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Full Length Article

Performance evaluation of different harvesting methods and cultivation media on the harvesting efficiency of microalga and their fatty acids profile

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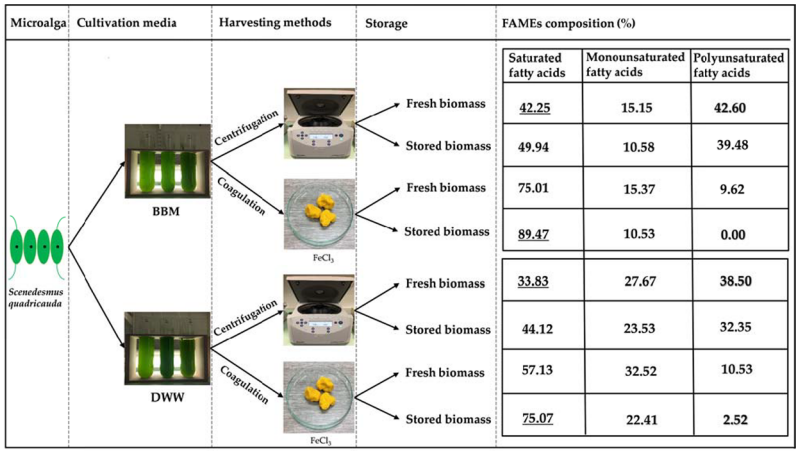
GRAPHICAL ABSTRACT



FAMEs composition (%)		
Saturated fatty acids	Monounsaturated fatty acids	Polyunsaturated fatty acids
42.25	15.15	42.60
49.94	10.58	39.48
75.01	15.37	9.62
89.47	10.53	0.00
33.83	27.67	38.50
44.12	23.53	32.35
57.13	32.52	10.53
75.07	22.41	2.52

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ABSTRACT

Harvesting techniques directly affect the quality and quantity of microalgal biomass as well as the total cost of final products. In this study, the harvesting efficiency of freshwater microalga, *Scenedesmus quadricauda*, from synthetic medium (Bold's Basal Medium, BBM) and real wastewater (dairy wastewater, DWW) was studied using ferric chloride ($FeCl_3$). For this purpose, the effects of three variables namely solution pHs (3, 7, and 11), coagulant concentrations (100, 400, and 700 mg/L), and reaction times (5, 10 and, 15 min) on the harvesting efficiency of microalgae were evaluated. The highest harvesting efficiencies from BBM and DWW media were achieved as 95.75% (pH 11, 700 mg/L coagulant concentration, and 10 min reaction time) and 87.11% (pH 7, 700 mg/L coagulant concentration, and 15 min reaction time). The harvested microalgal biomasses were subsequently used for the lipid extraction. The analysis of lipids profile showed that the cultivation media (BBM and DWW), harvesting methods (coagulation and centrifugation), and biomass storage time (one day and two months) significantly affected the percentages of saturated fatty acids (SFAs) and poly unsaturated fatty acids (PUFAs) in microalgal lipids. The findings of this study revealed that the harvesting methods and cultivation media are two major factors that remarkably influenced the harvesting efficiency of microalga and the fatty acids of microalgal lipids as well.

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1. Introduction

Microalgae are bioresources that can be used as sustainable feedstocks for the production of various valuable bioproducts including biofuels. Although microalgae (with high growth rate and lipid yield) have been highlighted as a promising feedstock for biodiesel production (by transesterification [1] and pseudo-catalytic transesterification methods [2,3]), but the process is not considered economically feasible yet. Production of microalgal biomass in a cheap and suitable medium such as wastewater can reduce the cost of final products such as biodiesel. Although, cultivation of microalgae in wastewater offers environmental (wastewater treatment) and economical (reducing the cost of required freshwater and nutrients) merits, but harvesting of microalgal biomass from growth media is still an obstacle in the commercial production of microalgae, especially on large scale. According to the literature, the cost of microalgae harvesting is approximately 20–30% of the total expenses of biofuel production process [4]. This is due to the small size of microalgal cells (< 30 μm), low mass concentration, low specific gravity (close to cultivation media), and negative surface charge of microalgae [5,6].

Harvesting methods are not only important from economical point of view, but these can also affect the downstream process of microalgae (e.g. lipid extraction) [7]. Several techniques have been tested to maximize the harvesting efficiency of microalgae with varying degree of success [8]. Coagulation/flocculation, auto-flocculation, electrolytic process, bio-flocculation, flotation, electrical based processes, filtration, centrifugation, and foam fractionation are some of the commonly used methods for microalgae harvesting [9]. Among these, coagulation is an efficient and relatively cheap and simple separation method for harvesting the microalgae from larger scale including ponds and photobioreactors [10]. Dissolution of coagulants such as polyaluminum chloride, ferric, and magnesium chloride provides positively charged ions of Al^{3+}, Fe^{3+} and Mg^{2+}, which neutralizes negative charge of microalgal surface [11]. By spreading the coagulants among microalgal cell, flocs are formed and consequently, microalgae will be precipitated in a short time [12]. Although in many previous studies, different types of coagulants have been investigated for harvesting the microalgae, but most of them have been performed in synthetic media such as Bold's Basal Medium (BBM) [13]. Despite the importance of microalgae harvesting from wastewater, there are less reports on microalgae coagulation in real wastewater. The characteristics of real wastewater and formulated (synthetic) media are different, which can affect the coagulation efficiency of microalgae, even with the use of same coagulant. In addition, dosage of the coagulant/flocculant, pH value of culture media, reaction time, ionic strength, biomass concentration, zeta potential, degree of salinity, and mixing speed are some of the physicochemical and operation parameters that affect the coagulation efficiency of microalgae from a culture media [7,10].

Assessment of the quality and quantity of fatty acids of microalgae, harvested by coagulation, is another research gap in the field of microalgal biodiesel production that has been rarely studied. This is important because fatty acid methyl esters (FAMEs) (the main compound of biodiesel) are synthesized by transesterification of triglycerides extracted from the microalgal biomass. Triacylglycerols as the dominant fraction (90% to 98%) of lipids are composed of one molecule of glycerol [$C_3H_8O_3$] and three molecules of fatty acids [R-COOH], where 'R' represents a long hydrocarbon chain and 'COOH' is carboxyl functional group. The features of hydrocarbon chain, such as number of carbon and degree of saturation, directly control the properties of biodiesel such as cetane number, oxidation stability, flash point, cold flow property, calorific value, viscosity, and density [14]. Metal-based coagulants such $FeCl_3$ can significantly affect the lipids profile of microalgal biomass harvested by coagulation. As an underlined hypothesis of this study, these effects are expected to be more intense in stored biomass as compared to the fresh biomass due to oxidative ability of iron [7]. In the present study, the lipid profile of microalgae was compared between biomass harvested by coagulation and centrifugation (as control) harvesting methods.

Overall, the main aims of this study were to (i) compare the coagulation efficiency of a selected microalga species (*Scenedesmus quadricauda*), cultivated in synthetic medium (BBM) and real wastewater (dairy wastewater, DWW) using the same coagulant ($FeCl_3$), (ii) examine the effect of coagulant concentrations, pHs of the medium and reaction times on the coagulation efficiency, (iii) simulate the pilot-scale conditions of microalgae coagulation in 4 L column photobioreactors, (iv) study the effect of cultivation media (BBM and DWW), harvesting methods (centrifugation and coagulation) and biomass storage time (short term and long term) on fatty acids profile of microalga. The findings of this study present new information about the efficiency of microalgae harvesting from wastewater via coagulation as well as short- and long-term influences of coagulant on microalgae lipids profile.

2. Materials and methods

2.1. Microalga strain and cultivation conditions

The stock of freshwater microalga, *Scenedesmus quadricauda*, was purchased from Culture Collection of Algae and Protozoa (CCAP, Scotland, UK). Microalgae was cultivated in BBM and DWW. BBM was prepared according to CCAP instructions that have been reported in our previous work [15]. DWW was collected from a local factory. The characterization of DWW and storage conditions have been reported in our previous study [16]. Microalga was cultivated for 12 days under continuous illumination with 90–100 μmol photon/m^2/s. The optical density (OD) of microalga cells was measured at 680 nm using a UV–Visible spectrophotometer (UV-2401PC). The OD concentration of microalga was converted to dry mass by the linear formula as follows:

$$\textit{Scenedesmus quadricauda}\ \text{dry mass (g/L)} = a \times OD_{680} - b \quad (1)$$

where a and b are the constants of formula and OD_{680} is the optical density of microalga in 680 nm. Accordingly, the same concentration of microalga solutions as 0.58 g/L in BBM and DWW were prepared for the coagulation experiment. The lower concentrations were concentrated by centrifugation and the higher concentrations were diluted by the addition of BBM or DWW to the culture.

2.2. Coagulation experiments

The interactions of three variables viz., coagulant concentrations (100, 400, and 700 mg/L), solution pHs (3, 7, and 11) and reaction times (5, 10, and 15 min) on the harvesting efficiency of microalgal biomass from BBM and DWW were investigated through 27 experimental units (Table 1). Coagulation experiments were performed in 15 mL polypropylene tubes containing 10 mL of microalga culture. For this purpose, three highly concentrated stock solutions of $FeCl_3{\cdot}6H_2O$ with concentrations of 48.40, 193.61, and 338.82 g/L were prepared. The pH of cultivation media (BBM and DWW) was adjusted to pH 3, 7, and 11 and equal volume of 9.90 mL was added to each 15 mL polypropylene. Then 100 μL of the highly concentrated solutions of $FeCl_3$ was added to 9.90 mL of BBM or DWW containing microalga to provide 100 mg/L, 400 mg/L and 700 mg/L of coagulant (Fe^{3+}), respectively. Thereafter, the tube was vortexed for 30 sec and kept vertically unmoved for certain reaction time. After the intended contact time (5, 10, and 15 min), a sample was taken from the middle of solution and the optical density of microalga was measured by UV-spectrophotometer. Optical density of microalga was converted to dry weight according to Eq. (1) and consequently, the harvesting efficiency of microalga was calculated according to Eq. (2) as follows:

$$\text{Harvesting efficiency}(\%) = \frac{C_i - C_e}{C_i} \times 100 \quad (2)$$

Table 1
Experimental design of microalga harvesting from BBM and DWW media by coagulation: a1 = pH 3, a2 = pH 7, a3 = pH 11, b1 = 100 mg/L coagulant concentration, b2 = 400 mg/L coagulant concentration, b3 = 700 mg/L coagulant concentration, c1 = 5 min reaction time, c2 = 10 min reaction time, and c3 = 15 min reaction time.

Run	Interactions	Run	Interactions	Run	Interactions
1	a1b1	10	a1c1	19	b1c1
2	a1b2	11	a1c2	20	b1c2
3	a1b3	12	a1c3	21	b1c3
4	a2b1	13	a2c1	22	b2c1
5	a2b2	14	a2c2	23	b2c2
6	a2b3	15	a2c3	24	b2c3
7	a3b1	16	a3c1	25	b3c1
8	a3b2	17	a3c2	26	b3c2
9	a3b3	18	a3c3	27	b3c3

where C_i and C_e are the concentrations of microalga before and after the addition of coagulant to BBM and DWW media. Each experimental unit was performed in duplicate and the mean values were used to plot the figures by MATLAB software (R2017b).

2.3. Pilot scale harvesting of microalga

The coagulation of microalga with $FeCl_3$ was investigated in 4-liter vertical column photobioreactors (vcPBRs) to evaluate the performance of harvesting method on a large-scale. For this purpose, vcPBRs with 1.4 m length and 62 mm inner diameter were used for the cultivation and coagulation of microalga. Each column had two inlets at the bottom for aeration and taking sample and one outlet on the top for the evacuation of gas. The vcPBRs were filled with BBM and DWW media (separately) and aerated with atmosphere air (0.04% CO_2 concentration). After 12 days of cultivation, the concentration of microalga in BBM and DWW was adjusted to 0.58 g/L, as explained in section 2.1. The middle levels of coagulant concentration (400 mg/L) and solution pH (7) were selected for the coagulation experiment in vcPBRs. Coagulant was added from the top of vcPBRs and mixed with the media via aeration for 30 sec. Then, aeration was stopped and the coagulation in each vcPBRs was monitored visually by taking photos at predetermined time intervals (0, 5, 10 and 15 min). Microalgal biomass, harvested by the coagulation and centrifugation, was preserved for the next steps of lipid extraction and fatty acids analysis.

2.4. Lipids extraction and fatty acids analysis

Lipid extraction from microalgal biomass was performed according to Bligh and Dyer method with minor modifications [17]. The lipids were extracted from the fresh (one day after harvesting) and stored (two months after harvesting) biomasses of microalga, cultivated in BBM (MA-BBM) harvested by centrifugation, microalga cultivated in BBM (MA-BBM) harvested by coagulation, microalga cultivated in DWW (MA-DWW) harvested by centrifugation, and microalga cultivated in DWW (MA-DWW) harvested by coagulation. Lipids were extracted from 50 mg freeze-dried microalgal biomass by the addition of 7.5 mL chloroform and methanol in the ratio of 1:2. The mixture was vortexed for one min and sonicated for 30 min. The supernatant was separated and the procedure was repeated with the half volume of solvents (1.25 mL chloroform and 2.5 mL methanol). In the next step, all supernatants were collected in one tube and 2 mL 1% NaCl was added to the extracted solution and agitated for five min. Subsequently, the mixed solution was centrifuged to separate the dark green phase including microalgal lipids. Finally, the solution was kept in an oven at 55 °C for 24 h to evaporate chloroform and microalgal lipids were collected.

To analyze the fatty acids profile, microalgal lipids were converted to fatty acid methyl esters (FAMEs) by acid-catalyzed transesterification method. 2 mL methanol, 0.092 g sulfuric acid (98%) and C17:0 were added to the extracted lipid [18]. The mixture was kept in a water bath at 55–60 °C for 30 min and vortexed for five min. To collect the FAMEs, 1 mL hexane was added to the mixture and vortexed for one min. 200 μL of supernatant containing FAMEs was added to Certified Vial Kit 9 mm (Fisherbrand™) to analyze the fatty acids profile of microalgal lipid by gas chromatography (GC). The analysis of FAMEs was performed in a chromatograph Agilent Technologies 7890A equipped with a capillary column of fused silica DB-Wax (0.1 mm × 10 m, 0.1 μm standard film) and a flame ionization detector (FID). Helium was used as a carrier gas at a flow rate of 30.34 cm/s. The temperatures of both injector and detector were set at 250 °C. The oven temperature was initially set at 40 °C for 0.5 min and programmed to increase to 195 °C and 205 °C at a rate of 25 °C/min and 3 °C/min, respectively. Finally, the temperature was set at 240 °C for 4 min. Supelco 37 Component FAME Mix (Sigma-Aldrich) was used as external standard.

2.5. Statistical analysis

The data were analyzed using IBM SPSS Statistics 23 (IBM corporation). The normality of data and the homogeneity of variance were confirmed with Kolmogorov–Smirnov and Levene's tests, respectively, before statistical significance analysis. Two-way ANOVA was applied to investigate the significant effect of three independent variables viz., coagulant concentrations, pHs of the medium and reaction times on the coagulation efficiency (dependent variable) at probability level < 0.05. Mean values were analyzed by a multiple-dominant Duncan test to identify the significant differences between experimental values.

3. Results and discussion

3.1. Microalga harvesting from BBM and DWW media

3.1.1. Effect of pH

Figs. 1(a) and 2(a) represent the simultaneous effects of different pHs (3, 7, and 11) and coagulant concentrations (100, 400, and 700 mg/L) on the harvesting efficiency of *S. quadricauda* from BBM and DWW media, at constant reaction time (10 min). In the BBM medium, with the lowest (100 mg/L) and the highest (700 mg/L) coagulant concentrations, the harvesting efficiency increased as pH increased from 3 to 11. As can be seen from Fig. 1(a), the highest harvesting efficiency was obtained as 95.75% at pH 11 with coagulant concentration of 700 mg/L. In DWW medium with 100 mg/L and 400 mg/L coagulant concentration, the harvesting efficiency decreased from 80.89% to 9.37% and from 66.66% to 23.99%, respectively as pH increased from 3 to 11. While in DWW medium with 700 mg/L coagulant, the maximum harvesting efficiency of 85.04% was observed at pH 7. Overall, as it can be seen from Table 2 that the harvesting efficiency increased significantly ($p < 0.05$) as the initial pH increased in BBM medium, whereas biomass recovery was negatively affected ($p < 0.05$) by the highest basic pH in the DWW medium.

Figs. 1(b) and 2(b) demonstrate the concurrent effect of the different pHs (3, 7, and 11) and reaction times (5, 10, and 15 min) on the harvesting efficiency of microalga from the BBM and DWW media, respectively, at the constant coagulant concentration of 400 mg/L. In the BBM medium, the maximum harvesting efficiency was obtained as 60.97% at pH 7 within 10 min, while the highest harvesting efficiency in DWW medium was achieved as 85.24% at pH 3 after 10 min. Thus, in the DWW medium, the harvesting efficiency decreased by increasing the pH in response to the reaction time, whereas the harvesting efficiency from the BBM medium at different reaction times increased as pH increased from 3 to 7 and then it decreased by increasing the pH from 7 to 11.

In DWW medium, the highest harvesting efficiency was obtained as 85.24% (10 min reaction time, pH 3, 400 mg/L coagulant

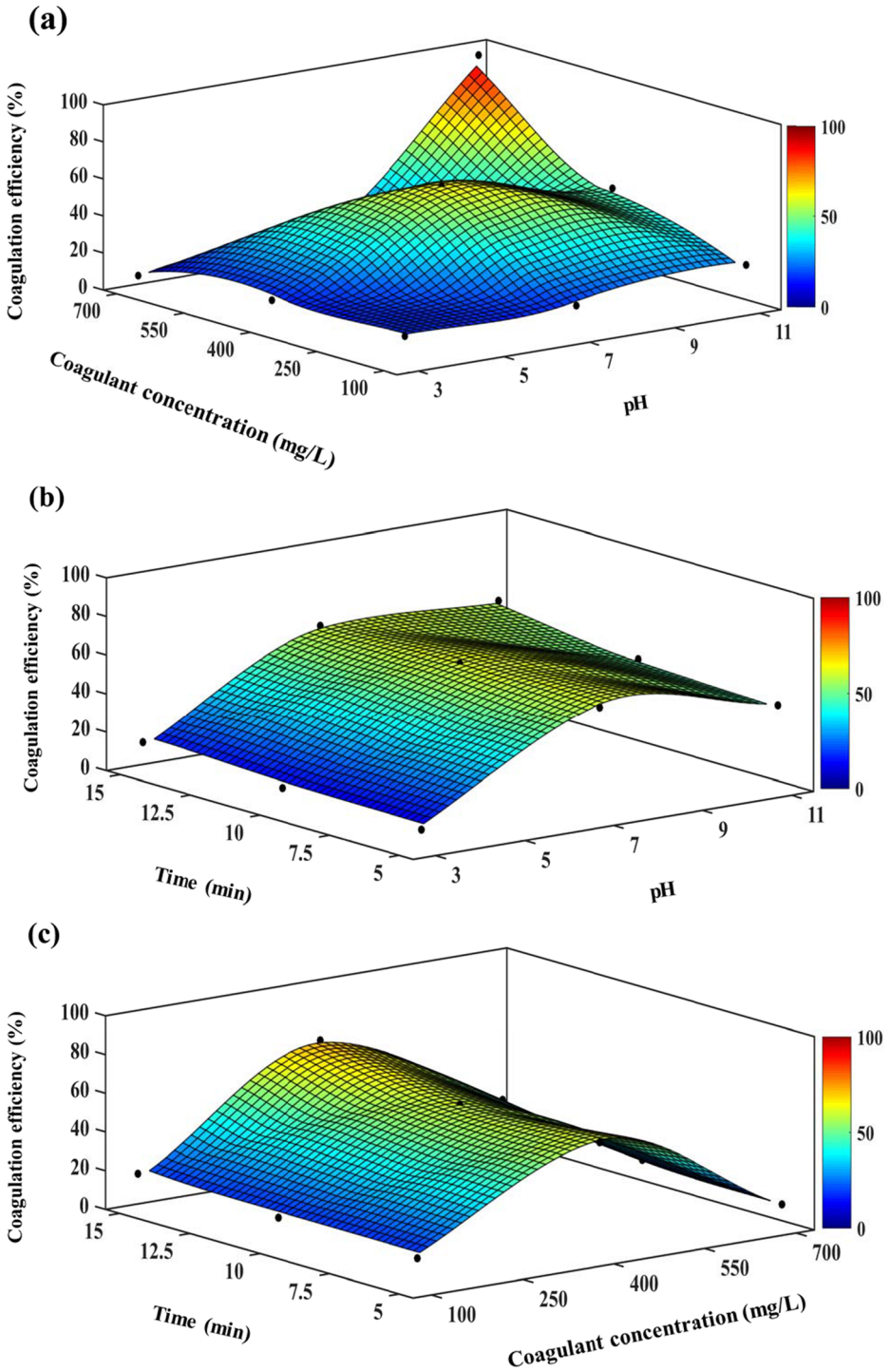

Fig. 1. The effects of variables viz., coagulant concentrations (100, 400, and 700 mg/L), pHs (3, 7, and 11), and reaction times (5, 10, and 15 min) on coagulation efficiency (%) of microalga from BBM medium: (a) interaction effects of coagulant concentrations and pHs at constant reaction time (10 min), (b) interaction effects of reaction times and pHs at constant coagulant concentration (400 mg/L), and (c) interaction effects of reaction times and coagulant concentrations at constant pH (7).

concentration) and 85.04% (10 min reaction time, pH 7, 700 mg/L coagulant concentration). At pH 3, the available protons in the medium react with the carboxylate ions of microalgal cells and flocculate them by the neutralization of their negative charges [19]. In addition, at pH 3, the abundant chemical form of dissolved iron is Fe^{3+}, which increases the coagulation efficiency of microalgal cells. Moreover, the presence of other divalent and trivalent cations in the medium, originated from milk, dairy production, and washing process may facilitate the formation of large flocs [20]. Similarly, Seo et al. [21] reported the maximum flocculation of *Chlorella* sp. KR-1 at pH 3 with 200 mg/L $FeCl_3$. Liu et al. [19] also stated that *Chlorocuccum nivale* UTEX 2765, *Chlorocuccum ellipsoideum* UTEX 972, and *Scenedesmus* sp. JNU-49 were harvested > 90% at pH 4 after 15 min. In another study, Kumar et al. [22] demonstrated > 99% harvesting of *Ascochloris* sp ADW007 from DWW medium at pH 3.69 after 5 min reaction time using $FeCl_3$.

The positively charged precipitates of ferric hydroxide are formed at pH < 8. These precipitates are attracted to the negatively charged

Table 2
The effect of different levels of pH, coagulant concentration, and time on harvesting efficiency (%) of *S. quadricauda* from the BBM and DWW media.

Medium	Factors				*P*-value		
		Coagulant concentration (mg/L)					
	pH	100	400	700	pH	$FeCl_3$	pH × $FeCl_3$
BBM	3	16.81 ± 0.34^{aA}	15.40 ± 0.30^{aA}	7.89 ± 1.70^{aB}	< 0.001	< 0.001	< 0.001
	7	17.70 ± 0.78^{aA}	61.28 ± 0.35^{bB}	14.70 ± 0.07^{bC}			
	11	24.04 ± 1.73^{bA}	44.60 ± 1.22^{cB}	95.75 ± 1.39^{cC}			
DWW	3	80.89 ± 1.29^{aA}	66.66 ± 9.60^{aA}	73.98 ± 0.27^{aA}	< 0.001	< 0.001	< 0.001
	7	5.48 ± 1.76^{bA}	62.99 ± 2.51^{aB}	85.04 ± 0.04^{bC}			
	11	9.37 ± 0.95^{bA}	23.99 ± 0.51^{bB}	38.29 ± 0.30^{cC}			
		Time (min)					
	pH	5	10	15	pH	Time	pH × Time
BBM	3	11.34 ± 0.07^{aA}	12.04 ± 0.10^{aA}	14.80 ± 2.17^{aA}	< 0.001	< 0.001	< 0.001
	7	59.15 ± 0.34^{bA}	60.97 ± 1.02^{bA}	59.68 ± 0.20^{bA}			
	11	44.72 ± 0.44^{cA}	47.63 ± 0.27^{cB}	56.99 ± 0.14^{bC}			
DWW	3	63.95 ± 1.56^{aA}	85.24 ± 0.71^{aB}	80.92 ± 0.85^{aC}	< 0.001	< 0.001	< 0.001
	7	61.72 ± 2.75^{aA}	65.08 ± 0.45^{bAB}	70.67 ± 2.92^{bB}			
	11	20.96 ± 1.80^{bA}	23.29 ± 0.21^{cA}	23.70 ± 0.24^{cA}			
		Coagulant concentration (mg/L)					
	Time (min)	100	400	700	Time	$FeCl_3$	Time × $FeCl_3$
BBM	5	16.79 ± 0.37^{a}	60.73 ± 2.72^{c}	12.76 ± 2.07^{a}	0.001	< 0.001	0.137
	10	17.00 ± 0.54^{a}	59.27 ± 1.87^{c}	14.96 ± 0.44^{a}			
	15	18.95 ± 0.65^{ab}	71.92 ± 2.58^{d}	25.09 ± 7.91^{b}			
DWW	5	2.72 ± 0.10^{a}	61.72 ± 2.75^{cd}	85.02 ± 0.27^{e}	0.008	< 0.001	0.110
	10	8.12 ± 0.88^{b}	60.16 ± 0.54^{c}	83.70 ± 1.93^{e}			
	15	8.94 ± 0.81^{b}	65.72 ± 4.07^{d}	87.11 ± 0.03^{e}			

Data are presented as mean ± Standard Deviation (SD) (n = 2).
Values in the same column with different small letters (a-c) are significantly different ($p < 0.05$) (pH × $FeCl_3$ and pH × Time interactions).
Values in the same raw with different capital letters (A-C) are significantly different ($p < 0.05$) (pH × $FeCl_3$ and pH × Time interactions).
Values with different small letters (a-e) are significantly different ($p < 0.05$) (Time × $FeCl_3$ interaction).

microalga cells and act as electrostatic bridge to bind microalgal cells together into bigger flocs [20]. Baharuddin et al. [23] used the residual of *Moringa oleifera* biomass after oil extraction as the coagulant for the harvesting of *Nannochloropsis oculata*. They reported the highest coagulation efficiency as 93.70% at pH 7 after 150 min. In another study, Wang et al. [24] applied 25 mg/L magnetic iron oxide and 0.1 mg/L cationic polyacrylamide as a bridging polyelectrolyte for harvesting the *Botryococcus braunii*. In their study, the highest coagulation efficiency was acquired as 95% at pH 7 and 10 min reaction time. Further, Aljuboori et al. [25] reported 87% coagulation efficiency by the addition of 9.54 g/L $ZnCl_2$ to the culture of *S. quadricauda* at pH 7.

In the BBM medium, the harvesting efficiency of *S. quadricauda* increased as the pH increased. The highest harvesting efficiency was achieved as 95.75% at pH 11 with maximum coagulant concentration of 700 mg/L. It might be related to the effect of cations such as magnesium in BBM medium. Vandamme et al. [26] studied the flocculation of *Chlorella vulgaris* from the synthetic WC medium by the increasing of solution pH to 11. Magnesium hydroxide is formed in the medium by increasing the pH. Some Mg^{2+} of magnesium hydroxide in the crystal structure are replaced with trivalent cations such as Fe^{3+} or Al^{3+}. Consequently, the crystal forms of layered double hydroxide with positive charges are developed in the medium [27], which participate in destabilization and flocculation of microalgal cells by neutralizing their negative surface charge and decreasing the energy barrier between them [26]. Castrillo et al. [28] stated that the harvesting efficiency of *Scenedesmus obliquus* and *Chlorella vulgaris* increased as the pH increased to higher than 10.8. Pérez et al. [29] also reported 100% biomass recovery of *Skeletonema Costatum* and *Chaetoceros Gracilis* microalgae species at highly basic pH of 11, 11.5, and 12. Moreover, Fan et al. [30] demonstrated the effective (> 90%) flocculation of *Chlamydomonas reinhardtii* at pH 11 by the addition of divalent cations such as Ca^{2+} and Mg^{2+}. Hence, cationic metal coagulants such as $FeCl_3$ with the ability to form metal hydroxide can be used efficiently for the harvesting of *S. quadricauda*.

3.1.2. Effect of coagulant concentrations

Figs. 1(a) and 2(a) depict the simultaneous effects of coagulant concentrations and pH on the harvesting efficiency of *S. quadricauda* from BBM and DWW media at constant reaction time (10 min). As it can be seen from Fig. 1(a), the harvesting efficiency of microalga from acidic, neutral and basic BBM solution was different in response to the coagulant concentration. Increasing the concentration of coagulant at pH 3 decreased the harvesting efficiency. At pH 7, the harvesting efficiency increased from 17.70% to 61.28% with the increase of coagulant concentration from 100 mg/L to 400 mg/L and then it decreased to 14.70% at 700 mg/L coagulant concentration (Table 2). At pH 11, by increasing the coagulant concentration from 100 mg/L to 700 mg/L, the harvesting efficiency increased from 24.04% to 95.75%. Fig. 2(a) shows the harvesting efficiency of microalga from DWW in response to increasing the coagulant concentrations at different pH. As can be seen from this figure, in acidic pH of 3, the higher coagulation efficiencies of 80.89% and 73.98% were observed at lower (100 mg/L) and higher (700 mg/L) coagulant concentrations as compared to 66.66% at the middle concentration (400 mg/L). In case of neutral and basic pHs, coagulation was increased from 5.48% to 85.04% (at pH 7) and from 9.37% to 38.29% (at pH 11) as coagulant concentrations increased from 100 mg/L to 700 mg/L.

Moreover, simultaneous effect of coagulant concentrations and reaction time on microalga harvesting was investigated at constant pH 7. Figs. 1(c) and 2(c) illustrate the effects of different coagulant concentrations and reaction times on the harvesting efficiency from BBM and DWW media, respectively. In the BBM medium (Fig. 1(c)), the same pattern of harvesting efficiency was observed in response to increasing the coagulant concentrations at different reaction times (5, 10, and 15 min). First, the harvesting efficiency increased as the coagulant concentration increased from 100 mg/L to 400 mg/L and then it decreased at the higher concentration of 700 mg/L. In DWW, the increase of coagulant concentrations from 100 mg/L to 700 mg/L improved the harvesting efficiency of microalga at different tested contact times (Fig. 2(c)). Hence, in the DWW medium, the highest harvesting efficiency was achieved as 87.11% by the maximum coagulant

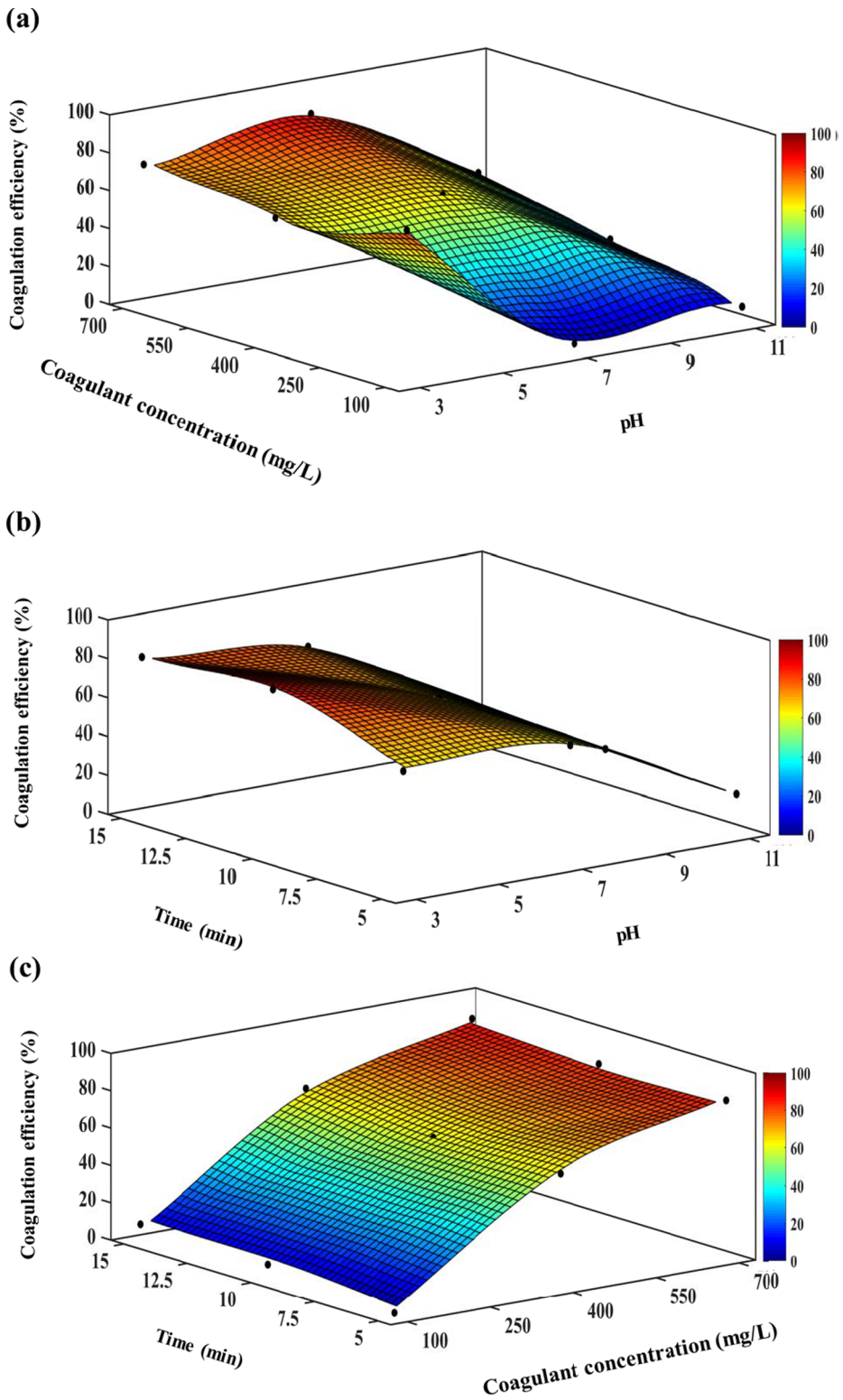

Fig. 2. The effects of variables viz., coagulant concentrations (100, 400, and 700 mg/L), pHs (3, 7, and 11), and reaction times (5, 10, and 15 min) on coagulation efficiency (%) of microalga from DWW medium: (a) interaction effects of coagulant concentrations and pHs at constant reaction time (10 min), (b) interaction effects of reaction times and pHs at constant coagulant concentration (400 mg/L), and (c) interaction effects of reaction times and coagulant concentrations at constant pH (7).

concentration of 700 mg/L after 15 min of contact time.

The results of this study showed that depending on the cultivation media (BBM and DWW), the pHs of media (3, 7, and 11), and the reaction time (5, 10, and 15 min), the increase of coagulant concentration might increase or decrease the harvesting efficiency. In some cases, the harvesting efficiency was minimum in the lowest coagulant concentration (100 mg/L) as compared to the higher concentrations (400 mg/L and 700 mg/L), among the experimental units. It might be related to the insufficient collision between coagulant ions and microalgal cells at the lower concentration of coagulant. Therefore, higher

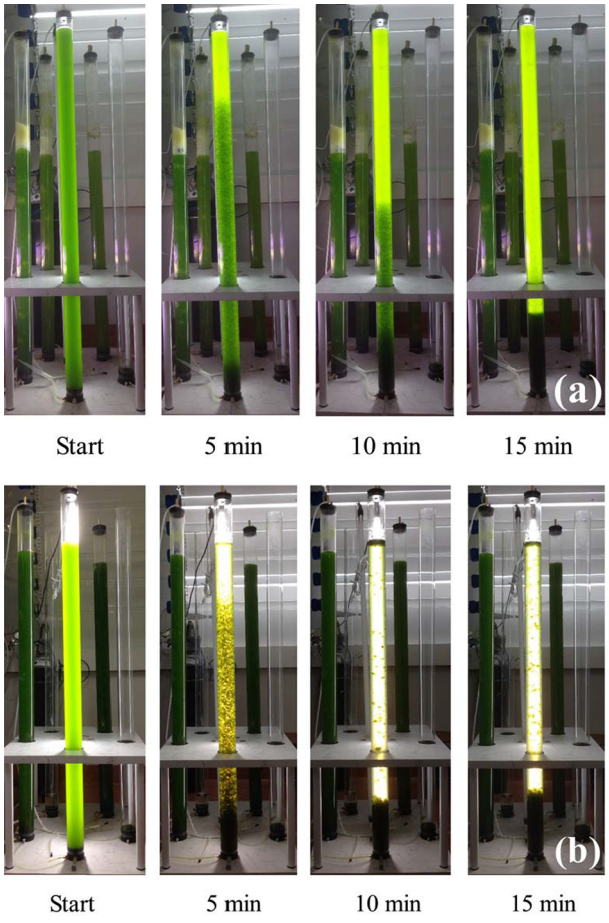

Fig. 3. Pilot-scale of microalga coagulation in 4 L column photobioreactors during 15 min reaction times: BBM medium (a) and DWW (b).

concentrations of coagulant are needed to neutralize the negative surface charges of microalgal cells. Improved harvesting efficiency at higher concentrations of coagulant could be related to the increase in electrostatic interaction, the main driving force of coagulation, between coagulants and microalgal cells, which destabilizes them and eventually generates more numbers of flocs [31]. Gupta et al. [11] showed that at pH 7, an increase in alum concentration from 50 mg/L to 300 mg/L resulted in an enhancement of the microalgal flocculation efficiency from 59% to 99%. Chatsungnoen and Chisti [32] also demonstrated that the harvesting efficiency increased as the initial flocculant concentration increased from 0 to 300 mg/L. The authors reported that the maximum recovery of freshwater microalga *Choricystis minor* with the initial biomass concentration of 1.0 g/L was attained as 95% by the addition of 250 mg/L of ferric chloride or 275 mg/L of aluminum sulfate.

Nevertheless, the results of this study revealed that the higher concentrations of highly charged conventional coagulant can be undesirable for the destabilization of microalgal cells, which is mostly caused by two reasons. First, the coagulant with high concentration may highly cover the microalgal surface without leaving enough vacant sites for electrostatic attraction between cells with negative charges and coagulants with positive charges. Second, electrostatic repulsion between positive charges of coagulants could decrease the harvesting efficiency of microalgae in the media with the high concentrations of coagulant [33].

3.1.3. Effect of reaction times

Figs. 1(b and c) and 2 (b and c) illustrated the effect of reaction time on the harvesting efficiency of microalga from BBM and DWW, respectively at different pH and coagulant concentrations. As can be seen from Fig. 1(b), increasing the reaction time from 5 min to 15 min did not significantly increase the harvesting efficiency from BBM medium at pH 3 (from 11.34% to 14.80%) and pH 7 (from 59.15% to 59.68%) ($p < 0.05$). However, in the BBM medium with the higher pH (11), the harvesting efficiency increased from 44.72% to 56.99% as the reaction time increased from 5 min to 15 min. Longer reaction time (15 min) also significantly increased the harvesting efficiency of microalga from BBM medium with the higher concentration of coagulant (700 mg/L) as compared with the lower concentration (100 mg/L). It is increased from 16.79% to 18.95% with 100 mg/L coagulant and from 12.76% to 25.09% with 700 mg/L coagulant as reaction time increased from 5 to 15 min (Fig. 1(c)). In DWW, different trends of harvesting efficiency were observed in acidic, neutral and basic solution in response to increase the reaction time (Fig. 2(b)). In DWW, at pH 3, the harvesting efficiency was increased from 63.95% to 85.24% and then decreased to 80.92% as reaction time increased from 5 min to 10 min and then 15 min, respectively. At neutral DWW medium, the harvesting efficiency increased gently from 61.72% to 70.67% in response to increase the reaction time from 5 min to 15 min. In DWW with pH 11, significant difference was not observed ($p < 0.05$) among different reaction times. The highest harvesting efficiency in DWW with different concentrations of coagulant was observed at the maximum reaction time of 15 min (Fig. 2(c)). The reason of different patterns of harvesting efficiency between BBM and DWW media in response to the different reaction times might be due to the variation in the constituents of these media [34].

In this study, the maximum harvesting efficiency of microalga from BBM (95.75%) and DWW (87.11%) was obtained within 10 min and 15 min. Similar to the findings of this study, Khadim et al. [35] reported up to 90% harvesting efficiency of *Dunaliella salina* after 15 min of reaction time by the addition of 1.0 mM potash alum to the medium, while increasing the reaction time longer than 15 min did not increase the coagulation efficiency. In the same study, 1.0 mM $FeCl_3{\cdot}6H_2O$ also resulted in 86% harvesting efficiency within 15 min of reaction time. In another study by Peng et al. [36], the highest coagulation efficiency of *Chlorella pyrenoidosa* was observed after 10 to 20 min of reaction time. Lee et al. [37] used the alkaline electrolyzed water (AEW) for harvesting the marine microalga *Tetraselmis* sp. and they evaluated the flocculation process for 25 min. They observed that all the formed flocs settled after 10 min of reaction time, regardless of the flocculant concentration. The results of the current study elucidated that ferric chloride was an efficient coagulant because it harvested microalgal cells from both media in a short time of 10 min to 15 min. However, the sizes of formed flocs are not persistent when time increases. Nan et al. [38] stated that flocs would become weaker and break under the mechanical stirring as time increases.

In the current study, a pilot-scale study of microalga coagulation was performed in 4-L column photobioreactors filled with BBM and DWW media. The pH of media was adjusted to 7 and the concentration of coagulant was kept as 400 mg/L in each column. Fig. 3(a-b) depicts the coagulation of *S. quadricauda* in BBM and DWW within 15 min after the addition of coagulant. As evident in this figure, the harvesting efficiency of microalga increased as the coagulation time increased. In both media, it reached to the maximum point after 15 min. The results of pilot-scale study were the same as the findings of batch-scale, therefore, it demonstrates that the findings of this study are applicable at large-scale also. Similarly, de Oliveira Corrêa et al. [39] reported that the coagulation efficiency of *Desmodesmus subspicatus* was around 99% in the jar test and when the experiment was scaled up using a 100-L flat plate PBR, the recovery efficiency was close to 98%. Yang et al. [40] also harvested microalgal biomass in a 60-L flat plate PBR by a natural flocculant, produced from *Moringa oleifera* seeds. The harvesting efficiency of *M. oleifera* was 81% for *C. vulgaris* and 92% for *Senedesmus obliquus*. In another study, Bhattacharya et al. [41] used a 10-L photobioreactor with working volume of 8 L to flocculate *Chlorella*

pyrenoidosa with *Aspergillus fumigatus*. The harvesting rate of C. *pyrenoidosa* increased up to around 90% as time of flocculation process increased.

3.2. Microalgal lipids profile

Microalgal cultivation media and harvesting methods can significantly affect the quality and quantity of microalgal lipids and consequently cost-effective biodiesel production from microalgae. In this study, the effect of microalgal cultivation media (BBM and DWW), harvesting methods (centrifugation and coagulation) as well as biomass storage time was investigated on microalgal lipid profile. Although, C16, C18, and their derivatives were the dominant fatty acids; but their percentages were significantly different depending on the cultivated media, harvesting methods, and storage time, as explained in the following sub-sections.

3.2.1. Effect of cultivation media

Table 3 represents the lipids profile of microalga, cultivated in BBM (MA-BBM) and DWW (MA-DWW) media. Apart from the harvesting of microalgal biomass by centrifugation or coagulation methods, lipid profile was different between MA-BBM and MA-DWW. In the case of harvesting of microalga by centrifugation, SFAs of MA-BBM were found as 42.25%, which were higher than the amount of SFAs in MA-DWW as 33.83%. On the other hand, monounsaturated fatty acids (MUFAs) values were higher in the microalgal lipids, centrifuged from DWW medium (27.76%) as compared to the microalgal lipids, centrifuged from BBM medium (15.15%). The similar trend of higher SFAs in MA-BBM and higher MUFAs in MA-DWW was observed in the microalgal biomasses, harvested by coagulation as well. The percentage of SFAs as 75.01% in MA-BBM decreased to 57.13% in MA-DWW, whereas the percentage of MUFAs as 15.37% in MA-BBM increased to 32.52% in MA-DWW. As can be seen from Table 3, the reason of decreasing SFAs in MA-DWW as compared to MA-BBM is mainly due to the decrease in the concentration of C16:0 and C18:0 fatty acids. On the other hand, increasing the concentration of MUFAs in MA-DWW as compared to MA-BBM is related to the increase in the concentration of C18:1. Two genes namely, stearoyl-ACP desaturase (SAD) and fatty acyl-ACP thioesterase A (FATA) are responsible for the synthesis of oleic acid (18:1 *cis*-9). The first one (SAD) synthesizes MUFAs by adding double bond to stearic acid, a SFA [42,43]. The second one (FATA) terminates the elongation of fatty acid chain by separating the synthesized monounsaturated acyl-ACP into free fatty acids and ACP [43]. Beside the culture conditions such as light, pH and temperature, the composition of cultivation media also has a significant effect on microalgal growth and lipid profile [44]. The concentration of nutrients such as nitrogen compounds in cultivation media may affect the activity of the aforementioned genes. Other researchers also have reported the diverse lipid profile of a certain microalga species, cultivated in different mediums. For instance, the value of unsaturated fatty acids of *Chlorella vulgaris*, cultivated in piggery wastewater, enhanced from 63% to 76% by increasing the concentration of wastewater [45]. Ji et al. [45] investigated the fatty acid profile of *Chlorella vulgaris*, cultivated in BG11 and different dilutions of monosodium glutamate wastewater (MSGW). Similar to the findings of this study, they reported the higher concentration of SFAs (71.97%) in BG11 and higher concentration of PUFAs (52.90%) in 50 times diluted wastewater.

3.2.2. Effect of harvesting methods

Harvesting of microalgal biomass from BBM and DWW was performed using centrifugation and coagulation (using $FeCl_3$). The results demonstrated that microalga coagulation by the iron chloride changed the composition of microalgal lipids significantly as compared to the centrifugation. As can be seen from Table 3, use of iron chloride for harvesting the microalgal cell remarkably increased the percentage of SFAs from 42.25% to 75.01% and 38.83% to 57.13% in MA-BBM and MA-DWW, respectively. Whereas, the percentage of PUFAs considerably decreased from 42.60% to 9.62% and 38.50% to 10.33% in MA-BBM and MA-DWW, respectively. Variation in the values of SFAs and PUFAs, due to the addition of NaOH as flocculant, to the medium of *Nannochloropsis oculata* was reported by Borges et al. [46]. In their study, the amount of C16:0 (SFA) in microalgal lipids increased from 32.70% (harvested by centrifugation) to 60.80% (harvested by NaOH flocculation) and the sum of C20:4 and C20:5 (PUFAs) decreased from 14.9% (harvested by centrifugation) to zero (harvested by NaOH flocculation). In another study, Gupta et al. [11] studied the coagulation efficiency and biochemical composition of *Scenedesmus* sp. by various flocculants. They stated that the reason of decreasing PUFAs concentration in the harvested microalgal biomass by alum as compared to the centrifugation could be related to the conversion of PUFAs to SFAS by oxidative cleavage of double bonds. Similar to the current work, in another study, use of $FeCl_3$ for harvesting the *Chlorococcum* sp. as compared to auto flocculation method increased the percentage of C16:0 from 39.7% to 44% [47]. The results of this study and review of literature show that method of microalgae harvesting can significantly affect the composition of their FAMEs. Therefore, selection of the

Table 3
The effect of cultivation media, harvesting methods, and biomass storage time on fatty acids profile of microalga.

FAMEs profiles	Percentage of fatty acids in FAMEs profiles (% of total FAMEs)							
	Cultivated in BBM				Cultivated in DWW			
	Centrifugation		Coagulation		Centrifugation		Coagulation	
	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored
C14:1	–	1.63	–	4.19	–	1.17	–	–
C16:0	31.64	34.35	50.44	60.31	26.39	30.66	41.49	51.98
C16:1	4.17	–	3.91	–	5.31	2.60	5.93	2.36
C17:1	2.07	1.43	–	–	5.61	3.02	–	–
C18:0	10.60	15.59	24.58	29.16	7.45	13.44	15.64	23.09
C18:1	8.91	7.51	11.46	6.34	16.84	16.77	26.59	20.05
C18:2w6	13.18	10.15	4.1	–	19.50	15.08	7.95	2.52
C18:3w3	29.42	29.33	5.49	–	19.00	17.27	2.38	–
Σ SFAs[1]	42.25	49.94	75.01	89.47	33.83	44.12	57.13	75.07
Σ MUFAs[2]	15.15	10.58	15.37	10.53	27.76	23.53	32.52	22.41
Σ PUFAs[3]	42.60	39.48	9.62	0.00	38.50	32.35	10.33	2.52

1. SFAs: Saturated fatty acids.
2. MUFAs: Monounsaturated fatty acids.
3. PUFAs: Polyunsaturated fatty acids.

appropriate harvesting method is important for the production of biodiesel as it affects the degree of saturation of final products and consequently, the properties of biodiesel such as oxidative stability, cold flow properties, hydrocarbon emission, ignition quality and kinematic viscosity [48].

3.2.3. Effect of microalgal biomass storage time

The FAMEs composition in the lipids of harvested microalga from BBM and DWW media by centrifugation and coagulation methods was analyzed after one day (fresh) and two month (stored) of harvesting. Table 3 represents the percentage of eight fatty acids individually, and total amounts of SFAs, MUFAs and PUFAs. As it can be seen from this table, apart from the cultivation media and harvesting methods, a significant difference was observed in the lipids profile of fresh and stored microalgal biomass. In all cases, SFAs were significantly increased in the stored microalgal biomass; while the percentage of MUFAs and PUFAs were observed higher in the fresh microalgal biomass. The variations of FAMEs constituents between the lipids of fresh and stored biomasses were more remarkable in harvested microalga by coagulation method as compared to the centrifugation method. For example, the difference in percentages of SFAs in the fresh and stored biomasses of MA-BBM harvested by centrifugation, MA-BBM harvested by coagulation, MA-DWW harvested by centrifugation and MA-DWW harvested by coagulation followed the order as: 7.69%, 14.46%, 10.29%, and 17.94%, respectively. The results of this study showed that the cultivation media, harvesting methods and biomass storage time

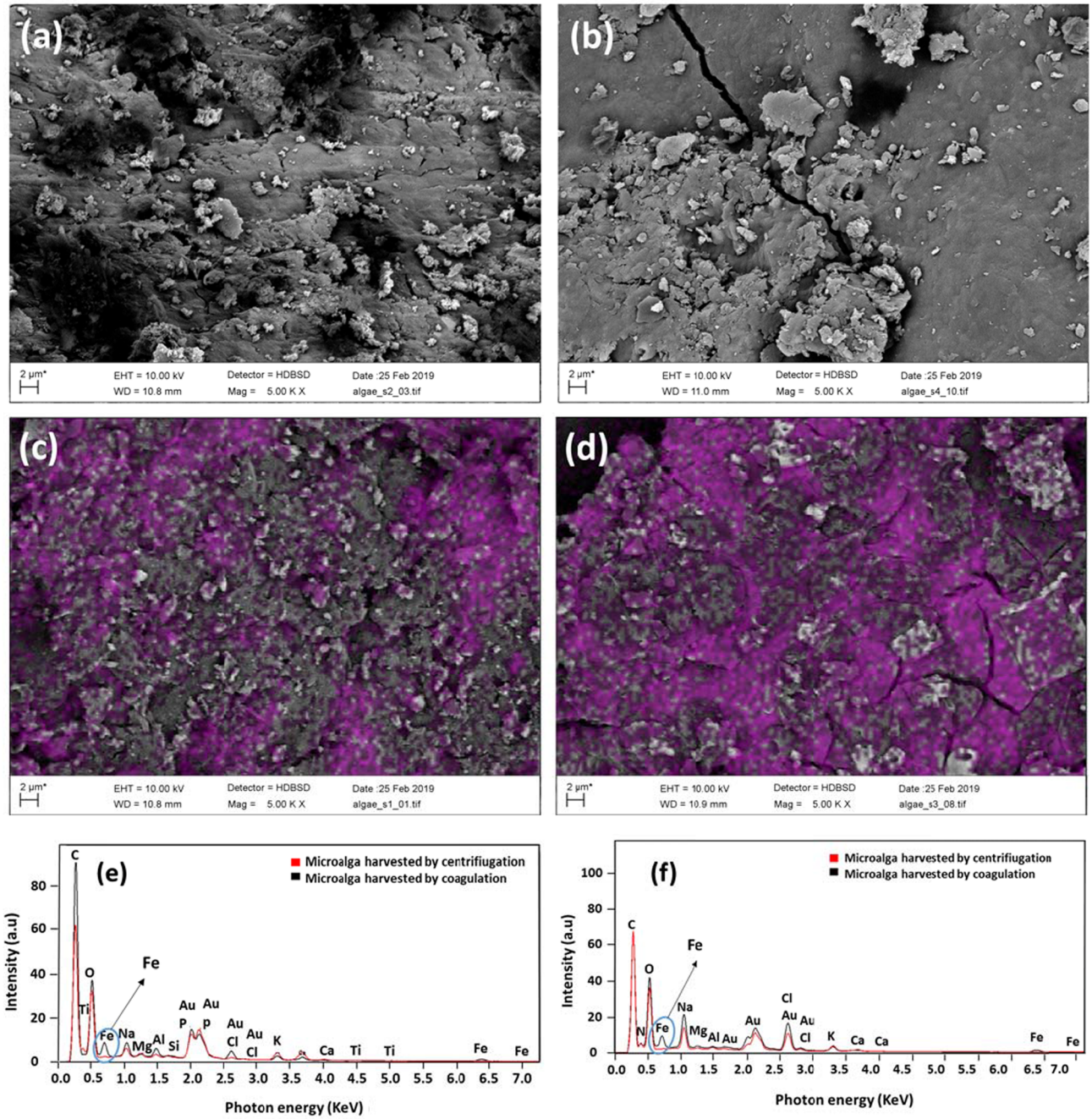

Fig. 4. Scanning electron microscopy (SEM) images of harvested microalga by centrifugation (a): MA-BBM and b): MA-DWW), the elemental mapping images of harvested microalga by coagulation (c): MA-BBM and d): MA-DWW), and the EDS spectra of microalga harvested by centrifugation and coagulation (e): MA-BBM and f): MA-DWW).

have remarkable synergic effects on the lipids profile of a certain microalga species. The values of SFAs as 42.25% and 33.83% in the fresh biomasses of MA-BBM and MA-DWW harvested by centrifugation changed to 89.47% and 75.07% in the stored biomasses of MA-BBM and MA-DWW harvested by coagulation, respectively. In another study, Welladsen et al. (2014) investigated the effect of storage conditions (refrigerator and freezer) on microalgal nutrition profiles [49]. In agreement to our results, they found > 90% decrease of omega-3 fatty acids of diatoms after two months of storage. The reason of this observation might be related to the oxidation of PUFAs and subsequently, decreasing their concentration during storage [50]. In this study, storage of microalgal biomass for two months decreased the amount of PUFAs noticeably from 42.60% in the fresh biomass of MA-BBM harvested by centrifugation to zero in the stored biomass of MA-BBM harvested by the coagulation and from 38.50% in the fresh biomass of MA-DWW harvested by the centrifugation to 2.52% in the stored biomass of MA-DWW harvested by the coagulation. Notable decrease of PUFAs in the stored microalgal biomass harvested by the coagulation as compared to the fresh biomass harvested by the centrifugation method is related to the presence of iron. Scanning electron microscopy (SEM) images, the elemental mapping images, and the EDS spectra of MA-BBM and MA-DWW harvested by the centrifugation and coagulation are presented in Fig. 4. The elemental mapping images clearly showed the uniform distribution of iron on the surfaces of microalgal biomasses harvested by the coagulation (Fig. 4 (c and d)). The higher concentration of iron on the surface of microalgal biomasses harvested by the coagulation as compared to the centrifugation was confirmed by the EDS spectra (Fig. 4 (e and f)). Balasubramanian et al. [51] detected 9.7% (before washing the biomass) and 1.5% (after three times washing with deionized water) of iron content in the harvested microalga by ferric chloride coagulation. They stated that the transition metals like iron, catalyze the free radical oxidation of triglycerides and increase the amount of free fatty acids. Our results clearly showed the effect of iron on changing the microalgal lipids profile even in fresh harvested microalgal biomass; however, the storage of harvested microalga containing iron intensified the variation of microalgal lipid profile.

4. Conclusions

The results of this study revealed that the same coagulant ($FeCl_3$) could have different behavior towards the coagulation efficiency of a certain microalga species (*S. quadricauda*) from different cultivation media. This finding confirmed that the characteristics of cultivation media have a significant effect on the performance of coagulants. The variables such as concentrations of coagulant, pH of medium, and reaction time may affect the coagulation efficiency of microalga. For example, the highest harvesting efficiency from BBM medium was found to be 95.75% at pH 11. While, in case of DWW (with the same concentration of 700 mg/L coagulant concentration), the highest harvesting efficiency was observed as 85.04% at pH 7 and increasing the pH to 11 decreased the coagulation efficiency to 38.29%. Coagulation of microalga in 4-L column photobioreactors within 15 min reaction time demonstrated that harvesting of microalga from DWW is applicable at larger-scale as well. Analysis of lipid profile of microalga proved that harvesting methods and cultivation media considerably affect the composition of the fatty acids in microalga. For instance, the amounts of SFAs in microalga biomass harvested by centrifugation (control) from BBM and in microalga biomass, harvested by coagulation from DWW, were found to be 42.25% and 57.13%, respectively. Whereas the percentages of PUFAs were observed as 42.60% and 10.33% in the former and latter media. In addition, two months storage of microalga biomasses, specially biomass harvested by $FeCl_3$ (containing iron) significantly changed their lipid profile. Therefore, depending on the cultivation media, harvesting methods, and storage time, different fatty acids profile can be obtained from the lipids of a certain microalga species. It is recommended for the future studies to compare the effect of other harvesting methods on the lipids profile of microalgae. Also, it is suggested to evaluate the effect of several shorter and longer storage times on lipid profile of harvested microalgal biomass.

CRediT authorship contribution statement

Ehsan Daneshvar: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing - original draft, Writing - review & editing. **Mohammad Javad Zarrinmehr:** Data curation, Formal analysis, Software, Validation. **Masoud Kousha:** Software, Writing - original draft. **Amit Bhatnagar:** Funding acquisition, Project administration, Supervision, Resources, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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