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Microalgae strain separation using electrophoresis

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ABSTRACT

The aim of this study is to demonstrate the potential application of electrophoresis for separation of microalgae to select single cells of microalgae for the development of pure cultures of the desired strain. A mixed culture of *Chlorella vulgaris* and *Scenedesmus* sp. was used in this study. A serial arrangement of mega-bore capillary tubes was designed to separate *Chlorella vulgaris* from *Scenedesmus* using different parameters such as size, charge, and morphologies under an electric field in very short time (30 min). The mixture of both species was successfully separated into several fractions with high resolution. *Chlorella vulgaris* was separated from *Scenedesmus* with 94 % purity. The characterization techniques were applied on each fraction to identify algae species and their properties. Finally, cell viability was examined after separation by reculturing the last separated fraction. This work presents a simple, fast, and effective method for microalgae separation with potential application at large scales.

1. Introduction

Microalgae are photosynthetic microorganisms widely found in marine and fresh water environments [1]. They are an important source of proteins, fatty acids, and oils [2]. Because of this, microalgae have been widely used in biofuel production, wastewater treatment as well as the food and pharmaceutical industry [3]. There are a large number of microalgae strains in nature with their unique properties and industrial application. One of the main problems is strain selection from samples in the natural environment [4]. Strain selection is the most important step in culturing microalgae for a specific application. Heterogeneous mixed strains cause competition for nutrients that leads to an overall reduction in biomass and quality of cultivation process [4,5]. Pure strains of microalgae are required to produce microalgae products for various applications. To obtain pure strains, it is necessary to separate the desired strain from a culture with multiple species. Separation techniques are performed to select the desired strain of microalgae from the culture medium. Some manual microalgae separation techniques such as micropipette [6], serial dilution [7], and plate coating [8] are time-consuming, labor intensive, and sometimes inefficient. The centrifugal is type of separation method which is simple and easy to use [9], however, it cannot achieve high resolution separation among the cells with similar size and density. The filtration method may cause clogging and contamination resulting in cell size heterogeneity [10]. Cell sorting using flow cytometry can also be used for efficient single-cell identification based on fluorescent staining of a certain molecule on the surface of algae strain [11]. However, this method requires time-consuming sample preparation, expensive equipment, and high operation costs. There is a need for an efficient, low-cost, and convenient microalgae separation method to obtain purified strains.

Electrophoresis separates charged molecules under an applied electric field. Smaller particles migrate through the column faster than larger particles. Moreover, high-resolution separations can be achieved. Electrophoresis has been demonstrated to be an effective tool in the separation of various biomolecules, macromolecules, and nanomaterials, as well as single cells [12–15]. Capillary electrophoresis (CE) has been used for efficient single-cell separation, but it is difficult to operate at large scale [16]. There are several examples in the literature showing the use of dielectrophoresis (DEP) in separating microalgal cells using non-uniform electric fields based on the dielectric properties of different cells [17,18]. DEP in microfluidic chips can separate cells into micro-scale channels. Wang et al. [17] separated *Chlorella* and *Closterium* using DEP in microfluidic chips with a separation efficiency of more than 90 %. After separation, the cell viability of microalgae was examined by fluorescence staining method. Han et al. [19] used DEP-based microfluidic to isolate microalgae with high lipid content. Huang et al. [20] separated lipid-rich microalgae by a new sampling technique under an inverted fluorescence microscope using a micromanipulator. These microfluidic systems have the advantages of small size, simplicity of operation, and low cost. However, these techniques require complex networks of microscale structures including branched channels and post arrays.

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Herein, a novel method is proposed for the first time for the separation of microalgae cells using zone electrophoresis into mega-bore capillary tubes. Multiple capillary tubes were arranged in an array to further improve the overall system throughput. The advantage of this approach is that cells are separated in a uniform electric field under low separation voltage based on their size, charge, and morphology. The separation is relatively fast compare to traditional strain separation techniques, such as serial dilution. As compared with CE or DEF, it has a simpler setup. Multiple mega bore capillaries can be used simultaneously, allowing for higher throughput analysis of the samples. Another important advantage is the potential of this technique for large-scale production of pure strains in relatively short time without any cell inactivation caused by applying low voltage. Conventional electrophoresis technique such as CE or DEF require nanoliters of growth media for separation that results in single cells of microalgae resolution in minute amounts making it very challenging to collect these minute fractions for further evaluations and analysis. On the other hand, in serial arrangement of capillary tubes, presented in this work, the loading would be in microliters scale, at least 1000 times more loading capacity as wells as having several tubes in parallel that increases the high throughput capacity of this separation method. The combination of several orders of magnitude higher loading capacity and high throughput potential increases the scale of the separation from analytical/laboratory scale to large-scale separation to obtain considerable quantity of pure strains appropriate for further characterization or re-culturing.

The main challenges in electrophoresis technique such as DEP or CE are cell viability after separation because of applying high voltage, which may cause damages or induce deformation in the cells. Here, we have addressed these challenges by showing that the separated microalgae cells are viable.

In different phase of the microalgae growth, there is a gradual variation in shape and size of microalgae, ranging from spherical to elongated cylindrical shapes [21]. Electrophoresis has been reported in cell cycle analysis [22]. Electrophoresis can also be used for shape-based separation of microalgae. The heterogeneous mixture of different microalgae strains with different shapes are oriented in a specific direction based on the charge density of the surface functional groups. However, the microalgae cell sizes and shapes can vary depending on their growth phase or levels of intracellular lipid content [23]. Despite all this, the unique capability of electrophoresis in separation based on the ratio of charge to size make it an attractive technique for microalgae research.

In this work, two strains of microalgae, *Chlorella vulgaris* (*C. vulgaris*) and *Scenedesmus* sp. were selected as the samples on the basis of their different sizes and shapes, making them suitable choices for the proof of concept to show the possibility of electrophoresis being used as a separation technique for the purpose of strain selection. This study shows the potential application of electrophoresis for the separation of microalgae compared to traditional separation techniques which are time-consuming and laborious.

2. Materials and methods

2.1. Preparing microalgae culture

C. vulgaris and *Scenedesmus* were cultivated in BG11 culture medium under sterile conditions at a temperature of 25 ± 2 °C, light intensity of 3000 Lux, and continuous aeration [24]. The BG11 medium was composed of 1.5 g $NaNO_3$, 20.0 mg Na_2CO_3, 36.0 mg $CaCl_2{\cdot}2H_2O$, 7.5 mg $MgSO_4{\cdot}7H_2O$, 6.0 mg $(NH_4)_5[Fe(C_6H_4O_7)_2]$, 6.0 mg $C_3H_5O(COOH)_3$, 1.0 mg EDTA, 40.0 mg $K_2HPO_4.2H_2O$, and 1 mL of the trace elements stock solution all in one liter distilled water. The trace elements stock solution contained 494.0 mgL^{-1} $Co(NO_3)_2{\cdot}6H_2O$, 79.0 mgL^{-1} $CuSO_4{\cdot}5H_2O$, 39.0 mgL^{-1} $NaMoO_4{\cdot}2H_2O$, 222.0 mgL^{-1} $ZnSO_4{\cdot}7H_2O$, 1.8 gL^{-1} $MnCl_2{\cdot}4H_2O$, and 2.8 gL^{-1} H_3BO_3. The growth of microalgae was evaluated by optical density measurement at 750 nm. Then, the microalgae at the stationary growth phase were harvested for the experiments. After cultivation of two strains, microalgae strains were mixed with equal proportions (containing 55 % *C. vulgaris* and 45 % *Scenedesmus*) with volume ratio of 1:1.5 of *C. vulgaris* to *Scenedesmus* to be used for further experiments.

2.2. Instruments

The zeta potentials, ζ, of the samples were measured using ZetaSizer (Nano-ZS) (Malvern, United Kingdom). The electrophoresis device was provided from Bio-Rad Laboratories (Hercules, USA). The UV–vis absorption spectra were obtained using Photonix Ar 2015 UV–vis spectrophotometer (Photonix, Iran). The FT-IR spectra were recorded using FT-IR Spectrometer model Thermo Nicolet Avatar 370 scanning from 4000 to 500 cm^{-1} (Thermo Nicolet, America). The field-emission scanning electron microscopy (FE-SEM) images of the samples were recorded using TESCAN-XMU (Czech Republic).

2.3. Size separation

The electrophoresis experiments were carried out using 75 mm long mega-bore capillary tubes (internal diameter of 1.1 mm) arrayed in a set of several tubes for simultaneous and high-throughput fractionation in one stage. Using this arrangement, instead of nanoliters of growth media, several microliters of growth media containing mixed strains were loaded onto each mega bore capillary tube. Although 50 μm internal capillaries used for CE offers an excellent resolution in separation, there should be thousands of CE experiments in order to collect enough fractions for further characterizations. Using mega bore capillaries, by sacrificing high resolutions achieved by CE, the sample loading increases resulting in high enough fractions being collected for further characterizations.

The electrophoretic separation of microalgae was performed in phosphate buffer at three pH values of (H_3PO_4, pH 2.4), (NaH_2PO_4, pH 7.4), and (Na_2HPO_4, pH 11.4).

The electrophoretic experiments of microalgae were also carried out using 25 and 50 mM phosphate buffer at constant pH 7.4. The ionic strength (I) was calculated using the following equation:

$$I = \frac{1}{2}\Sigma c_i z_i^2 \tag{1}$$

where c_i is ion concentration and z_i is ion charges. The 25 mM phosphate buffer was prepared by mixing 6.0 mM NaH_2PO_4 and 18 mM Na_2HPO_4. The ionic strength was calculated to be 60 mM and kept constant throughout the experiments. The 50 mM phosphate buffer was prepared by mixing 10 mM NaH_2PO_4 and 40 mM Na_2HPO_4 with ionic strength of 130 mM.

Then, 5 μL of mixture of both microalgae strains suspension was loaded in each capillary. Then, the capillary tubes were placed inside the electrophoresis chamber (Hercules Bio-Rad Laboratories) filling with buffer. The separation was performed for about 30 min under 100 V separation voltage. After separation, a syringe plunger was used to extrude the last fraction of algae into glass vials for further characterization of separated strains.

The resolution was calculated by the following equation [43]:

$$R_s = \frac{\Delta t_r}{w_{av}} \tag{2}$$

where Δt_r and w_{av} are the difference between two peaks' retention times and the average peak widths, respectively.

Then, the percent purity of the two strains in each fraction was calculated using the following equation [25]:

$$Purity_{N_i}(\%) = \frac{N_i}{N_j + N_i} \times 100 \tag{3}$$

where, the N_i and N_j are the number of cells i and j in each fraction, respectively. The number of microalgae cells in each fraction was obtained by a hemocytometer.

The electrophoretic mobility of microalgae was calculated from the algebraic sum of the μ_{ep} and that of the EOF, μ_{eo}, [26]:

$$\mu_a = \mu_{ep} + \mu_{eo} \tag{4}$$

where μ_a is called apparent mobility, which can be measured using a neutral marker moving at the same velocity with EOF, this makes it possible to simultaneously separate cationic, neutral, and anionic analytes. In this experiment, curcumin was used as neutral marker. μ_{ep} or μ_{eo} can be calculated using the following equation [27]:

$$\mu_{ep} = \frac{lL_t}{t_m V} \tag{5}$$

where L_t and V are the total length of the capillary tube (7.5 cm) and the applied voltage (100 V), respectively. The t_m is migration time, or time in which a cell migrates a distance l from the injection to the detection points (effective length of the migration).

Electrophoresis experiments were performed in triplicates in order to calculate the electrophoretic mobilities and zeta potentials of separated strains and the results were reported as mean values with their respective standard deviations (SD).

2.4. Cell viability experiment

The number of viable cells was measured using a spectrophotometer. For this purpose, the last separated fraction was re-cultivated in BG11 medium until exponential growth phase was reached and the growth rate was monitored by measuring optical density (OD) at 570 nm.

3. Results and discussions

3.1. Surface properties of microalgae

The surface properties, such as surface charge, plays a key role in the effectiveness of electrophoretic separation of microalgae. Based on the potential measured at different distances from algae surface, the surface charge of microalgae can be divided into two parts: surface potential and zeta potential (ζ) [28]. Due to the unequal distribution of the permeant ions on two sides of the cell membrane, a potential difference is created inside and outside the cell membrane known as Donnan potential (Ψ_D) or surface potential [28] (Fig. 1).

The membrane surface of microalgae is covered with various functional groups such as carboxylic (-COOH) and amino ($-NH_2$) groups playing an important role on surface charge characteristics of the microalgae [29]. Surface charges of microalgae can cause attraction of counter ions from the surrounding electrolyte solution to near the algae surface and form electric double layer (EDL) composed of a Stern compact layer and a diffuse layer [28]. Unlike rigid particles, microalgae are coated by an ion-permeable surface layer of polyelectrolytes, where EDL is formed both internally and externally. The potential created at the interface between the compact layer and the diffuse layer (slipping plane) is called zeta potential (ζ) [30] (Fig. 1). It is often used to characterize the surface potential of the cell. Zeta potential is affected by various parameters, including ionic strength, pH of the medium, and strains of microalgae [31,32]. As the zeta potential of microalgae varies in different strains, it causes a variation in electrophoretic mobilities of different strains of microalgae to be exploited in electrophoretic separation [28,29]. The number of surface functional groups is significantly different among various strains of microalgae [33]. The cell walls of microalgae contain high percentage of proteins, providing different functional groups with significant influence on electrophoretic migration of different strains of microalgae. Since, the intercellular lipid

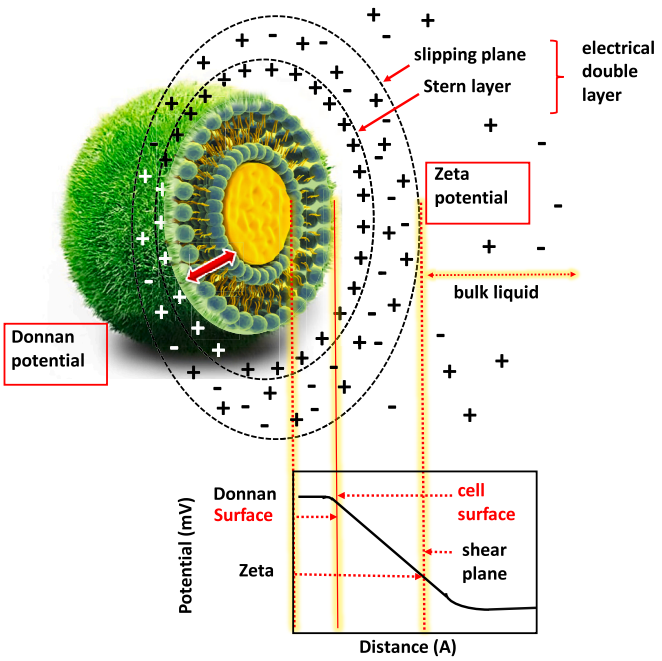

Fig. 1. Schematic of the Donnan and zeta potential of a microalgae.

synthesis creates some changes in the component of cell wall at different phases of algae growth, the number of surface functional groups is so diverse at various phases of algae growth that imposes significant variations in electrophoretic mobilities for efficient separation [1]. Based on the studies reported by Li et al. [34], the algae at the stable phase contains the highest functional groups. Thus, the stable phase can be considered as the optimal phase to harvest microalgae from culture medium for electrophoresis experiments. However, the effects of growth phase on surface properties of microalgae should be investigated in detail to obtain best separation efficiency.

Different strains migrate at a specific velocity under an applied electric field. The electrophoretic migration velocity, v_{ep}, of an ion can be expressed as [27]:

$$v_{ep} = \mu_{ep} E \tag{6}$$

where μ_{ep} is the electrophoretic mobility of the ion, which depends on the size and charge of the ionic species and E is field strength.

The second effective force in electrophoresis is electroosmosis [27]. Electroosmotic flow (EOF) is the liquid flow caused by the contact of an ionic solution with a charged solid surface in the presence of an electric field. Due to the ionization of the surface silanol groups, the silica surface of glass tubes in contact with an aqueous electrolyte solution has an excess of negative charge. An EDL is generated at the inner surface of a glass tubes. The cationic counter-ions in the diffuse layer are solvated, as these ions migrate toward the cathode, they drag solvent along with them creating the EOF.

3.2. Optimization of different parameters affecting electrophoretic separation

3.2.1. pH

The microalgae growth and electrophoretic separation are two different processes. During microalgae growth pH is increasing to values of up to pH 9 due to low buffering capacity of the growth media, which in turn affects the growth of microalgae. However, in the separation process by electrophoresis, the electrolysis is carried out in electrodes which generates acid (in the anode) and base (in the cathode). Therefore, electrophoresis systems need to be well buffered to compensate the electrolysis. The buffer capacity is high enough to keep the pH almost

constant despite the fact that the pH of the photobioreactor may change in the growth media and is not buffered. The functional groups on the surface of algae can be protonated or deprotonated at different pH values [35].

At low pH, the functional groups on the surface of microalgae are protonated and the net surface charge is positive. On the other hand, at high pH values, the ionizable groups such as carboxylic acids are deprotonated and the net surface charge becomes negative. The surface charge is electrically neutral at the isoelectric point (pI, $\zeta \approx 0$ mV) [36]. Several groups reported the pI of *Chlorella vulgaris* and *Scenedesmus* at pH ranges of 2.9–3.5 [37,38]. The possible reason for the different pI values can be ascribed to cellular metabolic activity [29]. The electrophoretic separation of microalgae was performed at pH ranges of 2–11. The results are shown in Fig. 2. At low pH 2.4 both amine and carboxyl groups at surface are protonated as COOH and NH_3^+ creating a positive surface charge. Thus, microalgae cells migrate toward the cathode. At low pH, the surface charge density of microalgae is low. On the other hands, at low pH, the electro-osmotic flow (EOF) direction is toward the anode. Electrophoretic migration of positively charged cells is in the opposite direction of electroosmosis. Hence, μ_{ep} and μ_{eo} have the opposite signs, resulting low separation efficiency (Fig. 2a).

At pH 7.4, these surface groups are deprotonated as COO^- and NH_2 resulting in a negative surface charge. On the other hand, the silanol groups in glass capillary tubes are deprotonated as Si—O that is the origin of electroosmotic mobility toward the cathode. At high pH, the surface charge density of microalgae is high. Thus, algae cells migrate to the anode (positive direction) and are separated with high resolution (Fig. 2b). Higher pHs (11.4) will significantly increase the EOF toward the cathode. Microalgae cells migrate toward the cathode with higher EOF that results in lower resolution (Fig. 2c).

Finally, at the intermediate pH (around pH 3), carboxyl groups are deprotonated (COO^-) while the amine groups are protonated (NH_3^+) resulting in surface charge neutralization known as the isoelectric point. These values are in accordance with the previous studies reported as point of zero charge for algae to be around pH 3.

Thus, the best separation efficiency and highest resolution were obtained at high pH values. A pH of 7.4 was, therefore, selected as optimum pH for the other experiments. Also, pH 7.4 is the physiological pH suitable for various biological applications.

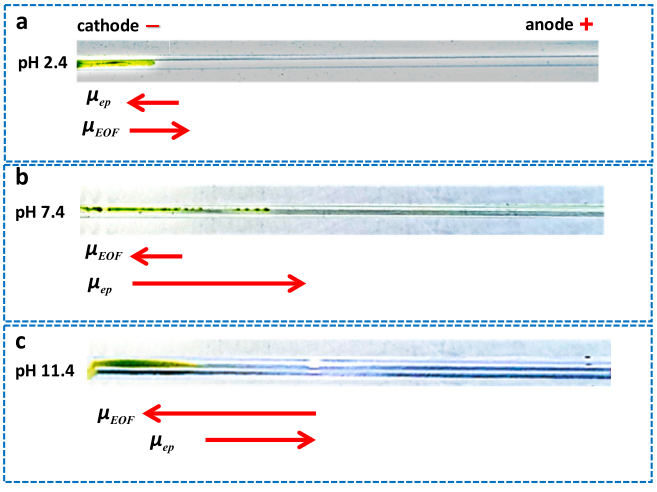

Fig. 2. Electrophoretic size separation of microalgae at different pH values. a) at pH 2.4, μ_{ep} is toward the cathode and μ_{EOF} is toward the anode, both μ_{ep} and μ_{EOF} are low. b) at pH 7.4, μ_{ep} is toward the anode and μ_{EOF} is toward the cathode and $\mu_{ep} > \mu_{EOF}$, thus, microalgae cells migrate toward the anode and are separated efficiently; c) at pH 11.4, μ_{ep} is toward the anode and μ_{EOF} is toward the cathode and $\mu_{ep} < \mu_{EOF}$, thus, microalgae cells migrate toward the cathode and exit from the end of the capillary tube.

3.2.2. Ionic strength

Zeta potential of a particle is highly dependent on the ionic strength of the electrolyte solution. The thickness of the double layer on microalgae surface is significantly related to the ionic strength of the medium [39,40]. The electric double layers around the algae cells is compressed at the higher ionic strength of medium, reducing the zeta potential of algae. The quantity of κ corresponds to the inverse thickness of double layer can be defined as follows [40]:

$$\kappa = 3.288\sqrt{I} \tag{7}$$

where $1/\kappa$ is the thickness of double layer as a function of ionic strength, I. The ionic strength of the electrolyte solution was optimized at 60 mM using phosphate buffer. The phosphate buffer with higher ionic strength (130 mM) did not provide the optimized fractionation (Fig. 3). The higher ionic strengths partially neutralize the surface charge of microalgae because of adsorption of counter ions which led to suppression of the double layer around the microalgae cells [26].

3.3. Characterization

FT-IR spectra of *C. vulgaris* and *Scenedesmus* are shown in Fig. 4. Some ionizable functional groups like O—H and N—H stretching (~3367 cm^{-1}), P═O (1248 cm^{-1}), and O–C–O (1024 cm^{-1}) show higher intensity for *C. vulgaris* compared with *Scenedesmus*, which means that the density of functional groups for *C. vulgaris* is higher than that of *Scenedesmus*.

To estimate the surface charge density, the zeta potential of both *C. vulgaris* and *Scenedesmus* species was separately measured at optimum pH of 7.4. The average values of zeta potential of *C. vulgaris* and *Scenedesmus* were − 21.2 (±0.9) and − 19.2 (±0.4) mV, respectively. From the results obtained from zeta potential measurements, it is plausible to say that *C. vulgaris* contains the highest number of ionizable functional groups than *Scenedesmus*. This observation was confirmed by FT-IR spectroscopy.

3.4. Electrophoretic separation

Fig. 5a shows visual electrophoretic separation of microalgae. A typical electropherogram of microalgae is also shown in Fig. 5b. The mixture of both *C. vulgaris* and *Scenedesmus* are separated into several fractions. The last fraction (F3) was separated with highest resolution of (Rs =12) from other fractions (calculated by Eq. (2)).

After separation, three different fractions with lowest (F_1), intermediate (F_2), and highest (F_3) mobilities were collected for further characterization. The purity of each fraction was confirmed by characterization techniques.

The morphological characteristics of the separated fractions was detected using FE-SEM and optical microscopy. FE-SEM images of microalgae before separation shows mixture of both species including *C. vulgaris* and *Scenedesmus* (Fig. 6a). *C. vulgaris* shows small and spherical cells with average diameter between 1 and 3 μm in stationary phase, while *Scenedesmus* is observed as spindle-shape cells with average length of 5–7 μm in stationary phase. After separation, the population of *C. vulgaris* has increased compared to *Scenedesmus* (Fig. 6b) so that the last fraction (F3) shows much higher *C. vulgaris* than *Scenedesmus* (Fig. 6c).

Fig. 7 shows the light microscopy images and distribution of both species in several fractions. Identification and counting of microalgae cells in each fraction was obtained by a hemocytometer. Bulk microalgae contain mixture of two microalgae strains with cell number of 6.6 × 10^3 and 5.4 × 10^3 of *C. vulgaris* and *Scenedesmus,* respectively (containing 55 % *C. vulgaris* and 45 % *Scenedesmus*). The results show that the purity percentage of *C. vulgaris* (calculated by Eq. (3)) has increased as compared with *Scenedesmus*. So that, the last collected fraction (F3) contain 94 % *C. vulgaris* and 6 % *Scenedesmus*, respectively. These

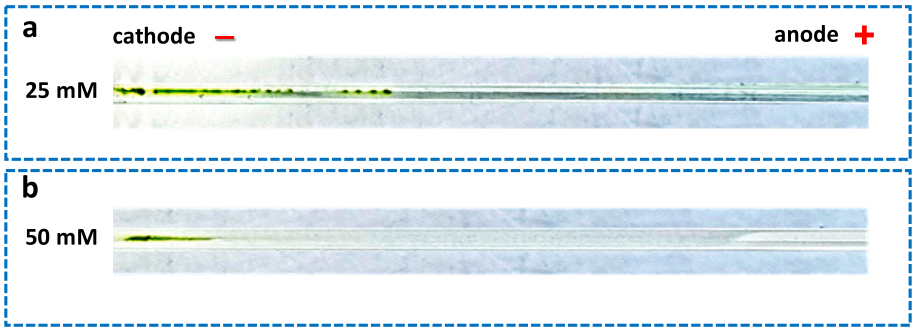

Fig. 3. Electrophoretic size separation of microalgae at different ionic strengths at pH 7.4. a) 25 mM phosphate buffer (I: 60 mM); b) 50 mM phosphate buffer (I: 130 mM).

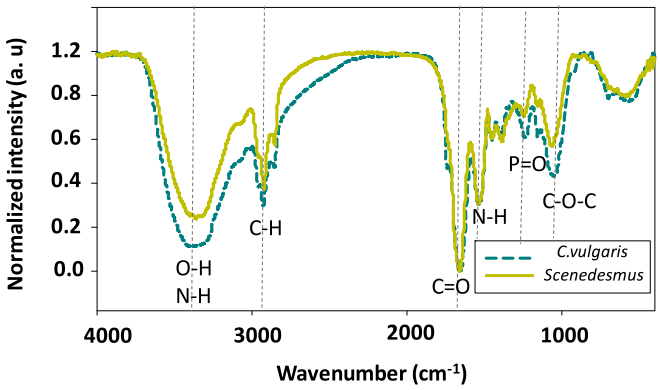

Fig. 4. FT-IR spectra of *C. vulgaris* and *Scenedesmus* microalgae.

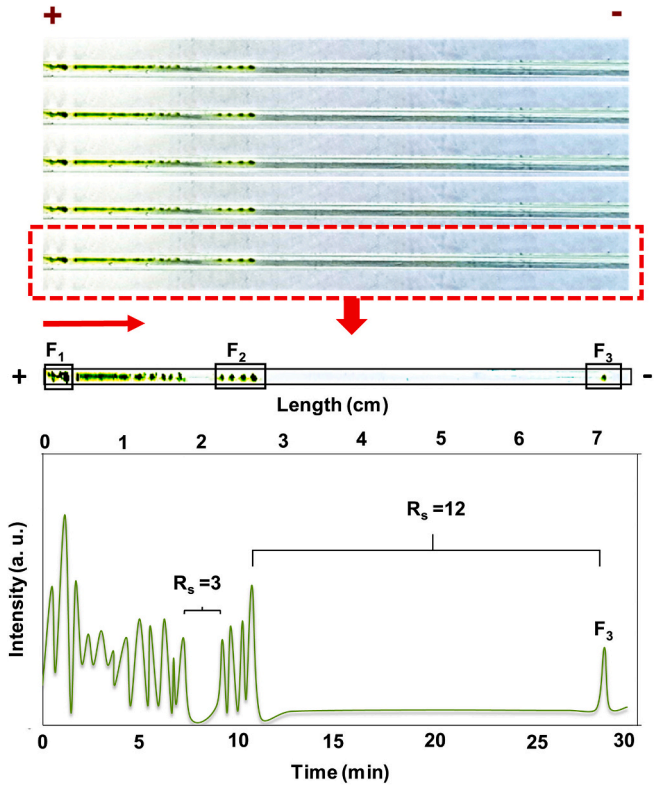

Fig. 5. a) Electrophoretic separation of mixed strain of microalgae including *C. vulgaris* and *Scenedesmus* into three fractions of F1, F2, and F3; b) corresponding electropherogram (obtained by PlotDigitizer software).

morphological characteristics indicated that the mixture of two algae strains separated with a 94 % efficiency.

Due to higher electrophoretic mobility, *C. vulgaris* was efficiently separated from *Scenedesmus* with high purity. The electrophoretic mobility (obtained from Eqs. (4) and (5)) for *C. vulgaris* (2.7 (±0.2) × 10^{-5} cm^2 s^{-1} V^{-1}) was about 13 times more than that of *Scenedesmus* (2.0 (±0.3) × 10^{-6} cm^2 s^{-1} V^{-1}) specie. Depending on the size, charge, and the density of ionizable functional groups on the surface, each strain of microalgae migrates with specific velocity under the applied electric field. Some reports showed that the concentrations of surface functional groups varies considerably in different species of microalgae [33]. The results of FT-IR spectroscopy and zeta potential measurements showed that *C. vulgaris* contains more ionizable functional groups.

On the other hand, *C. vulgaris* has spherical shape, so the surface charge is uniformly distributed on its surface. Unlike *C. vulgaris* with spherical shape, the electrophoretic mobility of long spindle-shaped *Scenedesmus* depends on its orientation relative to the applied electric filed. Due to the surface charge density, *Scenedesmus* cells are oriented in a specific direction. Hence, the electrophoretic separation of microalgae depends on the size, morphology, surface charge density, and the orientation relative to the electric filed. *C. vulgaris* with its smaller size, spherical morphology, and higher surface charge density has higher electrophoretic mobility and was effectively separated from *Scenedesmus*.

3.5. Cell viability

Applying high voltage may damage the cell wall of the microalgae [41]. The algae cell viability after electrophoresis separation was examined by measuring the optical density of the culture medium [42]. The results are shown in Fig. 8. The initial optical density was OD_{750} = 0.05 in all samples, after separation the optical density was increased. The results showed that, the applied voltage (100 V) did not have an effect on the viability of the microalgae cells, but showed positive consequences on biomass production in microalgae. This result has also been confirmed elsewhere [43,44]. Some reports showed electrical treatment of bacteria and microalgae can improve biomass production and the yield of high value products [43,44]. Kim et al. [45] showed the electrical treatments of *Haematococcus pluvialis* can increase dry weight and astaxanthin content of *H. pluvialis*. This strategy can be regarded as a suitable approach to enhance algae growth as well as biomass production.

4. Conclusion

In the present study, the electrophoresis was utilized to separate microalgae strains including *C. vulgaris* and *Scenedesmus* from mixed strains on the basis of their different sizes and shapes. Multiple mega-bore capillary tubes were arranged in an array to further improve the overall throughput of the system. The surface charge property of

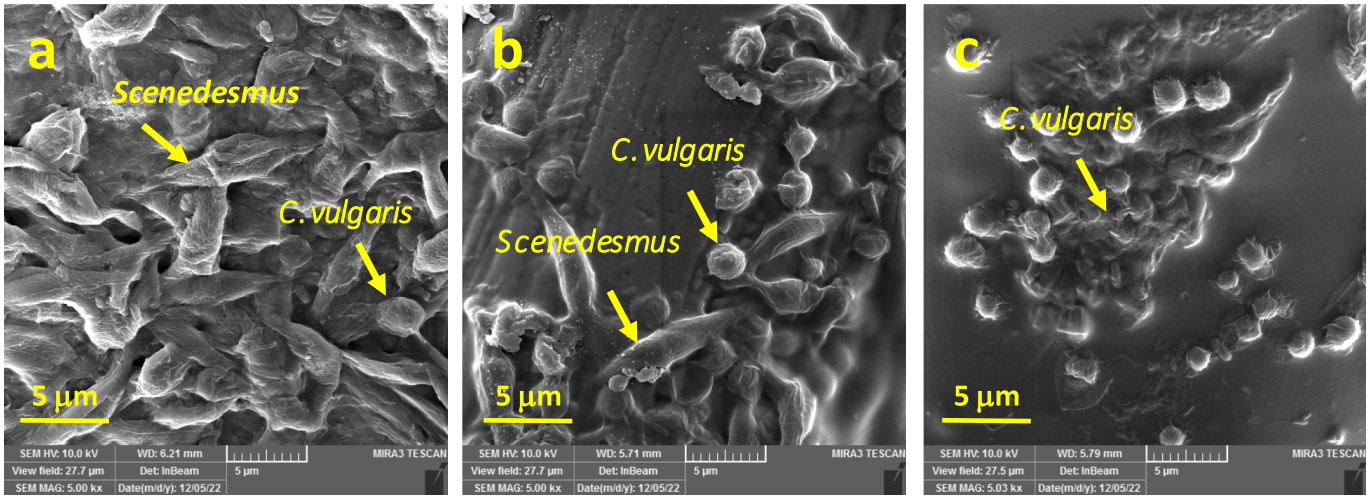

Fig. 6. FE-SEM images of a) F_1 (contain mixture of both *C. vulgaris* and *Scenedesmus*); b) F_2 (contain mixture of both *C. vulgaris* and *Scenedesmus*); c) F_3 (mostly contain *C. vulgaris*).

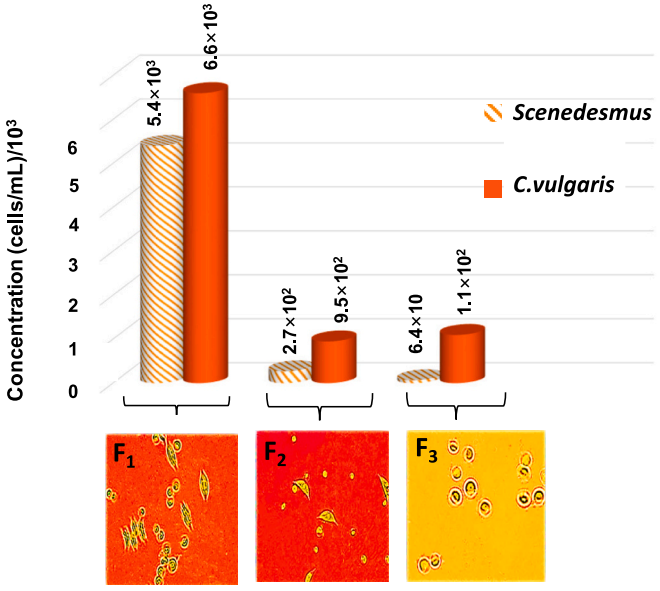

Fig. 7. Distribution of *C. vulgaris* and *Scenedesmus* species in different separated fractions including F_1 (contain mixture of both *C. vulgaris* and *Scenedesmus* before separation); F_2 (contain mixture of both *C. vulgaris* and *Scenedesmus* after separation); F_3 (mostly contain *C. vulgaris*), and corresponding light microscope images (100× magnification).

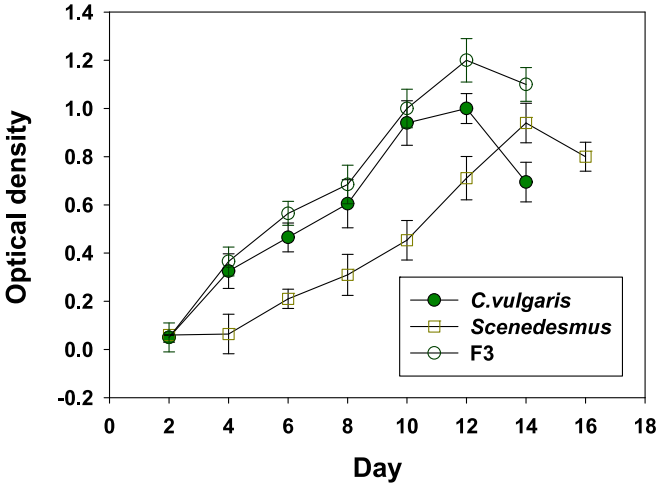

Fig. 8. The growth curve of *C. vulgaris*, *Scenedesmus*, and last separated fraction (F_3).

microalgae was used for electrophoretic separation. The results of surface characterization of microalgae showed that *C. vulgaris* contains more ionizable functional groups than that of *Scenedesmus*, thus, having higher electrophoretic mobility. High purity separation (94 %) of the *C. vulgaris* from *Scenedesmus* was achieved by optimizing some parameters such as pH and ionic strength. The microalgae cells were fractionated without loss in cell viability of species.

CRediT authorship contribution statement

Maryam Davardoostmanesh: Writing – original draft, Supervision, Investigation, Formal analysis. **Hossein Ahmadzadeh:** Writing – review & editing, Project administration, Conceptualization. **Soheila Samiee:** Software, Methodology, Investigation.

Author statement

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Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

Data will be made available on request.

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