

A simultaneous *Spirulina* biomass production and brine desalination in an auto trophic culture

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ABSTRACT

The simultaneous *Spirulina* biomass production and wastewater treatment have already been the subject of several investigations in the past; however, treatment of the brine effluent by photosynthetic microorganisms was rarely studied due to the brine's harsh condition as a medium for cellular growth. This study has investigated an auto trophic cultivation of *Spirulina* in various brine-based culture media, in order to illustrate its viability for simultaneous *Spirulina* biomass production and brine treatment. The laboratory experiments were conducted in a 3 l inverted pyramid photo bioreactor. Seven various brine compositions were analyzed as culture media and the Zarrouk culture medium was used as the control. To assess the process efficiency, the *Spirulina* dry biomass and brine treatment quality were monitored during the cultivation period. The total cellular density increased in the brine-based culture media during 16 days of cultivation, and reached up to 0.6 g·l⁻¹ in the diluted brine. Meanwhile, the electrical conductivity (EC) and the total dissolved solids (TDS) of the brine-based culture media decreased to 43.5% and 71%, respectively. By using brine and Zarrouk culture media, it was found that cellular density was lower in the brine culture medium when compared to the Zarrouk control culture medium. In conclusion, the inhibitory effects of the brine culture components could be the cause of such inhibition in cell growth. Cellular adaptation to the brine culture medium takes approximately two days during the initial lag phase of growth of the primary cultures. The results of this study, indicate the potential of *Spirulina* for brine desalination.

Keywords: Cyanobacteria biomass; Nutrient removal; Brine discharge; Tolerant microorganisms; Microalgae cultures

1. Introduction

The mega, medium, or small-scale desalination plants generate large volumes of brine on a daily basis. The brine discharged into the open water system is a potentially large cause of damage to the environment [1]. The discharged desalinated effluent is known to bring changes potentially harmful to the biotic and abiotic components of the water ecosystem [1]. In the coastal areas, seawater desalination plants are growing in numbers to provide reliable drinking water in the arid and semi-arid areas of the world, such as the Middle East as well as countries such as the USA and Australia, in addition to many other countries. The desali-

nation facilities are commonly composed of the main units of intake, pretreatment, and reverse osmosis units. They generate water plus a large volume of brine byproduct effluent [2]. The produced brine byproduct mainly consists of dissolved minerals such as magnesium, calcium, potassium, sodium, bromine, and chlorine. The safe management of these brine byproducts has to be considered for a sustainable discharge into the environment and in compliance with the regulatory discharge permit requirements of the local authorities [3–7]. Currently, the most common alternative approach for brine management is by releasing it into the ocean and seawater through discharge systems [8,9].

In order to cope with biological sustainable brine treatment processes, a brine adaptation is probably one of the most crucial steps, since it has a large impact on the effi-

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ciency of the overall brine treatment process. Brine includes high concentrations of inorganic ions with poor concentrations of organic compounds which cause stress and growth inhibition effects. The aim of brine treatment processes with various levels of salinity is removal of inorganic substances using photosynthetic micro organisms besides the physical and chemical methods [5,10,11]. The biosorption process takes place through several routes of adsorption, absorption and uptake by photosynthetic microorganisms [12–14]. The ions taken up may participate in cellular metabolism stored in cytoplasmic vacuoles. The main pathway for ion uptake is reversible passive adsorption on the cell surface, which is then followed by absorption through irreversible active routes inside of the cell membrane and cytoplasm [14]. Shahalam investigated the critical aspects of reject brine characteristics, inherent problems and special factors to be considered for treatment of such saline waters, including possible treatment solutions in the conventional wastewater treatment facilities [15]. In a study conducted by Angelo et al., they evaluated the effects of the dilution rate of brine on microalgae biomass productivity, lipid content and fatty acid profile during steady state cultivation of *Chlorella vulgaris*. Results showed that biomass productivity varied from 57 to 126 mg·l⁻¹·d⁻¹, when the dilution rate ranged from 0.1 to 0.3 d⁻¹ [4]. In the study conducted by Zarzo et al. regarding nutrient removal from brines by means of microalgae, they focused on the selection of species for nitrate removal in desalination brines, optimization of process parameters and possible commercial use. They found some species occurring in the surroundings of the desalination plants were able to survive in the brine. The isolated strain, was then cultivated in laboratory conditions of light/dark lighting at 22°C. A nitrate removal 45% and the highest biomass productivity was obtained by *Oocystis sp.* [10]. The reported results are surprising as they propose opportunities for application of brine as a culture medium for cultivation of photosynthetic micro organisms with potential for commercial application through the production of biomass as a food supplement, cosmetics, vitamins, and antioxidants, etc. [16]. Hypothetically, this applicability would have an environmentally and economically positive impact [8,11,17–21]. So far, the brine treatment process has been the subject of several researches that have used a number of species with brine as the sole source of nutrients [22].

In a study by El-Nadi and coworkers, they used an algae pond system for desalination of brine with salt concentrations ranging from 2000 to 40,000 ppm (2 and 40 g·l⁻¹). They reported 61% to 97.7% TDS removal under Egyptian climate conditions [23,24]. Another study investigated the electro dialysis of the backwash saline water in a batch culture system by *Chlorella vulgaris* [25]. In another research by Al-Tabbal et al., the effects of RO-reject waters were studied. They used several combinations of clay and zeolux and investigated their effects on plant growth. The results in this study indicated that the RO-reject water causes significant reduction in the growth-related parameters, and zeolux does not reduce the harmful effects of the RO-reject water [7]. However, further researches are required to address the technological and financial feasibility along with sustainability of biological brine treatment using photosynthetic microorganisms including cyanobacteria *Spirulina* [26].

Since brine treatment by photosynthetic microorganisms has high dependency on the brine composition, the goal of the present study was to evaluate the growth of *Spirulina*, a cyanobacterial species cultivated indifferent brine-based culture media. This study has assessed seven compositions of the brine culture media along with the Zarrouk culture medium (CM) as the control in a batch cultivation system using the inverted pyramid photobioreactor.

2. Materials

2.1. *Spirulina* strain and the culture medium preparation

The stock cultures of cyanobacterium *Spirulina* [27], maintained on slants were used in this investigation. The strain was sub-cultured in Zarrouk culture medium according to Sheykhi Nejad et al. [27]. The Zarrouk culture medium includes the following chemicals (all components are based on g·l⁻¹): NaCl, 0.92; NaNO₃, 2.5; K₂HPO₄, 0.50; K₂SO₄, 1.0; NaHCO₃, 16.8; CaCl₂·2H₂O, 0.05; KNO₃, 2.57; MgSO₄·7H₂O, 0.25; CaCl₂·2H₂O, 0.04; FeSO₄·7H₂O, 0.01, in addition to 1.0 ml·l⁻¹ of the micronutrient solution prepared by combining the following reagents: H₃BO₃, 2.86; MnSO₄·H₂O, 1.54; ZnSO₄·7H₂O, 0.22; NaMoO₄·2H₂O, 0.39; CuSO₄·5H₂O, 0.079, and CoCl₂·6H₂O, 0.038 (g·l⁻¹), plus 1.0 ml·l⁻¹ of the Fe-EDTA solution [(EDTA-Na₂, 29.8; FeSO₄·7H₂O, 24.9) (g·l⁻¹)] [28].

The cultured cyanobacterium, *Spirulina*, was illuminated under a fluorescent lamp (Pars Shahab, Iran) from both sides with a light intensity of 40 μE·m⁻²·s⁻¹, as measured by a light meter (TES 1332A Digital LUX Meter, Taiwan) at the room temperature of 25°C ± 2. Cultivation was conducted in 250 ml Erlenmeyer flasks containing 100 ml of culture medium. Mixing of the medium was provided using a shaker at a rotation rate of 220 rpm (Chimi Fan Azma, Iran). No aeration was used in the seed cultures. Subcultures were seeded by 0.03 g·l⁻¹ of dry cell weight (DCW) in sterile conditions.

2.2. Experimental setup

A 3 l handmade inverted pyramid shaped glass vessel photobioreactor with a 4 cm wide square bottom and 25 cm in length, with a 2 l working volume was used for all experiments. This configuration prevents the sedimentation of cells during cultivation because of its circulation pattern. The cultures were illuminated with four cool white fluorescent lamps (Pars Shahab, Iran) from both sides with a light intensity of 40 μE·m⁻²·s⁻¹, as measured by a light meter (TES 1332A Digital LUX Meter, Taiwan) at a constant temperature of 25°C ± 1. The temperature was kept constant using a previously developed control package that included a temperature probe, an AVR micro controller, and LabView software, 2013 (NI, USA), with one-minute interval measurements during the cultivation period [29]. Mixing of the cultures was carried out in the photobioreactor by bubbling with air (sparging), which was adjusted to an air flow of 350 ml·min⁻¹ by using an electromagnetic air pump (RESUN, ACO-006, China), mass flow meter, and a spargernozzle located at the bottom of the vessel. The cultivation was conducted for 15 days based on the preliminary experiments and selection of the best cultivation time period.

2.3. Culture media

Brine effluent was obtained from a local desalination plant (Bandar Lenge, Hormozghan province, Iran). The electrical conductivity of the brine was determined to be approximately $82 \text{ mS}\cdot\text{cm}^{-1}$ and was kept at the laboratory room temperature before use in the experiments. The chemical composition of the brine was analyzed in a respective laboratory facility (Keifiat Azmaye Jonoob Lab Co., Iran) and was found to contain bicarbonate 18.3; calcium 3700; carbonates 288.10; chloride 27047; phosphate < 0.05 ; magnesium 420; nitrate 0.66; potassium 884.7; sodium 14442.6; bromide 3.49; silicate 8.76, and sulfate 5074, respectively (all chemical concentrations are in $\text{mg}\cdot\text{l}^{-1}$, Table 1).

Table 1

The electrical conductivity of the brine effluent and the level of the major chemical elements in the present study and comparison with the typical seawater characteristics from another Persian Gulf country

| Parameter | Concentrated brine | Typical seawater, Kuwait* |
|--|--------------------|---------------------------|
| Electrical conductivity (EC), $\mu\text{S}\cdot\text{cm}^{-1}$ | 82000 | |
| Chemical oxygen demand (COD), $\text{mg}\cdot\text{l}^{-1}$ | 281.6 | |
| Total dissolved solids (TDS), $\text{mg}\cdot\text{l}^{-1}$ | 61542 | |
| Carbonate, $\text{mg}\cdot\text{l}^{-1}$ | 288.10 | |
| Phosphate, $\text{mg}\cdot\text{l}^{-1}$ | < 0.05 | |
| Chloride, $\text{mg}\cdot\text{l}^{-1}$ | 27047 | 23000 |
| Sodium, $\text{mg}\cdot\text{l}^{-1}$ | 14442.6 | 15850 |
| Sulfate, $\text{mg}\cdot\text{l}^{-1}$ | 5074 | 3200 |
| Magnesium, $\text{mg}\cdot\text{l}^{-1}$ | 420 | 1765 |
| Calcium, $\text{mg}\cdot\text{l}^{-1}$ | 3700 | 500 |
| Potassium, $\text{mg}\cdot\text{l}^{-1}$ | 884.7 | 460 |
| Bicarbonate, $\text{mg}\cdot\text{l}^{-1}$ | 18.3 | 142 |
| Strontium, $\text{mg}\cdot\text{l}^{-1}$ | – | – |
| Bromide, $\text{mg}\cdot\text{l}^{-1}$ | 3.49 | 80 |
| Borate, $\text{mg}\cdot\text{l}^{-1}$ | – | – |
| Fluoride, $\text{mg}\cdot\text{l}^{-1}$ | 0.65 | – |
| Silicate, $\text{mg}\cdot\text{l}^{-1}$ | 8.76 | 1.5 |
| Manganese, $\text{mg}\cdot\text{l}^{-1}$ | 0.195 | |
| Iron, $\text{mg}\cdot\text{l}^{-1}$ | 0.001 | – |
| Nitrate, $\text{mg}\cdot\text{l}^{-1}$ | 0.66 | |
| Nitrite, $\text{mg}\cdot\text{l}^{-1}$ | 0.01 | |

*<http://www.lenntech.com/composition-seawater.htm>

Table 2

Experimental design for the cultivation of the cyanobacterium *Spirulina* in the various culture media compositions. Numbers 1 to 8 shows the number of experimental culture runs

| Components | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|-----------------|---------------|-----|-----|-----|-----|-----|-----|-----|
| Zarrouk | 100%, control | 0 | 0 | 0 | 0 | 50% | 25% | 75% |
| Brine | 0 | 100 | 75% | 50% | 25% | 50% | 75% | 25% |
| Distilled water | 0 | 0 | 25% | 50% | 75% | 0 | 0 | 0 |

Eight types of culture media, also known as runs, were used in the experiments with chemical compositions listed in Table 2, along with Zarrouk CM as the control medium (run 1). The brine from the desalination process was used without modification as culture medium 2 (run 2). The other six culture media sets (runs) were prepared by combining brine, Zarrouk CM, and distilled water at different ratios. The media runs of 3, 4, and 5 were prepared by mixing the brine and distilled water. Run 3 was composed of brine (75% v/v) and distilled water (25% v/v) and run 4 was composed of equal volumes of brine (50% v/v) and distilled water (50% v/v). Run 5 included brine (25% v/v) and distilled water (75% v/v). Runs 6, 7, and 8 were a combination of brine and Zarrouk culture medium. Run 6 was composed of equal volume of brine and Zarrouk CM (50%/50% v/v), run 7 was a mixture of brine (75% v/v) and Zarrouk CM (25% v/v), and finally run 8 was composed of brine (25% v/v) and Zarrouk CM (75% v/v). The pH of all culture media was adjusted to 9.2 ± 0.2 .

2.4. Methods of analysis

Samples of 3 ml were taken in the course of cultivation and used for dry cell weight (DCW), EC, TDS, and salinity analysis. The biomass concentration was assessed following the measurement of 1.0 ml of dry cell weight after centrifugation at $10000 \times g$ for 5 min, discarding the supernatant, washing the biomass with distilled water, and drying at 105°C for 6 h on a dry plate incubator (Kia-Gen, Molecular Biology Company, Iran), and subsequently weighing with a Mettler Toledo AT261 Delta Range analytical balance (0.01 mg). In order to ensure and observe the dry cell weight measurement protocol and data reliability, a calibration curve of serially diluted culture samples (in distilled water) versus their DCW was constructed [30]. The morphology of the cells was monitored with a hemocytometer under a light microscope. The *Spirulina* culture broth parameters such as EC, TDS, and salinity were monitored with EC-470L (ISTEK, Korea) daily during the cultivation period. The estimation of the various chemical constituents in the culture media was carried out using the standard methods as described in the Standard Methods for the Examination of Water and Wastewater [31]. The chemicals that were subjected to testing included calcium, magnesium, iron, free ammonia, nitrate, chloride, fluoride, sulfate, phosphate, and silica.

2.5. Statistical analysis

The effects of various compositions of brine on *Spirulina* dry cell weight were assessed in a completely randomized

design with three replications. The resulting data were evaluated by analysis of variance (ANOVA) with a confidence level of 95% ($p < 0.05$), and differences between means were calculated using the general linear model (GLM) in order to determine if DCW was significantly different in the various culture media. The analysis was carried out using SAS/STAT software, version 9.00 (SAS Institute, USA).

3. Results

3.1. Nutrient and salinity removal

Fig. 1 depicts the electrical conductivity of the brine discharged from Bandar Lenge desalination plant in ppm ($\text{mg}\cdot\text{l}^{-1}$). The measurements were carried out for a month that began from August 10th to September 9th, 2016 in order to characterize the level of salinity in the effluent brine. The fluctuation of electrical conductivity, total dissolved solids, and water salinity of the desalination effluent brine ranged from 78100 to 80000 $\mu\text{S}\cdot\text{cm}^{-1}$ (average 78942.42 $\mu\text{S}\cdot\text{cm}^{-1}$) ($T = 31.2\text{--}32.0^\circ\text{C}$, average $T = 31.63^\circ\text{C}$), 55000 to 49000 $\text{mg}\cdot\text{l}^{-1}$, and 87.6 to 72.2 $\text{g}\cdot\text{l}^{-1}$, respectively (Table 1). The range of EC, TDS, and salinity of the discharged effluent brine shows the characteristic average range among the desalination plants' effluents that take in seawater in the Persian Gulf coastal areas [3].

The estimated percentage reductions of 17.36, 26.67, and 34.99% in the electrical conductivity was observed during the 5, 10, and 15 days of the cultivation period, respectively (Fig. 2). The electrical conductivity of the effluent brine was reduced to 53302 $\mu\text{S}\cdot\text{cm}^{-1}$ on day 15, which is relatively similar to that of the Persian Gulf coastal water [32]. The total dissolved solids concentration was consistently below 14,000 $\text{mg}\cdot\text{l}^{-1}$ at the final cultivation period, showing approximately a 71% reduction in the TDS.

3.2. *Spirulina* growth and biomass concentration

Fig. 3 shows cell growth and *Spirulina* biomass production in the various brine-based culture media. Results indicate that photosynthetic cyanobacterium *Spirulina*

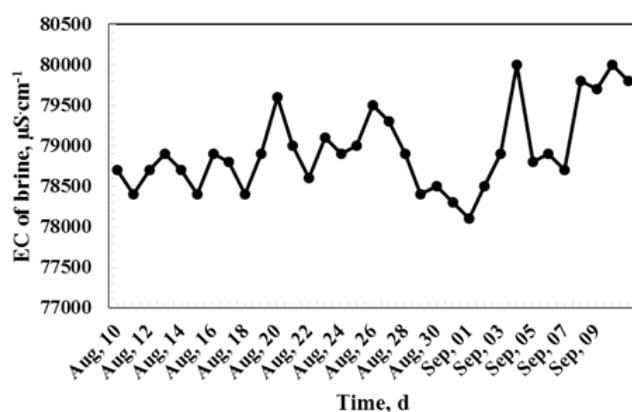


Fig. 1. The electrical conductivity of the brine discharged from the of Bandar Lenge desalination plant. The dot linear plot indicates the average total salinity of the effluent brine during the 31 days of measurement of the discharged brine from the facility.

was able to survive and grow in the various brine culture media throughout the entire cultivation period. The highest and lowest cellular biomass of 0.97 and 0.4 $\text{mg}\cdot\text{cm}^{-3}$ was obtained in the control (i.e., the Zarrouk culture medium) and in the brine culture medium, respectively. The general trend of cell growth in the cultures with brine supplemented with Zarrouk culture medium (runs 6 to 8) were relatively higher (approximately 0.7 $\text{mg}\cdot\text{cm}^{-3}$) when compared to the brine cultures supplemented with distilled water (runs 3 to 5) (approximately 0.5 $\text{mg}\cdot\text{cm}^{-3}$). Cell growth was slightly increased after diluting brine with distilled water. More-

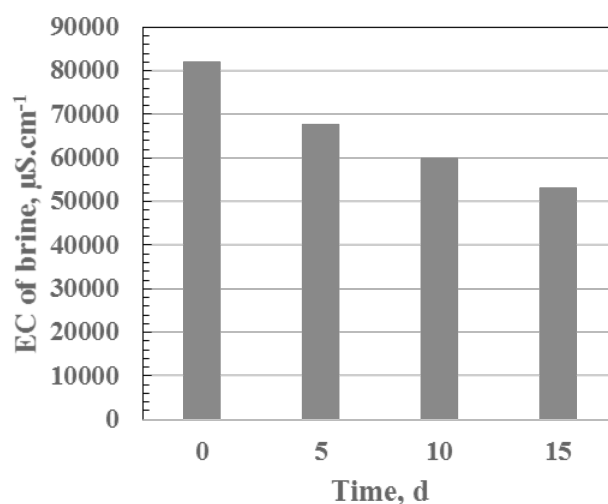


Fig. 2. The electrical conductivity (EC) measurement during different time intervals post-cultivation of the cyanobacterium *Spirulina*. The reduced electrical conductivity indicates consumption and consequently removal of ions from the surrounding medium by the microorganism.

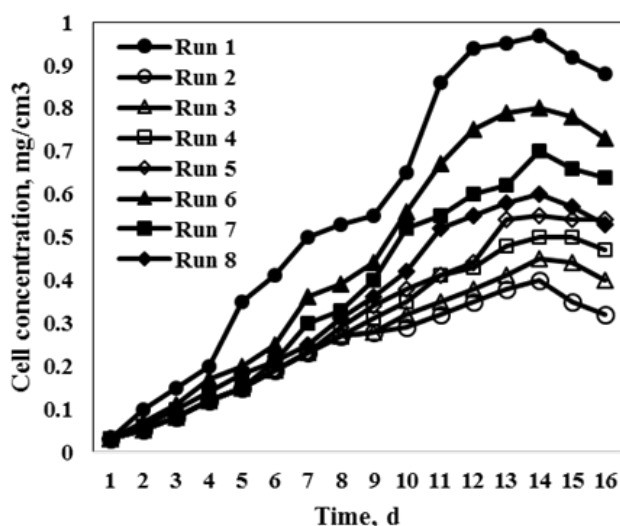


Fig. 3. *Spirulina* growth curves monitoring during the 16 days of cultivation. The highest growth rate could be observed in the running culture condition 1 which includes the Zarrouk control medium. Accordingly, the lowest growth rate was detected in the brine culture medium (i.e., run 2).

over, cell growth was lower in run 8 when compared to that of run 7. The mean biomass productivity varied from 0.4 to 0.97 mg·cm⁻³ with various adjustments of initial used brine. The variance between different treatments was significant indicating the influence of brine adjustment, thus recommending dilution and feed supplements for higher *Spirulina* biomass productivity. Adjustment of saline culture media for microalgae cultivation were also proposed in literature [4,33]. All experimental runs were performed with an initial cell density of 0.03 mg·cm⁻³.

Effluent brine was used as a culture medium in the production of cyanobacterium (i.e., *Spirulina*) biomass to overcome the costly feedstock in the production process associated with *Spirulina* cultivation systems. Similar to other photosynthetic microorganisms, cyanobacterium utilizes inorganic ions from the culture medium as nutrients, especially, ionic forms of nitrogen and phosphorous for their cellular metabolism [34]. Among the inorganic ions, nitrogen and phosphorous have been found to be removed effectively by a number of different species of cyanobacteria [18,35,36]. The results of this study also show a significant removal of the inorganic compounds by *Spirulina* as could be concluded from data presented in Table 3.

Among the cultures supplemented with the Zarrouk culture medium, the growth rate of *Spirulina* was slightly increased in run number 6, which is composed of the effluent brine supplemented with the Zarrouk culture medium at an equal ratio of 50:50%. The highest biomass production was obtained on day 13 of the cultivation.

The results of the statistical analysis are shown in Tables 4 and 5. Statistical analysis (i.e., analysis of the variance) indicated that there was a significant difference ($P < 0.05$) between the means of independent brine culture media and the dependent variables of the *Spirulina* DCW, (Table 4). The means of the DCW in various culture media

are shown in Table 4. Based on the least square deviation (LSD) results, the brine solution supplemented with the Zarrouk culture medium at a ratio of 50:50%, is the most effective culture medium among the seven brine-based culture media. The culture media that do not share a letter are significantly different in the level of probability equal or less than 0.05 (Table 5).

Table 4
The analysis of the variance for the *Spirulina* cultures in the various brine based culture media

| ANOVA | d_f | DCW |
|----------------|-------|---------|
| Culture medium | 7 | 0.052** |
| Error | 23 | 7E-3 |

* Significantly different in the probability level of 0.05.

Table 5
The mean DCW in the various brine based culture media

| Culture medium number | DCW |
|-----------------------|-------------------|
| 1 | 0.51 ^a |
| 2 | 0.17 ^b |
| 3 | 0.2 ^c |
| 4 | 0.22 ^c |
| 5 | 0.24 ^c |
| 6 | 0.36 ^d |
| 7 | 0.33 ^d |
| 8 | 0.31 ^d |

*Culture media that do not share a letter are significantly different in the probability level of $P < 0.05$.

Table 3
Physico-chemical parameters of the brine treated with the cyanobacterium, *Spirulina*

| Parameters | 0 th day | 5 th day | 10 th day | 15 th day | % of reduction |
|--|---------------------|---------------------|----------------------|----------------------|----------------|
| Appearance | colorless | colorless | colorless | colorless | – |
| Turbidity, NTU | 264 | 180 | 110 | 25 | 90.53 |
| Total dissolved solids (TDS), mg·l ⁻¹ | 1300 | 1200 | 1085 | 995 | 23.46 |
| Total suspended solids (TSS), mg·l ⁻¹ | 302 | 232 | 155 | 81 | 73.17 |
| Conductivity | 82000 | 67767 | 60127 | 53302 | 34.99 |
| pH, mg·l ⁻¹ | 5.26 | 5.62 | 6.75 | 7.65 | +31.24 |
| Alkalinity total, mg·l ⁻¹ | 824 | 675 | 570 | 440 | 46.60 |
| Total hardness, mg·l ⁻¹ | 1325 | 1000 | 672 | 454 | 65.73 |
| Calcium, mg·l ⁻¹ | 260 | 187 | 125 | 50 | 80.76 |
| Magnesium, mg·l ⁻¹ | 162 | 112 | 62 | 23 | 85.80 |
| Iron, mg·l ⁻¹ | 2.38 | 2.29 | 2.24 | 2.17 | 8.82 |
| Free ammonia | 70.56 | 47.14 | 24.62 | 6.20 | 91.21 |
| Nitrate, mg·l ⁻¹ | 1 | 5 | 8 | 13 | +92.30 |
| Chloride, mg·l ⁻¹ | 254 | 232 | 223 | 170 | 33.07 |
| Fluoride, mg·l ⁻¹ | 0.53 | 0.44 | 0.28 | 0.15 | 71.69 |
| Sulfate, mg·l ⁻¹ | 139 | 110 | 75 | 42 | 69.78 |
| Phosphate, mg·l ⁻¹ | 44.93 | 32.32 | 16.22 | 5.6 | 87.53 |
| Silica, mg·l ⁻¹ | 242.82 | 187.26 | 110.32 | 48.51 | 80.02 |

4. Discussion

The purpose of this study was to evaluate the possibility of cyanobacterium *Spirulina* biomass production in the desalination brine effluent. In addition, the ability of *Spirulina* cells to treat brine was assessed.

As was expected, the salinity of the brine culture media was decreased when *Spirulina* was cultivated in the various combinations of brine, distilled water, and Zarrouk culture medium; a result that supports bacterial influence on the brine desalination hypothesis. These results imply an alternative biological treatment for wastewater with high salinity such as brine and oilfield brine, etc.

Choonawala and Swalaha have previously reported the growth of *Spirulina* in the cooling tower brine effluent [18]. The present investigation has further expanded our knowledge about the biological treatment of inorganic substances from wastewater using photosynthetic microorganisms. However, this study has only shown a simple laboratory experiment for the brine treatment process. Exploring the biological brine treatment methods on the pilot and large scales would in practice encounter a number of challenges [37]. In addition, only a few photosynthetic microorganisms such as *Spirulina*, *Chlorella* and *Dunaliella* have been reported to grow in saline brine [23]. This is because of the osmotic effect that leads to growth inhibition in addition to the low nutrient content of the brine. Therefore, the adaptation methods such as brine dilution and the addition of supplements, or a combination of the treatments might be required before these systems are used for the treatment of such wastewaters.

The microscopic observations and growth of the *Spirulina* in the brine-based culture media show the ability of this microorganism in tolerating the high ionic strength of the culture medium. As most microorganisms are not able to tolerate the harsh conditions of the brine culture medium, the ability of *Spirulina* makes it a suitable candidate for further future studies [38]. The potential of photosynthetic cell growth in brine implies that a large amount of *Spirulina* biomass could be produced in the various saline wastewaters and brine effluents. The produced microalgal biomass has the potential to be used in the various pharmaceutical and food industries. The production of *Spirulina* biomass on either small or large-scales, has been the subject of many previous reports [20,28,39,40]. However, a large-scale brine treatment process operation has not been reported yet, which would be associated with a number of challenges that require being dealt with, including low yield.

The considerable growth of the *Spirulina* in the brine-based culture media with an electrical conductivity above 40000 $\mu\text{S}\cdot\text{cm}^{-1}$ in addition to the poor culture medium quality, may result in a lower contamination with other microorganisms, as well. The less contaminated culture may support higher *Spirulina* cell production in brine [41].

The results of this research have demonstrated that brine wastewater can be used in the biomass production process, which offers the use of various undrinkable water sources for biomass production as a nutrient supply. Development of an effective and low-cost culture medium implies a more feasible production process. However, with respect to the bio process, variable brine intake as a culture medium affects the process parameters and the production stability.

The simultaneous growth of *Spirulina* cells and reduction of culture medium ions during the cultivation period have indicated the ability of ion absorption by cells following adsorption, which can occur only in a short period of time [12]. In addition, the EC50 calculated for lead absorption by *Spirulina* demonstrated its strong ability to tolerate lead metal concentrations and possibly other ions [12]. However, more in-depth molecular substantiation through physiological studies such as the role of alkali-metal-cation transporters, surface sorption studies, and elemental analysis of the cells is required to reveal process pathways and mechanisms of sorption for further applications in brine treatment [13,42].

The effects of the input wastewater on cell growth have been the subject of investigations in a number of reports that have used different types of input wastewaters. The growth findings presented in this study show that cyanobacterium *Spirulina* has a salinity tolerance of 75 $\text{g}\cdot\text{l}^{-1}$. The same salinity resistance by *Spirulina* has also been reported by other researchers [8]. However, influent brine usually shows variance in the amount and composition of the mineral constituents that demands an adjustment for process stability improvement.

5. Conclusions

The preliminary results of the present study have confirmed that the photosynthetic cyanobacterium *Spirulina* has the potential to be used in the biological brine treatment process. This process combines nutrient removal and *Spirulina* biomass production which has the potential to be used as a feed stock for biodiesel and supplementary bioactive foods production, including many other potential applications. This ability was confirmed by significant inorganic nutrient removal through the desalination of brine, which encourages the employment of this cyanobacterium for the treatment of large volumes of discharged brine, especially brine discharged into coastal seawaters. In addition, for a simultaneous efficient biomass production and brine treatment, we are presently planning to perform further studies on continuous cultivation systems that were not the focus of this research and are considered as more favorable for the desalination industry. Further process optimization is in progress to improve biomass production and brine treatment efficiency for a safer discharge into the open waters.

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