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Optimal cultivation of *Scenedesmus* sp. microalgae in a bubble column photobioreactor

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The influence of several factors including salinity, *p*H, lamps with various light intensities and different water sources on the growth of isolated *Scenedesmus* sp. microalgae has been investigated. In the fabricated bubble column photobioreactor and under illumination of tungsten light at *p*H value of 8 and salinity of 3.6 psu, maximum growth of *Scenedesmus* sp. (3.71 g L^{-1}) has been obtained. The growth model for microalgae is investigated using Logistic model. The obtained data fits well with the projected model. The maximum specific growth rate, initial biomass concentration and apparent specific growth rate (x_{max}, x_0 and K_c) are found to be 3.5, 0.002 g L^{-1} and 0.63 d^{-1}, respectively. The extracted fatty acids from microalgae are analyzed by GC. It has been found that about 43.71% of oil content of microalgae is oleic acid (C18:1) which improves oxidative stability properties of biodiesel feed-stock.

Keywords: Extraction, Bubble column photobioreactor, Fatty acid, *Scenedesmus* sp. Microalgae, Biodiesel

Fossil fuels are in short supply as the global energy demands are progressively increasing. Use of fossil fuel may cause serious environmental pollution such as global warming, decrease in *p*H value of seawater and increase of acid rain. Air pollutions are inevitable results of fossil fuels combustion, thus several efforts have been made to omit the fossil fuels dependence. Recently, special attentions have been paid to alternative energy sources which are renewable, biodegradable, non-toxic and able to fix CO_2[1-3]. Among several renewable fuels, microalgal biodiesel is identified as suitable alternative fuel. The first generation of biodiesel was produced from some C4 plants (a plant in which CO_2 is fixed into a compound containing four carbon atoms before entering the Calvin cycle of photosynthesis) like corn and sugarcane. In contrast to C4 plants, microalgae are not considered as a part of human diet. It is reported that 1 kg of dry algal biomass utilizes about 1.83 kg of CO_2; thus, they can reduce appreciable amount of atmospheric CO_2[3-14].

In addition to biodiesel production, microalgae gained considerable attention due to high oil content, rapid growth, no necessity to allocate arable land and freshwater for cultivation and valuable products[6,9,13,15-17]. Demirbas and Fatih Demirbas[18] have reported that microalgal oil is an essential source of biodiesel feed-stock. The influential factors on microalgae growth and oil content are summarised as: type of nitrogen source, lack of nitrogen and silicon, high salinity, light intensity, ions content in growth media, cultivation time, CO_2 concentration and *p*H of the medium[17,19,20]. Furthermore, microalgal oil composition varies with respect to medium composition, temperature, *p*H and light intensity. Generally, microalgae are cultivated in open system for the purpose of low operation costs. However, contamination by other microorganisms, high necessity to land, CO_2 emission to atmosphere and water losses are considered as main disadvantages of open systems[21-24].

Photobioreactors have high biomass production capacity in comparison to open systems[22-24]. Photobioreactors are often fabricated with plastics, glasses and other transparent materials in the forms of tubular, flat plate and coil-type systems[17,19,24]. Among various configurations of photobioreactor, bubble columns have attracted more attention due to high potential for scale up[18]. In comparison to tubular photobioreactors, high gas flow rates in bubble column photobioreactors enhance biomass productivities[15].

The aim of present study was to fabricate a bubble column photobioreactor and evaluate the effect of

lights, water sources (tap water, diluted seawater and distilled water), *p*H and salinity on growth of *Scenedesmus* sp. microalgae under controlled temperature and light intensities. Finally, the extracted oil from harvested microalgae cells was analyzed by gas chromatography (GC) to identify the fatty acids. Then fatty acid analysis was used to investigate the potential of *Scenedesmus* sp. microalgae for biodiesel production.

Experimental Section

Microalgae selection and medium composition

Green microalgae, *Scenedesmus* sp. were isolated from Caspian Sea, Mazandaran province, Iran. BG11 medium was used for microalgae cultivation[25]. The culture medium was autoclaved for sterilization. The *p*H of medium was adjusted to 8. The inoculated medium was adapted to BG11 medium in a 1 L Erlenmeyer flask containing 250 mL culture media under natural illumination at 25°C for duration of one week. The culture was daily swirled to mix the medium as well as the culture. A SEM image of *Scenedesmus* sp. is shown in Fig. 1.

Photobioreactor design

Schematic diagram of the fabricated bubble column photobioreactor and a real image of it are depicted in Fig. 2. The photobioreactor was used to investigate effect of various process parameters on microalgae growth consisted of 4 columns with a total volume of 3 L. Columns with inner diameter of 44 mm, outer diameter of 50 mm and height of 50 cm were made of transparent Plexiglas (acid acrylic, Taiwan). Two ports were provided on top and bottom of columns. The ports were used for gas and medium inlet, outlet, and sampling.

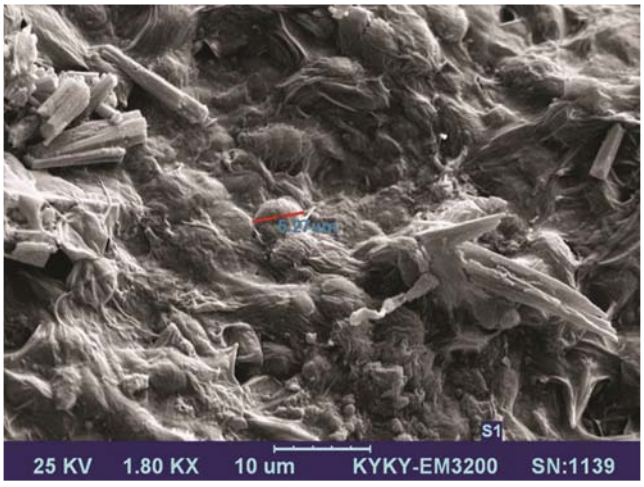

Fig. 1—SEM image of *Scenedesmus sp.* microalgae

Gas spargers were placed at the bottom of the columns for aeration. The air flow rate entering the photobioreactor was regulated using suitable valves. The photobioreactor was installed in a glassy cabin for desired temperature control. The cabin temperature was maintained at 25±0.5°C. The cabin temperature was also recorded with a display digital unit which was located on upper part of the photobioreactor column. The light intensity was set at 3000 lux and determined by a lux meter (MS 6610, Mastech, Taiwan). Light/dark periods were alternated for fixed period of 12 h. The cultivation period was 16 days.

Operational conditions

Effect of different types of lights, water sources (tap water diluted seawater and distilled water), *p*H and salinity on growth of *Scenedesmus* sp. microalgae under controlled temperature and light intensities were

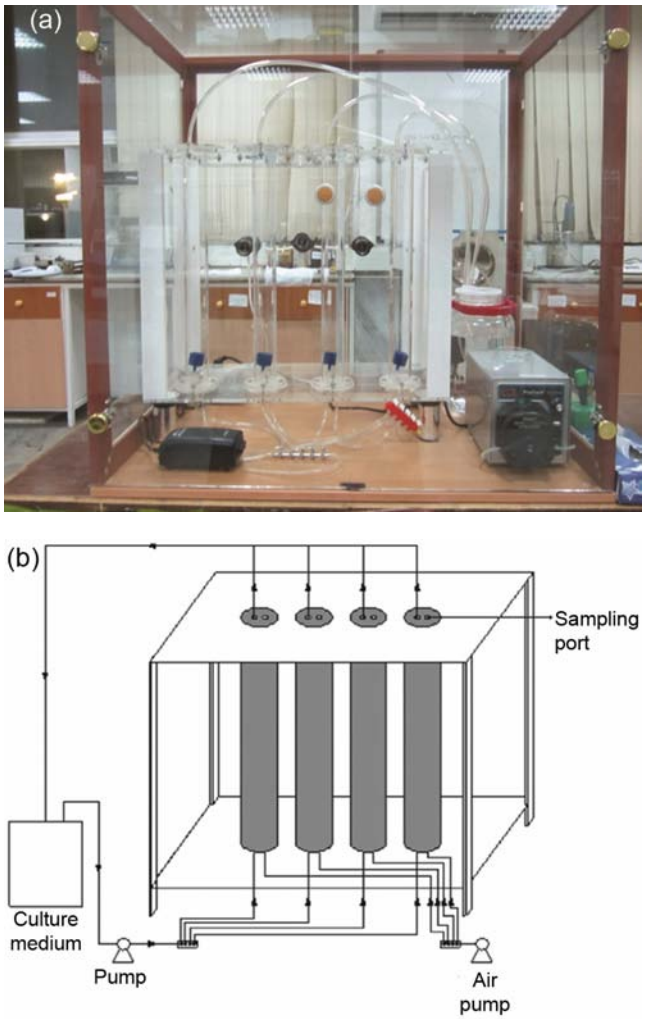

Fig. 2—a) Schematic diagram of the fabricated bubble column photobioreactor b) Image of the fabricated photobioreactor

evaluated. The effect of different types of light on microalgal growth was investigated using several lamps such as tungsten, fluorescent and compact fluorescent lamps under the light intensity of 3000 lux.

The salinity of water in sea and ocean based on definition of oceanographers is redefined as salinity in practical salinity unit (psu). Moreover, cultures with several *p*H values (7, 7.5, 8, 8.5 and 9) were prepared with distilled water and salinities in the range of 0-9 psu with increment of 1.8 psu. The *p*H values were adjusted by NaOH and HCl (0.1 M) solutions[25,26]. Culture with salinity of 0 and 9 psu were composed of pure distilled water and pure seawater, respectively. Tungsten light (100W) was used for column illumination.

Effect of different water sources such as diluted sea water, distilled water and tap water on algal growth was investigated while the photobioreactor was illuminated by tungsten lamps (100 W). For all experimental runs, temperature was maintained at 25±0.5°C and cultivation period was 16 days.

Extraction of oil from microalgal cells

After 16 days of cultivation, at the beginning of stationary phase, algal culture was harvested and centrifuged at 2000 rpm and 4°C for 5 min. Then algal broth was freeze dried (FDE-0350, humanlab instrument, Korea) at -48°C. A 500 mL soxhelt extractor was used to extract oil from algal dried cell using n-hexane as solvent[27]. The solvent and oil were separated by rotary evaporator (RE300, Stuart, UK).

Gas chromatography (GC-14A Shimadzu, Japan) was used to analyze the compositions of extracted microalgal oil. This device was equipped with a flame ionization detector (FID) and CP-Sill 88 capillary column (50×0.25 mm) with 0.2 μm film thickness. Helium was used as a carrier gas with a flow rate of 50 mL/min. Gas chromatography method consisted of following conditions under split less mode: the oven temperature was 190°C for 2 min, rose to 235°C for 20 min and was held at this temperature for 8 min. Sample size of 1 μL was injected to the GC. Fatty acid concentrations presented in samples were quantified by comparing their peak areas with those calculated from the standards. Fatty acid composition of microalgal oil is summarized in Table 1.

Growth characteristics

Optical densities of algal cells were determined by spectrophotometer (Analytik Jena Spekol 1500, Germany) at wavelength of 650 nm for every 24 h.

In order to measure the cell growth, optical density was determined and a calibration curve was developed based on cell dry weight and optical density. The regression equation is depicted as equation (1):

$$CDW = (0.6\ OD_{650} + 8\times10^{-3})\ R^2 = 0.99 \quad \ldots (1)$$

Specific growth rate was calculated by the following equation:

$$\mu = (LnOD_{(650)2} - LnOD_{(650)1})/(t_2 - t_1) \quad \ldots (2)$$

where μ is growth rate and OD is optical density.

One of the efficient and exact models which is enable to analyze the microalgal growth kinetic was found to be the Logistic model[20,28].

Simplified Logistic model with three constants K_C, a and b is described in equation (3)[20]:

$$x = a/(1+b \exp(-K_C\, t)) \quad \ldots (3)$$

where, x is biomass concentration, the constants a and b are x_{max} (g L^{-1}) and $((x_{max}/x_0) - 1)$, respectively. x_0 [g L^{-1}] initial biomass concentration and x_{max} [g L^{-1}] maximum biomass concentration. K_C is the apparent specific growth rate (d^{-1}). This model was applied for the obtained experimental data.

Results and Discussion

Effect of different types of lights on microalgae

Photosynthesis process was carried out at wavelengths of 400 to 700 nm[29,30]. Through photosynthesis process, red light (long wavelength of 700 nm) and blue light (short wavelength) are absorbed by chlorophyll; the most important pigment in *Scenedesmus* sp. green microalgae. Cell concentrations of microalgae illuminated by tungsten lamps (more red lights) and fluorescent and compact fluorescent lamps (more blue light) are shown in Fig. 3.

Microalgae growth decreased under the illumination of fluorescent and compact

Table 1—Fatty acid composition of microalgal oil

Content of fatty acid (%)	Fatty acid
0.91	C14:0
0.89	C14:1
40.48	C16:0
2.75	C16:1
4.27	C18:0
43.71	C18:1
1.53	C18:2
2.21	C18:3
0.58	C20:0
2.67	Others

fluorescent lamps since these lamps emitted UV lights as UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (180-280 nm) in addition of visible lights. These wavelengths diminish the photosynthesis efficiency and injure the DNA of microalgae[30]. It is important to note that biomass concentration under the illumination of tungsten lamps in photobioreactor was 3.23 g L^{-1}. This result is due to the function of chlorophyll a and b. The most significant process of photosynthesis is depended on chlorophyll a and b that absorb lights with wavelength of 700 nm (red lights) more than other wavelengths[30]. Therefore, applying tungsten lamps with more red light emissions and lower blue lights led to the highest amount of biomass concentration. However, lower intensities of blue light is essential in growth and metabolism of *Scenedesmus* sp. microalgae[31].

Effect of *p*H on microalgae growth

According to the microalgae physiology, any parts of the microalgae cell (tilacoid, chloroplast) carry out their vital functions in specific *p*H. Photosynthesis process is affected by *p*H of the media as well. Since the optimum value of *p*H is ranged between 8.2 to 8.7 for the growing microalgae, alkaline medium is preferred for algal cultivation[29,32]. High and low *p*H values decrease the photosynthesis rate. The high *p*H condition may change nutrients and trace metals absorption trend. On the other hand, the reactions of photosystem II would be confused in acidified media[33]. Low *p*H values play role as enzyme inhibitor in photosynthesis process[29,32]. At low *p*H values contamination of media by other microorganisms is inevitable[29]. The *p*H value of culture decreases when CO_2 diffuses in to the culture media via aeration while it rises through photosynthesis process due to CO_2 absorption of microalgae. However, growing algae in culture media enriched with CO_2 causes higher microalgal biomass.

Effect of several *p*H values on *Scenedesmus* sp. microalgae growth are shown in Fig. 4. According to the obtained results, biomass concentrations at *p*H values of 7, 7.5, 8, 8.5 and 9 were 0.84, 0.82, 3.23, 0.90 and 1.67 g L^{-1}, respectively. The maximum biomass concentration was obtained at *p*H value of 8 which was about 3.23 g L^{-1}. The *p*H of Caspian Sea water is 8.12.

Effect of salinity on microalgae growth

Salinity is one of the effective factors on microalgae growth since, natural environment of the *Scenedesmus* sp. microalgae (Caspian Sea), is saline[17]. Ruangsomboon has reported that cell concentration increased when salinity was maintained at 5 psu[19]. Figure 5 shows the effect of different salinity values on microalgae growth.

The obtained data for biomass concentrations of *Scenedesmus* sp. microalgae were 3.23, 2.63, 3.71,

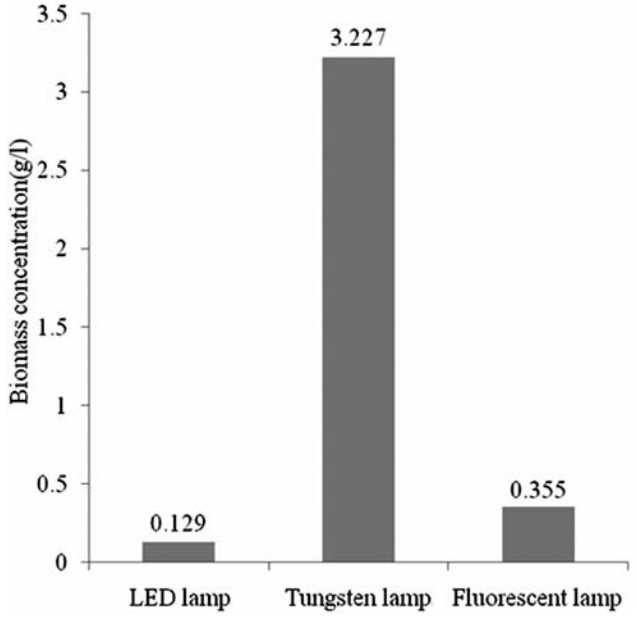

Fig. 3—Effect of different lights (tungsten lamps, fluorescent and compact fluorescent lamps) on *Scenedesmus* sp. microalgae growth in bubble column photobioreactor; maximum biomass concentration (3.23 g L^{-1}) under illumination of tungsten lamps

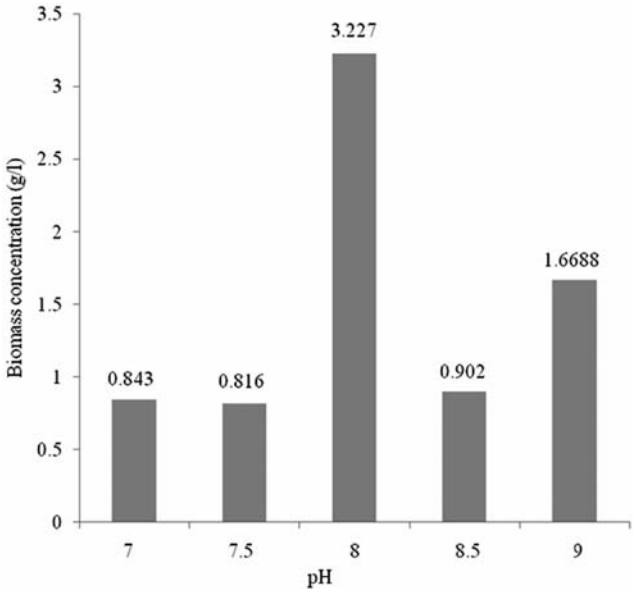

Fig. 4—Effect of different *p*H on *Scenedesmus* sp. microalgae growth in bubble column photobioreactor; maximum biomass concentration (3.23 g L^{-1}) at *p*H=8

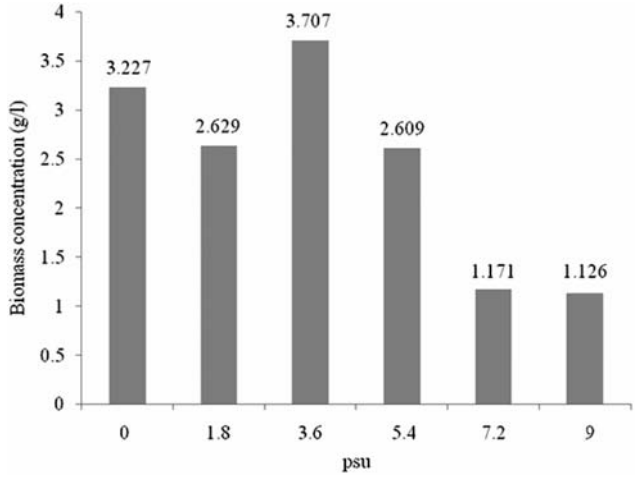

Fig. 5—Effect of salinity values of 0, 1.8, 3.6, 5.4, 7.2 and 9 psu on *Scenedesmus* sp. microalgae growth. The maximum biomass concentration (3.71 g L^{-1}) was obtained at 3.6 psu

2.61, 1.17 and 1.13 g L^{-1} at salinity values of 0, 1.8, 3.6, 5.4, 7.2 and 9 psu, respectively. The biomass concentration increased at salinity value of 3.6 psu. However, applying culture media with high salinity may cause reduction in biomass concentration of microalgae. Increasing salinity in media may cause physiological stress. CO_2 solubility, microalgal metabolism reaction and thereby photosynthesis rate may change under high or low salinities[34,35]. The osmosis phenomena could be excited in high salinity because of differences between salinity values of inner and outer algal cell. In addition, the osmotic phenomena may deteriorate the algal cell[36]. Moreover, fatty acids synthesis by microalgae maybe affected in cultures with various salinity values[37,38].

Effect of different water sources on microalgae growth

A comparison study between different water sources including tap water, distilled water and diluted sea water with the optimum salinity of 3.6 psu was carried out and the results are presented in Fig. 6. The lowest biomass concentration was obtained when tap water as water source was used. The reduction in biomass concentration might be occurred due to the presence of chloride ions which are inhibitor for microorganism growth. Microalgae biomass concentrations in distilled water and diluted sea water were 3.23 and 3.71 g L^{-1}, respectively.

Growth kinetics and fatty acids analysis

Specific growth rate (μ) was calculated according to equation (2) which was 0.36 d^{-1}. Data obtained from experiments carried out in bubble column photobioreactor with *p*H value of 8 and cell dry

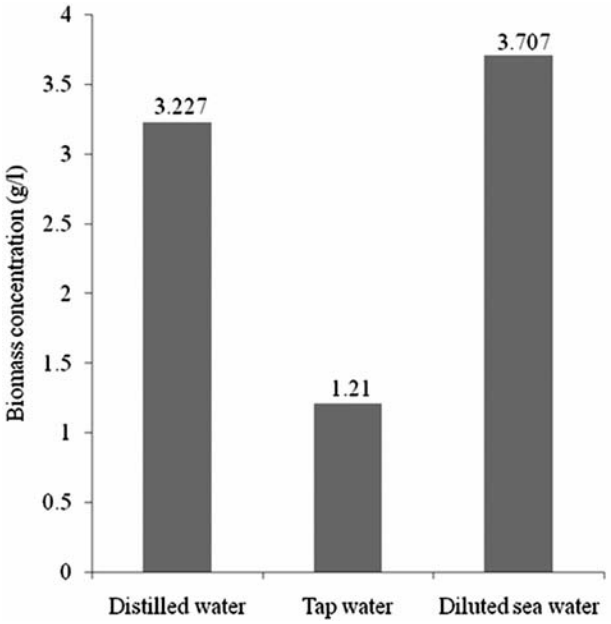

Fig. 6— Effect of different water sources (tap water, diluted seawater and distilled water) on *Scenedesmus* sp. microalgae growth, maximum biomass concentration with diluted sea water

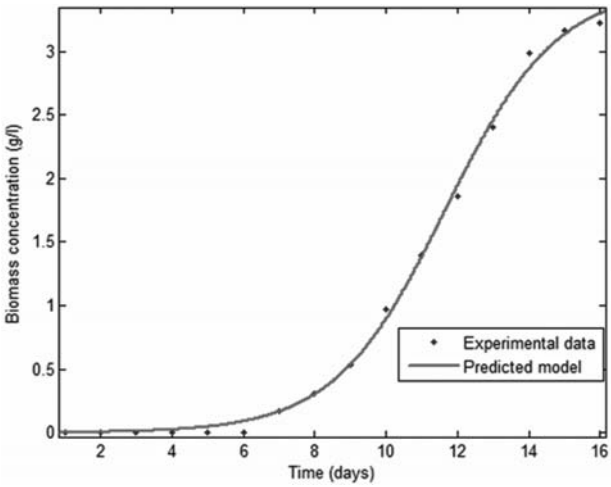

Fig. 7—Experimental data obtained from the growth of *Scenedesmus* sp. in fabricated bubble column photobioreactor, fitted with predicted Logistic growth model with R^2=0.99

weight of 3.23 g L^{-1} was well fitted with Logistic model as illustrated in Fig. 7.

Furthermore K_C, X_{max} and X_0 were calculated as 0.63 d^{-1}, 3.5 and 0.002 g L^{-1}, respectively. The predicted X_{max} (3.5 g L^{-1}) was close to experimental one (3.23 g L^{-1}). The obtained results were similar to data reported in the literature[20].

The extracted oil from *Scenedesmus* sp. was about 31% of the of algal biomass dry weight. Also, 46.24 and 51.09% of the oil content was consisted of saturated and unsaturated fatty acids, respectively.

Results of the GC analysis revealed that, C16:0 (40.48% of oil content) and C18:1 (43.71% of oil content) were the major components of *Scenedesmus* sp. oil. Moazami reported similar facts about *Nanochloropsis* sp. microalgae[16]. Also, Yeesang and Cheirsilp reported that C16:0 and C18:0 consisted major portion of fatty acids in microalgae isolated from freshwater sources in Thailand[17]. In another study C18:1 was the dominant fatty acids in *Botryococcus* sp., *Scenedesmus* sp. and *Chlorella Vulgaris*[27]. Qualified biodiesel with low temperature property and high oxidative stability could be attained by increasing unsaturated and saturated fatty acids respectively[16,39]. Approximately, equal synthesis of saturated and unsaturated fatty acid by *Scenedesmus* sp. with high percentage of oleic acid showed that isolated *Scenedesmus* sp microalgae had high potential for biodiesel production.

Conclusion

A bubble column photobioreactor has been fabricated to evaluate the influential parameters affecting the growth of *Scenedesmus* sp. microalgae which are isolated from Caspian Sea. The optimum *p*H and salinity are obtained by culturing the microalgae in the bubble column photobioreactor. The efficient lamps are found to be tungsten for photobioreactor illumination. The effects of different types of water on the algal growth are also investigated. The obtained data reveal that the highest biomass concentration (3.71 g L^{-1}) is achieved when diluted sea water is used as cultivation media. Growth parameters are well fitted with logistic model under the *p*H of 8 with specific growth rate of 0.36 d^{-1} and R^2 coefficient of 0.99. Also, extracted fatty acids are analyzed by GC method and C16:0 and C18:1 are identified as major components of *Scenedesmus* sp. microalgae and the most effective components for qualified biodiesel.

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