs) were then extracted using hexane/ chloroform (4:1, v/v), where hexane layer with extracted FAMEs was evaporated till dryness, then FAMEs were re-dissolved in 1 ml of hexane at time of measurement then characterized via gas liquid chromatographic analysis (Doan *et al.*, 2011). A gas chromatography (GC-QqQ/MS triple Quade) analysis system was an Agilent 7890A series GC system coupled with an Agilent 7000B QqQMS (Agilent

Technologies Inc., USA) which was run to identify the concentration of fatty acid fraction of the lipid extract. Individual peaks of FAMEs were identified by the comparison of the retention times and equivalent chain length values, by using the standard Supelco 37 component FAME Mix, (C4-C24) and quantified by area normalization.

Calculation of biodiesel properties from fatty acid profiles

The physical properties of biodiesel products were calculated to investigate the quality of the biodiesel extracted from *U. fasciata*. The fatty acids methyl ester profiles were used to estimate the Degree of Unsaturation (DU), Long Chain Saturation Factor (LCSF), Iodine Value (IV), Saponification Value (SV), the Cetane Number (CN), kinematic viscosity (υ), density (ρ), the Higher Heating Value (HHV), C18:3% (wt%) and weight percent of fatty acids with double bond higher than 4 Db≥4(wt%) according to Islam *et al*.,(2013) and Saravanan *et al*.,(2013).

Optimization of extraction parameters to enhance the lipid and biodiesel yields

About (1 g ± 0.001) of the dried algal biomass was weighted then added to solvent mixture (2:2:1, chloroform: methanol: water) in a predetermined sequence for oil extraction according to the modified method of Folch *et al*., (1957), following (Shaltout and Shams El-Din 2015) in a 250 mL screw cap bottle. The total extracted lipid yield (%w/w) was then quantified gravimetrically (Gutierrez *et al*., 2008). The batch extraction experiments were optimized for the extraction parameters by using one to one optimization model according to the following protocol: particle size of the dried alga ranged between (< 0.16 - 2.5 mm), solvent-to-solid ratio ranged from (5:1 to 100:1), extraction time (8-180 min) and shaking speed varied from (120 to300 rpm). Regarding the temperature, extraction was performed at different temperatures (25-60^{o}C) at 5^{o}C temperature intervals.

Kinetic study of lipid extraction

Considering the optimum extraction parameters achieved from the optimization experiments, the rate of extraction of *U. fasciata* lipid was determined at temperature of 55°C for particle size (< 0.16mm), solid to solvent ratio of 25:1 and extraction speed of 250 rpm. Extraction was performed for three hours in the following sequences, 8, 15, 30, 60, 90, 120, 150 and 180min.

Statistical analysis

Mean, variance, range, standard deviation, standard error, coefficient of variation and test of normality were performed according to Steel and Torrie (1980). Statistical analysis was done by descriptive analysis by using SPSS ver. 16 package. F-test, and L.S.D were produced by using the SAS software package, version 9.3 2008 (SAS, 2007).

Correlation analysis was used to determine the relationship between different parameters. Regression, correlation matrix, factor analysis, and cluster analysis were calculated among all traits by using SPSS ver. 16 package. The linear regression function between lipid, fatty acids, and fatty acids groups (SFA, MUFA and PUFA) and each of the studied extraction parameters was predicted according to kleinbaum and kupper (1978).

The following model was used to analyze the data obtained of the experiments.

$$Yij = \mu + \alpha i + e ij$$

Where Yij = the observation of lipid yield, fatty acids or fatty acid groups (SFA, MUFA and PUFA) with the extraction parameters; μ = overall mean; α =coefficient for the effect of iLine graphs which were produced by using curve expert version 1.13 software.

RESULTS AND DISCUSSION

Optimization of lipid extraction and biodiesel production from *Ulva fasciata*

In the current study, optimization experiments were done concerning different parameters namely; the particle size of the alga (< 0.16 to 2.5 mm), temperature (25°C to 60°C), solvent to solid ratio (R) (6:1 to 100:1), extraction time (8 to 180 min) and mixing intensity (120 to 300 rpm) to determine the optimum conditions (Tables 1-2) (Figs. 1-5) in order to achieve our goal of increasing lipid yield, reducing biodiesel cost, and enhancing at the same time the biodiesel quality (Siddiquee and Rohan, 2011).

The particle size of the dried alga is a critical parameter for the oil extraction from the biomass. The low extraction yield in case of larger particles diameter is attributed to the difficulty for the solvent to penetrate into the core of the biomass to leach the lipid. Consequently, the increase in lipid yield as size decrease is attributed to the increase of the specific surface area of grained alga interacting with the solvent mixture (Suganya and Renganathan, 2012).

The results of the present study agreed with Suganya and Renganathan (2012), where the lipid yield increased by decreasing the biomass particle size from 2.50 to <0.16 mm (10.33mg g^{-1} to 27.05 mg g^{-1}) (Table1). Suganya (2013) found similar results, where optimum particle size for extraction of lipid from *Enteromorpha compressa* was 0.15 mm, whereas for *Caulerpa peltata* the yield increased 6 folds by decreasing particle size from 0.84 to 0.11mm.

Table 1: The effect of different parameters; particle size (< 0.16 mm to 2.5 mm), temperature (25°C to 60°C), solvent to solid ratio (R) (6:1 to 100:1), extraction time (8 to 180 min) and mixing intensity (120 to 300rpm) on lipid yield (mg g^{-1} dried alga).

Particle size (mm)	**Total lipid weigh**	**Temperature (^oC)**	**Total lipid weight**	**Solvent to solid ratio (v/w)**	**Total lipid weight**	**Time (min)**	**Total lipid weight**	**Mixing intensity (rpm)**	**Total lipid weight**
< 0.16	27.05	**25**	18.03	**6:1**	16.99	**8**	21.76	**120**	24.23
0.16	22.98	**30**	23.83	**7:1**	17.67	**15**	24.32	**150**	25.89
0.2	21.23	**40**	24.01	**8:1**	20.78	**30**	24.89	**180**	27.31
0.32	16.97	**50**	24.87	**9:1**	22.71	**60**	28.33	**220**	28.05
0.4	15.94	**55**	27.72	**10:1**	23.76	**120**	29.65	**250**	28.84
0.5	15.80	**60**	26.84	**15:1**	26.38	**150**	29.65	**280**	27.69
0.8	13.92	-	-	**25:1**	28.84	**180**	30.71	**300**	22.45
1.5	12.02	-	-	**50:1**	13.38	-	-	-	-
1.6	11.60	-	-	**100:1**	4.14	-	-	-	-
2.5	10.33	-	-	-	-	-	-	-	-

Similarly, the cumulative TFAs were progressively improved by reducing particle size from 2.5 to < 0.16 mm (530.72 to 1124.84 µg g^{-1}) (Table 2). However, ΣSFAs and MUFAs followed the same pattern, while PUFAs had no significant change between different particle sizes (Table 2). Considering the fatty acid profile, palmitic acid (C16:0) was the dominant one at all particle size, with the highest concentration at size of < 0.16 mm (768.42 µg g^{-1}), followed by behenic acid (C22:0) (89.67µg g^{-1}) and oleic acid (18:1) (74.66 µg g^{-1}) (Fig.1).

By raising temperature from 25^o C to 55^o C, the lipid yield was enhanced from 18.03 to 27.72 mg g^{-1}(Table 1). The contents of TFAs improved from 849.17 µg g^{-1} to 1144.80µg g^{-1}, then decreased to 825.81 µg g^{-1} at 60^{o}C (Table 2). ΣSFAs followed the same pattern accompanied by a decrease in MUFAs and PUFAs (Table 2).

Table 2: Effect of changing *U. fasciata* particle size, extraction temperature, solvent to solid ratio, extraction time and mixing intensity on biodiesel properties compared with ASTM D 6751-02 and EN 14214).

Biodiesel properties		**DU**	**LCSF**	**IV ($gI_2100g^{-1}fat$)**	**SV (mg KOH g^{-1})**	**CN**	**TFA wt (µg g^{-1})**	**SFA (%)**	**MUFA (%)**	**PUFA (%)**	**Kinematic viscosity (υ) ($mm^2 s^{-1}$)**	**Density (ρ) ($g cm^{-3}$)**	**HHV ($MJ kg^{-1}$)**	**C18:3 (wt%)**	**Db≥ 4 (wt%)**
Biodiesel Standard EN (14214)		-	-	≤120	-	≥51	-	-	-	-	3.5–5.0	0.86–0.9	NA	≤12	≤1
Biodiesel Standard ASTM D6751−02		-	-	NA	-	≥47	-	-	-	-	1.9–6.0	NA	NA	-	-
min/max		Max	max	max	max	min	min	min	max	max	max	max	min	max	max
Threshold value		-	-	120	-	47	-	-	-	-	-	0.9	-	12	1
Particle size (mm)	**<0.16**	15.87	23.48	16.50	200.32	73.85	1124.84	86.65	10.84	2.52	4.72	0.87	39.88	0.59	0.74
	0.16	18.49	23.07	19.42	200.03	73.36	912.59	84.77	11.96	3.27	4.72	0.87	39.92	0.71	1.10
	0.32	19.54	21.94	20.62	200.36	73.03	886.54	83.87	12.71	3.42	4.68	0.87	39.92	0.72	1..19
	0.40	19.15	23.22	21.05	200.18	73.12	834.25	84.16	12.54	3.30	4.71	0.87	39.92	0.76	1.42
	0.80	17.70	22.24	18.70	200.82	73.24	802.18	85.11	12.07	2.82	4.68	0.87	39.93	0.81	0.79
	1.25	15.63	21.43	19.03	201.96	73.02	626.63	88.67	7.04	4.29	4.63	0.87	39.97	0.99	1.73
	1.6	17.89	22.46	20.94	201.45	72.84	583.98	86.49	9.13	4.38	4.66	0.87	40.00	1.05	1.67
	2.5	22.99	21.99	27.00	201.11	72.56	530.72	83.97	9.08	6.96	4.67	0.88	40.15	1.62	1.87
Extraction temperature(^oC)	**25**	24.58	23.69	27.82	198.92	72.83	849.17	79.91	15.59	4.50	4.73	0.87	39.94	0.73	2.21
	30	23.50	24.05	24.08	199.19	72.70	852.48	80.06	16.37	3.57	4.74	0.87	39.94	0.87	1.15
	35	22.41	24.78	23.66	199.15	72.98	855.36	81.35	14.89	3.76	4.75	0.87	39.93	0.85	1.34
	40	22.44	25.01	24.14	198.91	72.94	906.40	81.48	14.59	3.93	4.76	0.87	39.93	0.90	1.52
	45	21.64	23.48	22.29	199.73	72.85	914.57	81.67	15.03	3.30	4.72	0.87	39.90	0.76	1.11
	55	17.82	23.14	20.06	200.48	73.33	1144.80	85.52	11.14	3.34	4.69	0.87	39.85	0.68	1.49
	60	15.96	24.60	18.51	200.42	73.74	825.81	87.98	8.08	3.94	4.72	0.87	39.92	0.82	1.52

Com. Table : 2

	6:1	36.10	20.21	39.39	198.31	70.70	403.50	71.67	20.56	7.77	4.70	0.88	40.21	1.96	2.59
Solvent to	**7:1**	26.04	24.29	28.12	198.40	72.68	524.20	79.45	15.06	5.49	4.78	0.87	40.10	1.17	1.86
solid ratio (R)	**8:1**	24.54	23.06	27.95	199.24	72.58	566.32	80.43	14.60	4.97	4.73	0.87	40.05	1.11	2.19
	9:1	24.28	20.56	27.29	199.96	72.43	687.16	80.64	14.44	4.92	4.67	0.87	39.97	0.91	2.11
	10:1	24.17	23.24	27.85	199.04	72.89	793.99	80.73	14.38	4.89	4.73	0.87	39.95	0.81	1.24
	15:1	21.66	23.25	22.54	199.45	72.98	818.91	82.00	14.33	3.66	4.73	0.87	39.94	0.79	1.23
	25:1	16.81	23.28	17.85	200.69	73.34	1137.76	86.05	11.09	2.86	4.69	0.87	39.85	0.68	0.99
	50:1	20.60	20.63	22.05	200.47	72.84	737.84	83.42	12.57	4.02	4.67	0.87	39.95	0.85	1.40
	100:1	36.66	22.47	41.39	198.43	70.44	335.63	70.98	21.37	7.64	4.72	0.88	40.29	1.85	3.00
Extraction time	**60**	17.58	23.06	18.48	200.66	73.21	1148.94	85.25	11.92	2.83	4.68	0.87	39.85	0.68	0.98
	120	16.26	24.53	17.21	199.88	73.82	1265.77	86.47	10.81	2.72	4.74	0.87	39.87	0.65	0.98
	150	15.98	24.60	16.42	199.75	73.89	1345.77	86.49	11.03	2.48	4.75	0.87	39.87	0.63	0.81
	180	16.45	25.07	16.63	199.38	73.98	1328.07	85.88	11.79	2.33	4.77	0.87	39.87	0.53	0.79
Mixing intensity	**120**	23.54	23.95	23.90	199.04	72.74	937.07	79.72	17.01	3.26	4.74	0.87	39.92	0.77	1.09
(rpm)	**150**	24.11	24.90	26.02	198.72	72.86	983.30	79.76	16.38	3.87	4.76	0.87	39.91	0.89	1.64
	180	24.22	24.41	26.66	198.83	72.81	1019.50	79.84	16.09	4.06	4.74	0.87	39.90	0.95	1.82
	220	22.65	24.55	22.83	198.98	72.95	1028.53	80.42	16.51	3.07	4.75	0.87	39.91	0.79	0.98
	250	17.07	23.23	17.75	200.64	73.33	1141.16	85.70	11.53	2.77	4.69	0.87	39.86	0.70	0.88
	280	22.22	25.80	22.76	198.74	73.11	958.39	81.10	15.59	3.32	4.78	0.87	39.92	0.88	1.05
	300	24.89	25.58	25.38	198.25	72.82	918.79	78.49	18.14	3.37	4.78	0.87	39.94	0.73	1.21

However, palmitic acid (C16:0) was the dominant one, recording the highest concentration (768.42 µg g^{-1}) at optimum temperature (55°C).Considering MUFAs, the oleic acid (C18:1c) was the dominant at all temperatures, except at 60°C**.** The PUFAs, cis- 4,7,10,13,16,19-docosahexaenoic acid (C22:6) was dominant with relatively high concentration (10.27 µg g^{-1}) at 55°C (Fig. 2).

In fact, temperature plays a significant role in the extraction process. The synergism of lipid yield with temperature is attributed to the increase in the dissolution capacity of the solvent system and lipid volatility (Mamata, 2000). Nevertheless, the extraction process improved until approaching to the temperature of the solvent boiling point (Richardson and Harker, 2002), which is 64.7°C for methanol and 61.2°C for chloroform at 1 atm. But beyond the boiling point of the solvents causes a decrement in diffusion coefficient and the solubility, hence do not permit further extraction of lipid (Moaddabdoost and Kordi, 2010). In the current study, optimum temperature; following Folch method is 55°C and not the room temperature as suggested by Folch *et al*. (1957). This finding is in a good agreement with Suganya (2013) and Jeong and Park (2015).

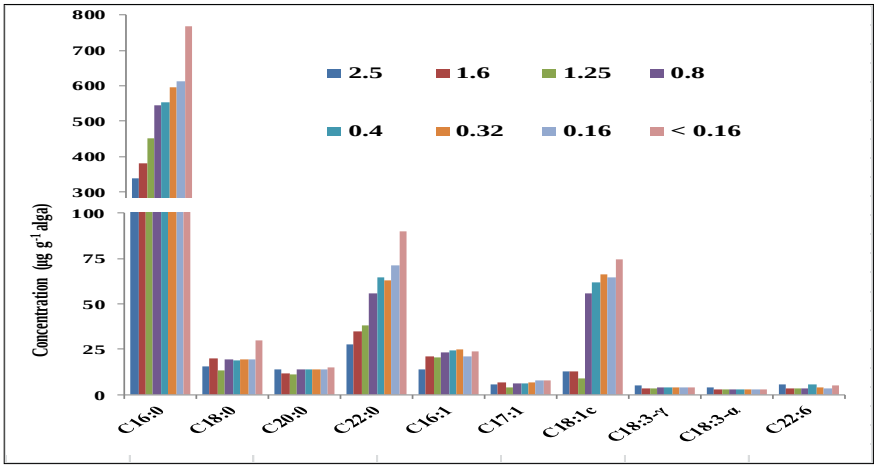

Fig. 1: Effect of *U. fasciata* particle size on concentration of FAMEs (µg g^{-1}alga).

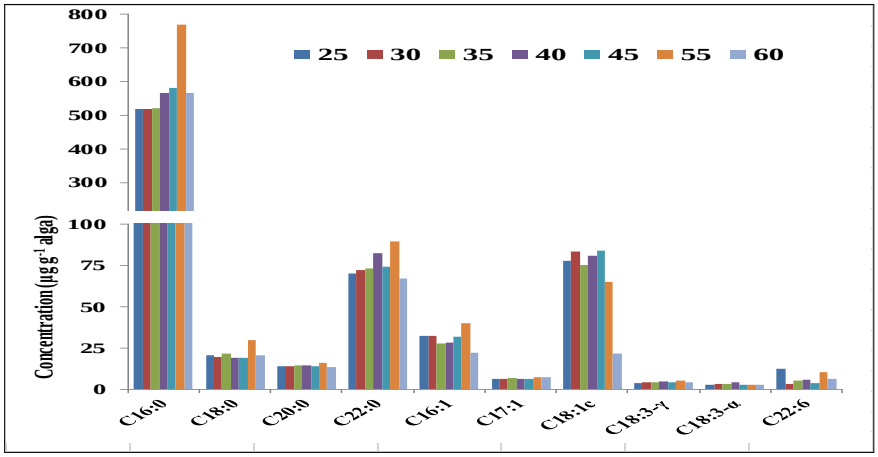

Fig. 2: Effect of extraction temperature on concentration of FAMEs (µg g^{-1} alga)

By increasing the solvent to solid ratio from 6:1 to 25:1 (v/w), the lipid yield increased from 16.99 to 28.84 mg g^{-1} (Table 1). Similarly, TFAs improved from 403.50 µg $g^{-1}$to 1137.76 µgg^{-1}, and then decreased by further increasing the ratio to 50:1 and 100:1(v/w) (Table 2). On the other hand, ΣSFAs and ΣMUFAs showed the inverse pattern, recording the lowest values at ratio of 25:1 (11.09 and 2.86%),

respectively and then increased by further increasing the ratio to 50:1 and 100:1(v/w). Palmitic acid (C16:0) was the dominant one at all ratios, recording the highest concentration (768.42 μgg^{-1}) at 25:1 (Fig. 3). Similarly, oleic acid (C18:1c) (MUFA) and cis- 4,7,10,13,16,19-docosahexaenoic acid (C22:6) (PUFA) were dominant at all ratios (Fig. 3). This may be due to that a greater amount of solvent allows forming a greater number of Van der Waals interactions (non-polar solvent) and hydrogen bonds (polar solvent) with polar and non-polar lipids. Further increase in ratio (R) leads to a small increment in the amount of the extracted lipids, but it raises the cost of separation of the solvent. Moreover, the concentration gradient between the solid and the liquid phase becomes greater, which favors mass transfer till saturation of solvent with extracted lipid. At this point extracted lipid is the maximum and no further effect can be achieved (Sayyar *et al*., 2009).Further increasing solvent to solid ratio (R) to 50:1 and 100:1(V/W), decreased the lipid yield (Suganya and Renganathan, 2012). In contrary to Blight and Dyer (1959) method, our results concluded that increasing the solvent to biomass ratio from 6:1 to 25:1 will increase the quantity of the produced biodiesel with no further increase beyond 25:1.

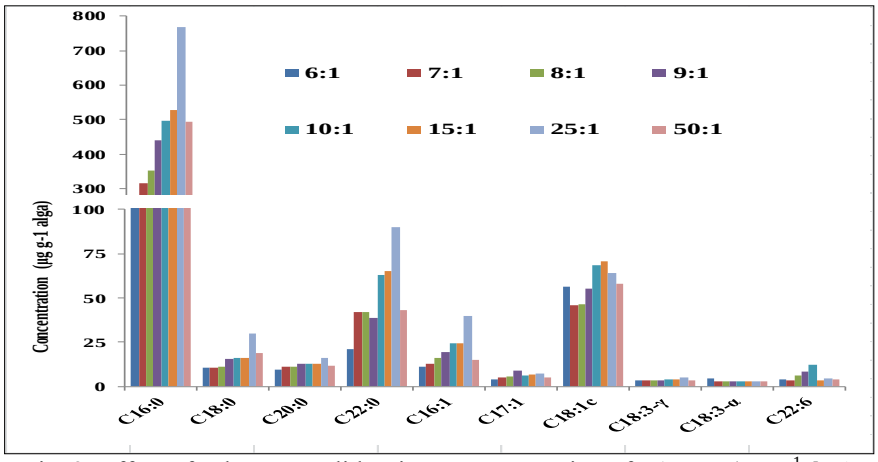

Fig. 3: Effect of solvent to solid ratio on concentration of FAMEs (μg g^{-1}alga)

The extraction time is identified as one of the governing parameters in the lipid extraction processes, where the solvent extraction needs sufficient time to extract lipid and intracellular fatty acids from cell wall (Mubarak *et al*., 2016).

In the current study, the lipid extraction yield increased by 30% as time increased from 8 to 60 minutes. Doubling time of extraction (120 min.) was accompanied by insignificant increase in lipid yield (1.32 mgg^{-1}) (Table 1). Thus, by considering the energy and cost prospective, 60 minutes can be identified as optimum time for lipid extraction from *Ulva fasciata* at these conditions. Suganya and Renganathan (2012) identified 140 minutes as an optimum extraction time, with no further increase for lipid by increasing time.

Concerning the cumulative TFAs, they improved from 1148.94 to 1345.77 μgg^{-1} by increasing extraction time duration from 60 to 150 min, respectively. Saturated fatty acids (ΣSFAs) followed the same pattern, while there was no significant change in MUFAs between 60 and 120 minutes, while increasing time till 180 minutes led to slight increase to 11.79% (Table 2). Noticeably, increasing time of extraction didn't show any pronounced effect on PUFAs concentrations (Table 2 & Fig. 4).

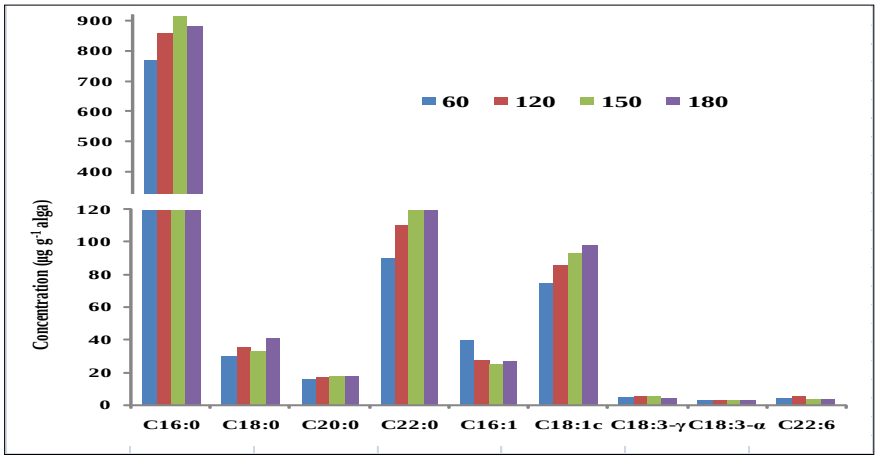

Fig. 4: Effect of extraction time on concentration of FAMEs (µg g^{-1} alga)

The mixing intensity (R.P.M) is another important parameter for lipid extraction process. There is a synergism between lipid yield and mixing intensity from 120 to 250 rpm (24.23 to 28.84 mgg^{-1}) (Table 1) which can be attributed to the increase in agitation of the solvent, which increases the eddy diffusion and therefore increases the transfer of lipid from the algal biomass to the bulk of the solvent mixture (Abdul-Nabi, 2011). Further increase of the mixing intensity to 280 and 300 rpm coincided with a decrease in the extracted lipid (Table 1). This may be due that the algal particles become mostly in contact with H_2O layer rather than equilibrium between the two layers of the solvent mixture. In this trend, Suganya and Renganathan (2012) showed an increase in lipid yield of *U. lactuca* from 7.81% to 9.36 % by increasing mixing intensity from 200 to 500 rpm, with no significant increase by further increase in mixing intensity to 600 rpm.

In fact, the cumulative TFAs and ΣSFAs improved by increasing the mixing intensity from 120 to 250 rpm, respectively (Table 2). Thereafter, both decreased by further increasing in mixing intensity above 250rpm.On the other hand, ΣMUFAs content showed irregular pattern, while the concentration of ΣPUFAs remained almost stable (Table 2) (Fig. 5).

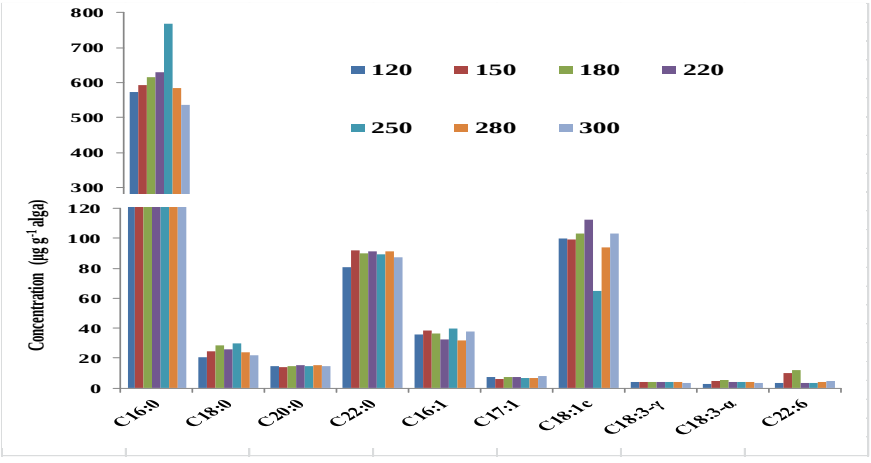

Fig. 5: Effect of mixing intensity on concentration of FAMEs (µg g^{-1} alga)

The physico-chemical properties of the produced biodiesel are necessary to determine the efficiency and suitability of the produced biodiesel, as well as they are used as a confirming tool for screening of the optimum conditions (Islam *et al*., 2013).

Comparing the results of CN to the ASTM D6751-02(≥47) and EN14214 (≥51) standards for biodiesel, its value was higher than that of both standards. In fact, the highest value of CN (73.85, 73.33, 73.34, 73. 98 and 73.33) was almost recorded at particle size of <0.16mm, extraction temperature 55^{o}C, (R) 25:1 v/w, extraction time at 180 min and mixing intensity from at 250 rpm (Table 2).

The second important physical property of biodiesel is kinematic viscosity (υ). It is a measure of resistance to flow of a liquid due to internal friction of one part of a fluid moving over another (Knothe, 2005). However higher viscosity leads to poorer fuel atomization (Hasimoglu *et al*., 2008), whereas lower value was preferable to ensure that an adequate fuel supply reaches injectors at different operating temperatures (Knothe, 2002). Actually, several structural factors influence (υ), such as hydrocarbon chain length, degree of unsaturation and double bond orientation (Moser, 2009). Longer hydrocarbon chain lengths and lower degrees of unsaturation result in higher kinematic viscosity (Zheng *et al*., 2011), whereas the double bond, with trans configuration gives higher viscosity than cis one (Knothe, 2005).

In the current study, particle size had a negligible effect on the kinematic viscosity, since its values were almost equal at all particle sizes (4.63-4.72 $mm^2 s^{-1}$) (Table 2). On the other hand, all the recorded kinematic viscosities were probably the lowest at 55^{0}C, 25:1, 60 min, and 250 rpm and in the acceptable limit (1.9-6.0 and 3.5-5mm^2s^{-1}) for ASTM D6751-02 and EN14214 standards, respectively (Table 2).

Fuel density (ρ) is a key property that affects engine performance. Since fuel injection pumps meter fuel by volume not by mass, so that, a greater or lesser mass of fuel is injected depending upon its density. Moreover, a denser fuel contains a greater mass in the same volume. Therefore, the air-fuel ratio and energy content within the combustion chamber are influenced by fuel density. In addition, biodiesel density is strongly affected by the degree of unsaturation of FAMEs and chain length fatty acid. The higher unsaturation increases the density, while higher chain length leads to lower fuel density (Refaat, 2009).

In the present study, there was negligible effect of all studied parameters on density. On the other hand, the values of density were low and fit perfectly with that of EN14214 standard for biodiesel, which means that the produced biodiesel are highly suitable for ignition engines (Table 2).

Higher heating value (HHV) is the amount of heat produced by the complete combustion of a unit quantity of fuel. This property is obtained when all products of the combustion are cooled down to the temperature before the combustion and the water vapor formed during combustion is condensed. The higher heating value of a fuel synergizes with carbon number in fuel molecules and also with the ratio of carbon and hydrogen to oxygen and nitrogen (Demirbas, 1997). As the FA carbon chain increases in length (for a constant unsaturation level) the mass fraction of oxygen decreases, so the heating value increases (Demirbas, 2008).

Neither the U.S. nor European biodiesel standards include a specification for heating value. The FAME-derived HHVs of the investigated parameters were found to comply with the set range of regular biodiesel (39.8–40.4 MJ kg^{-1}), which is normally 10% to 12% less than obtained for petroleum-derived diesel (46 MJ kg^{-1}), due to biodiesel substantial oxygen content (Sanford *et al*., 2009) (Table 2).

One of the most important biodiesel physical properties is the iodine value (IV), which is a measure of the average amount of unsaturation and is determined by measuring the amount of I_2 that reacts by addition to carbon–carbon double bonds per 100 g of lipid (AOCS, 2005). The European biodiesel standard, EN 14214, has a maximum IV specification of 120 gI_2 $100g^{-1}$fat (Moser, 2009).

The maximum IV values (27.00, 27.82, 41.39, 18.48, 26.66 gI_2 $100g^{-1}$fat) were recorded at particle size of 2.5 mm, 25°C, 100:1, 60 min, 180 rpm, respectively, accompanied by the highest degree of unsaturation (Table 2),while minimum IV value (16.5, 18.51, 17.85, 16.42, 17.75 gI_2 $100g^{-1}$fat) was recorded at <0.16 mm, 60°C, 25:1, 150 min, 250 rpm, accompanied by the lowest degree of unsaturation, respectively (Table 2). All the recorded iodine values were lower than the maximum limit identified by EN 14214 standards. This is attributed to high percentage content of saturated fatty acids (SFAs%) rather than unsaturated fatty acids (USFAs%).

The contribution of polyunsaturated FAME with respect to fuel stability has been recognized by many researchers. Ramos *et al.* (2009) defined a parameter called the degree of unsaturation (DU) that weighted polyunsaturated species twice as much as mono-unsaturated ones. The limitation of unsaturated fatty acids is necessary due to the fact that heating higher unsaturated fatty acids results in polymerization of glycerides, which can lead to the formation of deposits or to deterioration of lubrication properties (Hoekman *et al.*, 2012), resulting in low biodiesel quality. In the current study, the lowest DU (15.87, 17.82, 16.81 and 17.07) was recorded at particle size <0.16 mm, 55^{o}C(R), 25:1 and 250 rpm, respectively. However, neither ASTM D6751-02 nor EN14214 standards identified certain specifications for degree of unsaturation (DU), MUFAs%, PUFAs% or SFAs%, but according to Knoth (2009) higher degree of unsaturation than 137 meant that the oils are out of the acceptable limits of the EN standard.

The saponification value (SV) of the biodiesel has an inverse relationship to the average molecular weight of the fatty acids (Knoth, 2002). The SV of the esters is higher than that of oil due to the fact that, the process of transesterification removes the heavier glycerol molecules from the oil, consequently reducing the average molecular weight of the methyl esters (Wagutu *et al*., 2009).

In the current study, the saponification values showed no great difference, with decreasing particle sizes from 2.5 mm to <0.16 mm (Table 2). By increasing extraction temperature (25^{o}C to 55^{o}C), SV increased from (198.92 to 200.48 mg $KOHg^{-1}$), respectively (Table2). As shown in Table (2), maximum SV was (200.69, 200.66 and 200.64 mg $KOHg^{-1}$) at solvent to solid ratio of 25:1 v/w, at 60 min. extraction time and at 250 rpm, respectively (Table2). According to Sanford *et al*., (2009), the saponification value (SV) for many feedstock was in the range 185–210 mg $KOHg^{-1}$. According to the Italian standard (UNI 10635), the SV was identified to be ≥170 mg $KOHg^{-1}$. Thus, all SV values for the studied parameters were within limits of UNI 10635 standard. The higher values of saponification for *U. fasciata* biodiesel indicated the high volatility and low density of produced biodiesel, which is desirable for burning the biodiesel smoothly and in avoiding misfire (Azeem *et al*., 2015).

It is clear that, Linolenic acid C18:3% improved from 1.62% to 0.59% by decreasing the particle size from 2.5 mm to < 0.16 mm, whereas, its lowest percentage (0.68%) was recorded at both 55^{o}C (Table 2) and at solvent to solid ratio 25:1 v/w (Table 2).On the other hand, it varied from (0.53-0.68%) at extraction time 60-180 min (Table 2), whereas its lowest value was 0.70% at mixing intensity of 250 rpm against the highest one (0.95%) at 180 rpm (Table 2). These values were within the limits of The European Biodiesel Standard, EN 14214 to be ≤ 12%.

However, the percentage of polyunsaturated fatty acid methyl esters containing double bonds (≥4d.b %) was set by the EN 14214 to be in the produced biodiesel ≤ 1%. In the current study, percentage of PUFAs (≥4d.b%) was slightly higher than 1 % at all particle sizes, all extraction temperatures and mixing intensities, except for 0.80

and <0.16 mm, 250 rpm (Table 2). However, the same pattern was followed by changing solvent to solid ratio (6:1 to 100:1), except for 25:1 v/w, where it recorded 0.99% (Table 2). On the other hand (≥4d.b%), decreased from 0.98% to 0.79% at 60 min to 180 min extraction time, respectively (Table 2).

The long chain saturation factor (LCSF) is a critical parameter controlling cetane number and IV of the biodiesel, where high LCSF is desirable for biodiesel cetane number and other physicochemical properties of the produced biodiesel. In contrast, the longer the biodiesel carbon chains, the worse are their low-temperature properties (Francisco *et al*., 2010). In addition, when a liquid biodiesel is cooled, the alkyl esters of stearic and palmitic acids are the first to precipitate and, therefore, they typically constitute a major share of material recovered from clogged biodiesel fuel filters (Francisco *et al*., 2010). Neither ASTM D6751- 02 standard nor EN14214 standard include specifications for long chain saturation factor (LCSF). In the current study, the lowest value (23.06, 23.14 and 23.23) was recorded at 60 min, 55^{o}C and 250rpm, respectively, whereas it was maximum (23.48) at <0.16 mm and relatively high (23.28) at (R) 25:1 (Table 2).

In conclusion, the optimum lipid extraction conditions for biodiesel production from *U. fasciata* with high quantity and quality were; <0.16 mm algal particle size, 55^{o}C extraction temperature, 25:1 v/w solvent to solid ratio, 60 min extraction time and mixing intensity at 250 rpm.

Production of biodiesel from *Ulva fasciata* at the optimum conditions

Applying the optimum lipid extraction conditions for biodiesel production from *U. fasciata* by using chloroform: methanol: H_2O (2:2:1) solvent mixture without any pretreatments resulted in lipid yield 28.84 mg g^{-1}. This result was comparable to the extracted lipid from *Ulva fasciata* (30 mg g^{-1}) recorded by Khan and Fatima (2015), while Abirami (2012) and Trivedi *et al.*(2013) reported lower lipid contents in *U. fasciata* (8.9 and 18.3mg g^{-1}), respectively. On the other hand, Ahmed *et al.* (2015) recorded higher lipid content in *U. fasciata* (41.6 mg g^{-1}).

Considering the fatty acids profile, Valeem *et al.* (2011) recorded lower number of fatty acids (23) than that of the current study (35 fatty acids) in *U. fasciata*, with SFAs (85%) and PUFAs (2.83%) (Table 3). On the other hand, Suganya and Renganathan (2012) found that SFAs in *Ulva lactuca* were (79.82%), while the unsaturated fatty acids were (20.18%). Furthermore, Suganya *et al.* (2013) reported that SFAs% and PUFAs% of *Enteromorpha compressa* were 62.95% and 37.05%, respectively. In contrast, Kumari *et al.*(2011) reported higher concentrations of PUFAs against SFAs (291.6 and 58.15 μg g^{-1}), respectively, using Blight and Dyer method in extraction.

As presented in Table (4), the physicochemical properties of biodiesel produced from *U. fasciata* at optimum extraction conditions laid within the acceptable limits of both the ASTM D 6751-02 and EN14214 standards for biodiesel.

Table 3: Fatty acid profile of *U. fasciata* (µg g^{-1}) and % to total FAMEs at optimum extraction conditions.

Fatty Acid				Fatty Acid				Fatty Acid				Fatty Acid			
Common name	**Abbrev.**	**Conc.**	**%**	**Common name**	**Abbrev.**	**Conc.**	**%**	**Common name**	**Abbrev.**	**Conc.**	**%**	**Common name**	**Abbrev.**	**Conc.**	**%**
Carpoic acid	**C6:0**	0.39	0.03	**Stearic acid**	**C18:0**	29.61	2.58	**Oleic acid**	**C18:1c**	74.66	6.5	**cis-11,14,17-Eicosatrienoic acid**	**C20:3 cis11,14,17**	3.48	0.3
Caprylic acid	**C8:0**	5.31	0.46	**Arachidic acid**	**C20:0**	15.83	1.38	**Elaidic acid**	**C18:1t**	2.5	0.22	**cis-11,14-Eicosadienoic acid**	**C20:2**	3.18	0.28
Capric acid	**C10:0**	1.48	0.13	**Heneicosanoic acid**	**C21:0**	3.46	0.3	**cis-11-Eicosenoic acid**	**C20:1**	3.46	0.3	**cis-4,7,10,13,16,19-Docosahexaenoic acid**	**C22:6**	4.53	0.39
Undecanoic acid	**C11:0**	0.6	0.05	**Behenic acid**	**C22:0**	89.67	7.8	**Erucic acid**	**C22:1**	2.23	0.19				
Lauric acid	**C12:0**	2.39	0.21	**Tricosanoic acid**	**C23:0**	4.09	0.36	**Nervonic acid**	**C24:1**	3.46	0.3	**cis-13,16-Docosadienoic acid**	**C22:2**	3.46	0.3
Tridecanoic acid	**C13:0**	1.35	0.12	**Lignoceric acid**	**C24:0**	11.46	1.00	**γ-Linolenic acid**	**C18:3-γ**	5.01	0.44	**ΣSFAs**		979.43	85.25
Myrisitic acid	**C14:0**	22.54	1.96	**Myristoleic acid**	**C14:1**	1.67	0.15	**α- Linolenic acid**	**C18:3-α**	2.77	0.24	**ΣMUFAs**		136.98	11.92
Pentadecanoic acid	**C15:0**	16.72	1.46	**cis-10-Pentadecenoic acid**	**C15:1**	2.07	0.18	**cis-5,8,11,14,17-Ecosapentaenoic acid**	**C20:5**	3.35	0.29	**ΣPUFAs**		32.53	2.83
Palmatic acid	**C16:0**	768.42	66.88	**Palmitoleic acid**	**C16:1**	39.9	3.47	**Arachidonic acid**	**C20:4**	3.44	0.3	**ΣTFAs**		1148.94	
Heptadecanoic acid (Margaric acid)	**C17:0**	6.1	0.53	**cis-10-Heptadecenoic acid**	**C17:1**	7.03	0.61	**cis-8,11,14-Eicosatrienoic acid**	**C20:3 cis8,11,14**	3.32	0.29				

Table 4: Biodiesel properties of *U. fasciata* biodiesel at optimum conditions compared with ASTM D 6751-02 and EN 14214

Biodiesel properties	**DU**	**LCSF**	**IV (gI_2 $100g^{-1}$fat)**	**SV (mg KOH g^{-1})**	**CN**	**TFA wt (µg g^{-1})**	**SFA (%)**	**MUFA (%)**	**PUFA (%)**	**Kinematic viscosity (υ) (mm^2 s^{-1})**	**Density (ρ) (g cm^{-3})**	**HHV (MJ kg^{-1})**	**C18:3 (wt%)**	**Db≥ 4 (wt%)**
Biodiesel Standard EN (14214)	-	-	≤120	-	≥51	-	-	-	-	3.5–5.0	0.86–0.9	NA	≤12	≤1
Biodiesel Standard ASTM D6751−02	-	-	NA	-	≥47	-	-	-	-	1.9–6.0	NA	NA	-	-
min/max	max	max	max	max	min	min	min	max	max	max	max	min	max	max
Threshold value	-	-	120	-	47	-	-	-	-	-	0.9	-	12	1
Control (at optimum extraction conditions)	17.58	23.06	18.48	200.66	73.21	1148.94	85.25	11.92	2.83	4.68	0.87	39.85	0.68	0.98

Kinetic study of lipid extraction

The kinetics and mechanism of lipid extraction from *U. fasciata* are based on a second-order model. To fit the second-order models to lipid extraction process, the following equation was employed, since this model was widely applied for the analysis of dissolution and leaching within inhomogeneous liquid-solid systems (Rakotondramasy *et al*., 2007).

$$t/C_t = 1/KC_S^2 + (1/C_S)\, t \qquad (1)$$

The rate of dissolution for the oil contained in the solid to solution can be described by the following equation:

$$dC_t/d_t = k(C_s-C_t)^2 \qquad (2)$$

where:

k = The second-order extraction rate constant (ml. $g^{-1}min^{-1}$)

C_s = The concentration of oil at saturation (g. ml^{-1})

C_t = The concentration of oil in the solution at any time (g. ml^{-1}), t (min)

The integrated rate for a second-order extraction was obtained by considering the boundary condition t = 0 to t and C_t = 0 to C_t:

$$C_t = C_s^2 k_t / (1+C_s k_t) \qquad (3)$$

Linear form of the equation (2) becomes:

$$t/C_t = (1/kC_s^2) + (t/C_s) \qquad (4)$$

The extraction rate can be written as the following

$$C_t/t = 1/[(1/kC_s^2) + (t/C_s)] \qquad (5)$$

The initial extraction rate, h, when t approaches 0 can be written as:

$$h = kC_s^2 \qquad (6)$$

Rearranging (4), the concentration of oil at any time can be obtained as:

$$C_t = t / [(1/h) + (t/C_s)] \qquad (7)$$

By plotting t/C_t versus t, the initial extraction rate, h, the extraction capacity, C_s and the second order extraction constant, k, can be calculated experimentally.

Regarding the changes in lipid yield with changing time (Fig. 6), it is worth to note that, at 8 minutes the yield was 21.76 mg g^{-1}. While at 30 minutes the yield was 24.89 mg g^{-1}, which increased till reach the maximum yield 28.33 mg lipid extracted per 1 g of the alga at 60 minutes. Based on the data in Figure (6), it is shown that, the extraction rate was fast at the beginning and was slowing until the end of the extraction process.

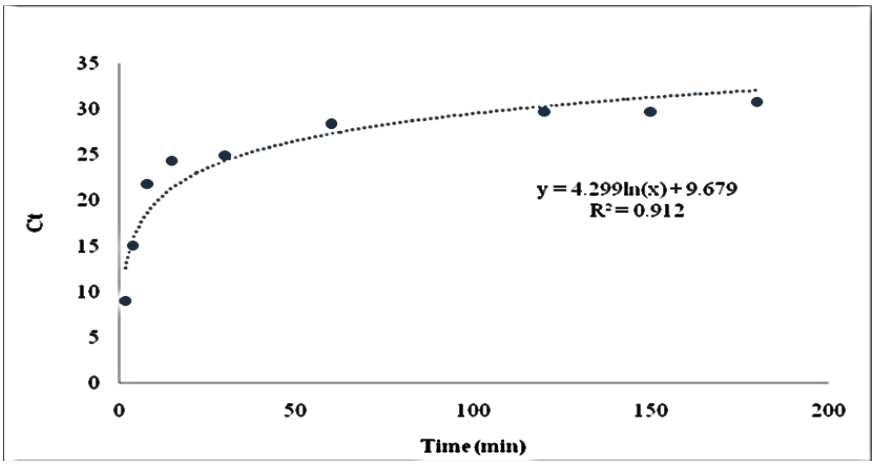

Fig. 6: Rate of lipid extraction (C_s g ml^{-1}) at different time (min).

As shown in Figure (7), the straight line curve proved and certified the assumption that the solid-liquid extraction of the algal lipid takes place in two subsequent stages.

The initial extraction rate (h), the extraction capacity (C_s), the second order extraction constant (k) and coefficient of determination (R^2) were calculated experimentally by referring to the linear plot in Figure (7).From graph t/C_t versus time, the slope is equal to $\mathbf{1/C_s}$ and intercept is equal to $\mathbf{1/ KC_s}$

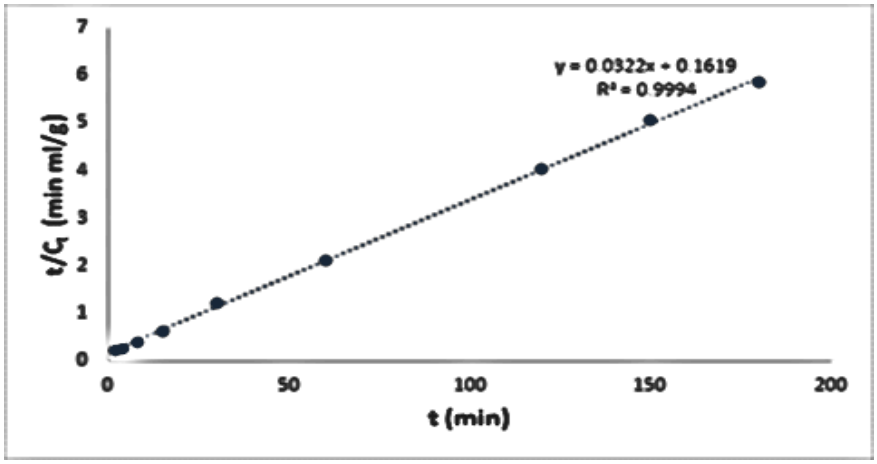

Fig. 7: Second order extraction kinetics of algal lipid extraction

The data are shown in Table (5), where the initial extraction rate (h) was 6.169 ml g^{-1} min^{-1}, the extraction capacity (Cs) was 31.036 g ml^{-1}, the second order extraction constant (k) was 0.0064 ml g^{-1} min^{-1} and the coefficient of determination (R^2) was 0.9994.

According to Sayyar *et al*., (2009), the extracted oil concentration was low at the first stage. Thus, oil diffuses rapidly from solute to liquid phase. Besides, the free oil on the surface of algae was solubilized when the solute was exposed to fresh solvent. Diffusion rate decreased as the time of extraction increased due to the high solute concentration in liquid at the second stage (Sayyar *et al*., 2009).

Table 5: linearization of second order kinetic model of solid liquid extraction of algal lipid from *U. fasciata*

Cs (g ml^{-1})	**K (ml g^{-1} min^{-1})**	**h (g ml^{-1} min^{-1})**	**R^2**
31.036	0.0064	6.169	0.9994

Statistical analysis:

The statistical calculation of the correlation significance of two way ANOVA represented in Table (6) indicated that, weight of extracted lipid, total fatty acids and the three fatty acids groups showed a high significant strong relationship at 0.01 confidence level, with variation in algal particles size, temperature of extraction (T), solvent to biomass ratio (R), time of extraction, mixing intensity (rpm), except monounsaturated fatty acids, which showed no significant correlation with mixing intensity.

Table 6: Analysis of variance for lipids, TFAs, ΣSFAs, ΣMUFAs and ΣPUFAs at different parameters

Source of Variation	Degree of freedom	lipid		Total fatty acids		ΣSFAs		ΣMUFAs		ΣPUFAs	
		Different algal particle sizes									
		MS (Mean square)	P-value	MS (Mean square)	P-value	MS (Mean square)	P-value	MS (Mean square)	P-value	MS (Mean square)	P-value
Between Groups	1	1330.216	9.1E-09	2867695	2.01E-10	2091737	2.03E-10	33846.57	8.25E-08	3542.693	8.32E-14
Within Groups	19	14.24349		16170.4		11805.72		426.9082		7.576468	
		Different extraction temperature									
Between Groups	1	1395.521	0.000901	2600301	9.38E-11	1738220	5.7E-10	21463.6	7.13E-06	430.4584	0.023739
Within Groups	11	68.91356		6073.477		5535.886		380.4918		64.26217	
		Different solvent to solid ratios									
Between Groups	1	105.1111	0.645657	1963054.638	2.2E-07	1263951.633	1.4E-06	25050.126	9.97E-06	264.7134	0.471553
Within Groups	17	480.6539		28481.84432		24158.29576		654.65249		488.2005	
		Different extraction times									
Between Groups	1	1146.104	0.004	2789766.87	9.83E-12	1867279.57	6.94E-11	24379.623	2.15E-06	275.4756	0.097224
Within Groups	13	92.33743		5633.21		5136.97		378.14629		86.242	
		Different mixing intensities									
Between Groups	1	105.11	0.65	1963054.64	2.2E-07	1263951.63	1.4E-06	25050.13	9.97E-06	264.71	0.47
Within Groups	17	480.65		28481.84		24158.29		654.65		488.20	

Statistical Model

At optimum conditions of lipid extraction, the particle size (PS) and the solvent to solid ratio (SR) denoted a very highly significance at 0.001 level of probability, while time of extraction (t) showed significance at 0.05 level of probability. In contrary temperature of extraction, (T) and mixing intensity (RPM) showed a poorly or non-significance at 0.01 level of probability (Table 7).

Table 7: Analysis of variance for lipids at optimum extraction conditions

Analysis of variance for lipids at optimum extraction conditions				
Source of variance	**Degree of freedom (df)**	**MS**	**F**	**Significance F**
Between groups	5	164.0503	7.158909	0.000114
Within groups	34	22.91554		
Total	39			
Analysis of variance for total fatty acids at optimum extraction conditions				
Between groups	5	176650.80	4.303493	0.004745
Within groups	29	41048.24		
Total	34			
Analysis of variance for saturated fatty acids at optimum extraction conditions				
Between groups	5	134738	3.970105	0.007267
Within groups	29	33938.14		
Total	34			
Analysis of variance for MUFAs at optimum extraction conditions				
Between groups	5	4363.755	6.405764	0.000402
Within groups	29	681.2232		
Total	34			
Analysis of variance for PUFAs at optimum extraction conditions				
Between groups	5	21.87803	1.350987	0.271421
Within groups	29	16.19411		
Total	34			

The Model describes the actual weight of effect of each extraction factor in the lipid extraction process, which was done using ANOVA multiple regression analysis. The final controlling equation of all the combined parameters was:

$$Lipid=26.24-6.909\times PS+0.06\times T-0.16\times SR+0.06\times t-0.023\times RPM \quad (with\ r = 0.72) \qquad (8)$$

The following models describe the contribution effect of each extraction factor in the total fatty acids, saturated fatty acids, MUFAs, PUFAs extraction process, which was done by using ANOVA multiple regression analysis by the following equations:

$$TFAs = 888.66-153.37\times PS-0.25\times T-2.37\times SR+4.73\times t-0.88\times RPM \quad (with\ r = 0.65) \qquad (9)$$

$$SFAs=609.07-116.45\times PS+0.73\times T-2.08\times SR+4.37\times t-0.53\times RPM \quad (with\ r = 0.64) \qquad (10)$$

$$MUFAs=232.20-36.19\times PS-0.86\times T-0.23\times SR+0.34\times t-0.32\times RPM \quad (with\ r = 0.72) \qquad (11)$$

$$PUFAs=47.38-0.72PS-0.11T-0.06SR+0.013t-0.04RPM \quad (with\ r = 0.44) \qquad (12)$$

CONCLUSION

It could be concluded that, optimum lipid extraction conditions for biodiesel production from *U. fasciata* with high quantity and quality were; <0.16 mm algal particle size, 55^{o}C extraction temperature, 25:1 v/w solvent to solid ratio, 60 min extraction time and mixing intensity at 250 rpm, using chloroform: methanol: H_2O (2: 2: 1) solvent mixture. On the other hand, lipid extraction kinetic study has proved to follow second-order model. The initial extraction rate (h) was 6.169 ml g^{-1} min^{-1}; the extraction capacity (Cs) was 31.036 g ml^{-1}; the second order extraction constant (k) was 0.0064 ml g^{-1} min^{-1} and the coefficient of determination (R^2) was 0.9994.

There is no conflict of interest.

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