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Photosynthetic free and immobilized cells of *Scenedesmus abundans* for desalination of seawater

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ABSTRACT

Saline water is considered to be a potential source of drinking water and a non-potable application. Current desalination techniques are energy intensive; hence, the need has arisen for an energy efficient alternative technique. New researches have paved the way for bio-desalination, that is, sustainable exploitation of photosynthetic microorganisms for desalination. This study evaluates the suitability of utilizing green alga *Scenedesmus abundans* in the form of free and immobilized cells for seawater desalination. For optimization study, different inoculums concentrations of free and immobilized cells such as 5%, 10%, 15%, and 20% were used to determine the salinity removal efficiency in 25 ppt of total dissolved solids (TDS) present in seawater. 10% of free cells and 15% inoculums concentration of immobilized cells worked well in the above seawater concentration. Further, effective and optimized concentration is used to analyze the desalination performance of different saline concentrations in terms of TDS such as 5, 10, 15, 20, and 25 ppt. The results showed that the removal efficiencies were higher with an increase in TDS concentration.

Keywords: Bio-desalination; *Scenedesmus abundans*; Total dissolved solids; Immobilized cells

1. Introduction

Water is an essential commodity to sustain life on the earth. Only 1% is accessible out of 3% of total clean water available on the earth. Increase in global population has increased water usage which in turn has led to the increase in demand for the development of water desalination techniques. Many of the existing conventional desalination methods, such as reverse osmosis, thermal process, solar desalination, etc., are expensive and energy-consuming [1]. Hence, it is a significant challenge to identify an alternative desalination technology supported by a renewable source to desalinate seawater.

The development of novel, low-energy biological desalination process using biological source is essential. A low-salt biological reservoir within seawater that can serve as an ion exchanger forms the core of the proposed "bio-desalination" process [2]. One of the essential bio-desalination techniques includes utilization of halophytes in the desalination process. Many salt-tolerant (halophytes) plants are identified for indirect desalination of water. In recent years, microalgae have been found to complete their life cycle within a wide range of salt levels and enhance the amount of desalination. Higher sodium uptake was observed in freshwater strain (*Anabaena* sp. L-31) when compared with sea strain *Anabaena torulosa*. The reason might be due to the higher sodium efflux capacity of the former. It has been found that 90% of the accumulated salt is bound to extracellular polymeric substances on the cell surface and the rest is internalized and osmotically active in the above strains [3,4]. It is also observed that salt-excluding mechanism is based on specific features called antiporters [5]. *Dunaliella salina*, microgreen algae can survive in 3 M NaCl due to its salt-blocking ability and is a typical example of salt-eliminating plants. The salt-removing strength is

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due to the presence of Na^+ and H^+ antiporter on the vacuole membrane and H^+/ATPase pumps on the plasma membrane [6]. Use of microalgae in biodiesel production is yet another important application. A geographic information system cost model predicts the ability of microalgae to thrive in unsuitable waters such as saline groundwater or seawater which makes it suitable for biodiesel production. The study has also predicted the use of freshwater, saline water in open-pond cultivation made possible by partial techno-economic assessment [7]. In another study, *Scenedesmus obliquus* cultured in brackish water reported highest lipid production (21%) at 8.8 g L^{-1} of NaCl concentration demonstrating the simultaneous desalination and biodiesel production process. The study also revealed that desalination rate and sodium chloride removal increased with increase in salinity [8].

Among the halophytes, *Scenedemus* and *Chlorella* sp. are considered to be the most active in stabilization ponds because of their extreme salt-tolerant trait [9]. *Scenedesmus* sp. grows well in saline water as it absorbs salts and makes use of them in metabolism. Reports show that cyanobacteria and photosynthetic bacteria act as ion exchangers and are found to remove sodium and chloride ions from seawater [10].

Algae are used in tertiary wastewater treatment. Biotreatment with microalgae especially *Scenedesmus* sp. and *Chlorella* sp. is found to be effective because of their photosynthetic capabilities whereby solar energy is converted into useful biomass [11]. Other advantages include the ability to grow algae in ponds with minimal nutritional input and maintenance. One of the limitations for the development of wastewater treatment systems based on microalgae is the harvesting and dewatering of algal biomass from its liquid environment at the end of the treatment process [12]. Experts have investigated wide range of technologies from sand filtration to high-speed centrifugation to be used at the end of the treatment process to harvest biomass [13,14]. Currently, researchers are focusing on non-suspended algae to avoid problems associated with harvesting [15]. One of the best techniques to treat wastewater is the immobilization of algae. Immobilization techniques involve the physical confinement or localization of cells (or enzymes) in a particular region of space to retain catalytic activity (viability in some cases) to permit repeated or continuous use. The advantages of using immobilized cells include the following: (1) increase in the cell retention time, (2) higher metabolic activity, (3) more flexibility in the reactor design, (4) accelerated reaction rates due to increased cell density, (5) increased cell wall permeability, and (6) no washout of cells and better operational stability [16].

It was reported that immobilized *Scenedesmus* sp. removed 100% phosphate in 2 h and 90% of ammonium in 4 h from a typical effluent in tertiary wastewater treatment [17]. Jiménez-Pérez et al. [18] reported immobilized cells of non-planktonic algal species, *Scenedesmus intermedius* and *Nannochloris* sp., isolated from different sources of pig manure could uptake phosphorous and nitrogen [18]. Commonly used polymers for immobilizing algae include carrageenan, chitosan, and alginate with alginate beads are being used most frequently because of the cell viability [19–22]. Recently, immobilized algal cells of *Chlorella vulgaris* are used in the cathode chamber of an algal fuel cell to achieve a combined process of biomass production, wastewater treatment, and electricity production at the same time [23].

El-Nadi et al. [24] first reported the removal efficiencies of different nutrients present in seawater by the direct addition of *Scenedesmus* sp. The study also used external feed every 7 d in continuous flow treatment system for the survival of algae. In another study, the growth potential of green alga *Scenedesmus* sp. was monitored in different salinity conditions, and their chlorophyll changes were analyzed [25]. This study uses strain of *Scenedesmus abundans* for biological desalination in the form of free and immobilized cells. The study also aims at optimizing the algal ratio to be added without any external supply of media and in reducing the desalination time with better efficiency.

The objective of the study is to compare the desalination performance of microalgae *S. abundans* in the form of free and immobilized cells in treating seawater. The optimized algal ratio for desalination performance of different saline concentrations was evaluated. Absorbance and number of cells were calculated to explain the activity of algae in seawater.

2. Materials and methods

2.1. Algae and culture conditions

S. abundans culture maintained in the Bio-Separation Laboratory, Department of Chemical Engineering, National Institute of Technology, Tiruchirappalli, India, was used for the study. Stock cultures of *S. abundans* were grown in BG 11 media containing the following constituents: sodium nitrate (1.5 g L^{-1}), dipotassium hydrogen phosphate (0.0314 g L^{-1}), magnesium sulfate (0.036 g L^{-1}), calcium chloride dihydrate (0.0367 g L^{-1}), sodium carbonate (0.020 g L^{-1}), disodium magnesium ethylenediaminetetraacetic acid (0.001 g L^{-1}), citric acid (0.0056 g L^{-1}), and ferric ammonium citrate (0.006 g L^{-1}). Final pH is maintained as 7. NaCl (10 g L^{-1}), vitamin B_{12} (1 g L^{-1}), and 20 mL of this solution (sterile filtered) was also added to 1,000 mL distilled water [26]. The cultures were maintained at a temperature of 30°C ± 2°C under a fluorescent lamp with a light intensity 3,100 Lux.

2.2. Preparation of immobilized algal beads

Algal culture in the logarithmic phase of growth was used for immobilization. Stocking beads with different concentrations of *S. abundans* microalgae were prepared by mixing sodium alginate (2.5%) with different volume percentages (5%, 10%, 15%, and 20%) of algal suspension. Beads of 4 mm diameter were obtained by dropping the algal alginate mixture into 100 mL of 2% $CaCl_2$ in distilled water at room temperature in a sterile condition. The beads were washed several times in autoclaved distilled water to remove any residual $CaCl_2$. Sodium alginate beads without algal cells served as a control.

2.3. Preparation of different concentration of free and immobilized microalgae

Different algae concentrations (v/v) such as 5%, 10%, 15%, and 20% at the exponential phase were taken for the study. For free algae and immobilized algae preparation, the required percentage amount of sample was centrifuged at 4,500 rpm for 10 min. The pellet formed was washed with distilled water and added directly to seawater to be analyzed

for free algae. For immobilized algae, the pellet was mixed with distilled water, and the procedure for bead formation was carried out.

2.4. Working procedure

The study was conducted in batch process. At every 4-h interval, the free algae and immobilized algae were replaced, and the salinity measurements were recorded. Optimization of different algae concentration was performed in both free and immobilized algae. The resultant best algae concentration was utilized in analyzing the salt-removal efficiencies at different saline concentrations (25, 20, 15, 10, 5, and 2.5 ppt total dissolved solids [TDS]).

2.5. Analytical methods

All the experiments were conducted at ambient temperature equivalent to 30°C ± 2°C approximately. Electrical conductivity, NaCl concentration, TDS concentrations, and pH of the saline solution were analyzed by water analysis kit (Cyber scan series 600, Eutech Instruments, Thermo Fisher Scientific Inc., Singapore).

Algae growth was monitored by measuring optical density at 680 nm by UV-3200 double beam spectrophotometer (Shimadzu, Japan). 1.5 mL of algal culture was taken at regular intervals from free cell cultures and 20 (approximately equal to 1.5 mL of free cell culture) beads were taken regularly from immobilized cell cultures, that is, during the 0th h (after the addition of algal cells to seawater) and analysis was carried out at the end of the 4th h. Both the samples were suspended in 10 mL of 0.1 M trisodium citrate separately. The cells were released from the beads after 15 min, and then the samples were centrifuged at 5,000 rpm for 5 min. The pellet formed was dissolved in distilled water and taken for absorbance measurement. The number of algal cells was measured by hemocytometer and digital cell counter. The biological desalination process of algae was also justified by the estimation of chlorophyll a content on hourly basis. Chlorophyll a was estimated by McKinney procedure [27].

3. Results and discussion

In this study, the desalination performance of *S. abundans* in the form of free and immobilized cells with different concentrations was studied. Seawater with a TDS concentration of 25 ppt was used for optimization of algae concentration. The experiments were carried out for 36 h. After optimizing algae ratio, the best volumetric ratio of free and immobilized algae cells was utilized in desalination of different concentrations of seawater such as 5, 10, 15, 20, and 25 ppt. The desalination performance pattern for different concentrations of free and immobilized cells of *S. abundans* show similar removal efficiency.

3.1. Optimization of hydraulic retention time of S. abundans

Many trial-and-error experiments were conducted to optimize the hydraulic retention time of algae in the form of free and immobilized cells for desalination process. The initial assessment performed on a daily basis found the rate of desalination to be ineffective. Hence, hourly basis assessment was preferred. Measurements of salinity removal and the number of cells were carried out on hourly basis after the addition of the required percentage of inoculums concentration. The efficiencies were found to be stable after the 4th h and the number of cells reduced drastically leading to reduction in algal activity. Hence, further replenishment of inoculum concentration was performed for every 4 h to maintain the algal activity. The same scenario was observed in immobilized cells of algae and so the experimentation hour was fixed as 4 h. The experiments were continued until the complete desalination efficiency was attained independent of the addition of inoculums concentration. Hence, the total experimentation period of 36 h was fixed by continuous replacement of immobilized cells and replenishment of free cells every 4 h.

3.2. Desalination performance of free cells of S. abundans

The desalination performance of *S. abundans* in the form of free cells is optimized by varying the algal concentration such as 5%, 10%, 15%, and 20%. The minimum ratio for effective desalination performance activity was seen in 10% algal concentration. The initial electrical conductivity and NaCl concentration for 25 ppt TDS solution were 45.38 mS and 33.63 ppt, respectively. The percentage removal efficiencies of NaCl and TDS concentrations were 11.38% and 9.34% at the end of the 36th h with continuous replenishment of culture every 4 h. 5%, 15%, and 20% of free algae cells concentrations showed low removal efficiency. When algal cells are exposed to high-salinity conditions, salinity stress occurs. The increase in external inorganic ion concentration disturbs the osmotic balance between the cells and the surrounding environment and forces water efflux from the cells thus leading to turgor pressure loss. Similarly, due to electrochemical gradients, increased ion concentration regulates the influx of inorganic ions into the cell thus leading to desalination process [28,29]. The loss of water and influx of ions lead to acclimatization process thereby activating the salt-tolerant species with new growth rate. In this study, growth rate was achieved initially over a period of time but declined at the end of 4th h due to the high ion concentration which might have impaired some solute proteins and biopolymer functions.

In addition, increase in algae concentration caused substrate limitation leading to improper activity and finally the death of algae. Hence, 15% and 20% ratio worked less when compared with 10%. Minimal inoculum concentration (5%) worked normally with its own capacity but not as 10%. Accordingly, the optimized concentration for desalination was fixed as 10% ratio for further levels of experiments. In all the proportions, the removal efficiencies were very high for the first batch of operating conditions (0–4 h). Further replenishment worked well but with lesser desalination capacity. The NaCl concentration induced a prompt response of algal culture leading to an effective increase in the reproductive capacity immediately after the initiation of salt stress [16]. The dead algal cells of the initial batches also affected the live cells growth and nutrient uptake capacity of further batches resulting in a negative response. Figs. 1(a)–(c) depict the decrease in electrical conductivity and percentage removal

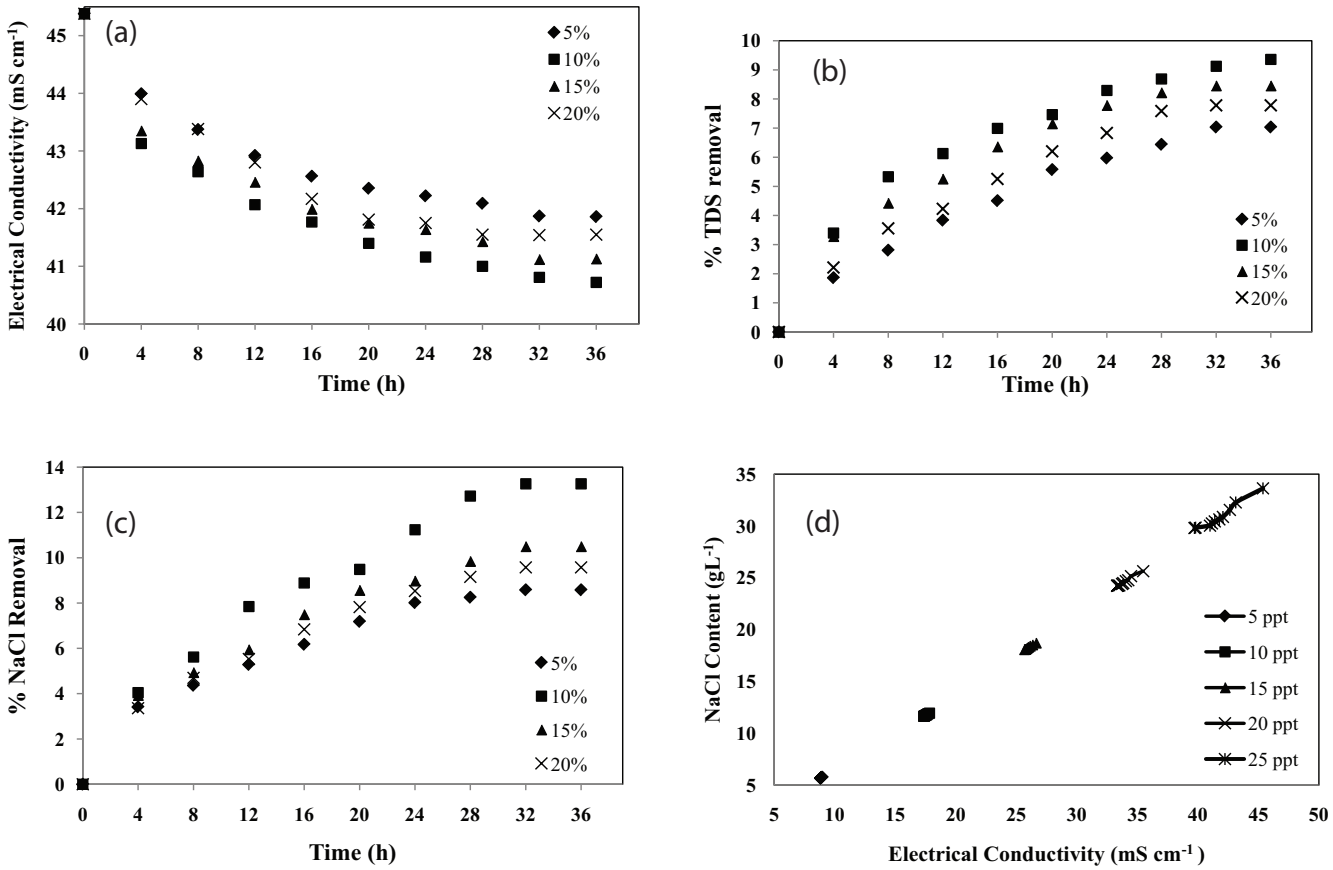

Fig. 1. (a) Electrical conductivity change, (b) TDS removal percentage, and (c) NaCl removal percentage for algal free cells at 25 ppt TDS, and (d) relationship between electrical conductivity and NaCl content for 10% algae.

rates of TDS and NaCl concentration for optimization of free cells of algae. The linear relationship between the conductivity change and the NaCl content reveals that the different concentrations of free cells of algae activity have the tendency to uptake NaCl content for its growth and development thus leading to simultaneous desalination process as shown in Fig. 1(d). The same linear relationship is also observed from Fig. 2(d) when the optimized algae concentration of 10% free cells is used in the desalination process of different saline concentration.

After optimization studies using free cells, the optimized concentration such as 10% algae ratio is used in the desalination process for different saline concentrations. Figs. 2(a)–(c) elucidate the desalination performance of 10% algae ratio in different TDS concentrations. The NaCl removal efficiencies on treatment with 10% algae ratio for 5, 10, 15, 20, and 25 ppt were 1.39%, 2.42%, 3.31%, 5.5%, and 11.38%, respectively.

In this study, after a specified reaction time, the algal activity attained equilibrium with the environment thus leading to the end of the desalination process. Higher inlet saline concentration, that is, 25 ppt worked well when compared with other saline concentrations. El Nadi et al. [24] proved that algae could be applied for biological desalination under natural Egyptian conditions. The removal rates depend on inlet saline concentration and also sunlight period and temperature. This study has also validated that desalination efficiency solely depends on inlet saline concentration.

3.3. Desalination performance of immobilized cells of S. abundans

NaCl removal in this study using immobilized cells of algae was relatively faster with high efficiency when compared with the less removal rates utilizing free cells of algae. 15% of algae ratio performed efficient desalination and was found to be optimum when compared with 5%, 10%, and 20%. The percentage removal efficiency of TDS and NaCl concentrations was 39.8% and 44.3%, respectively, at the end of 36 h of experimentation with the continuous replacement of immobilized cells every 4 h. Chevalier and Naue [30] and Lau et al. [31] have stated that small inorganic ions could pass freely through the counterparts of immobilized algae because nutrients must diffuse through the alginate pores to reach the algal cells. The removal rate of ions depends on the cell density and the number of active cells on the bead. Tam et al. [16] has found that immobilized cells of *Chlorella vulgaris* have higher physiological activity at low cellular density. The cellular metabolic activity of immobilized cells of *Chlorella emersonii* decreased as cell density increased [32]. Therefore, from the above observation, it is validated that denser concentration of beads would reduce the amount of light penetration inside the reactor and enhance the self-shading effects leading to growth limitation and decreased metabolic activities of the algal cell. Hence, in this study, the optimum concentration of immobilized cells in the treatment of seawater is found

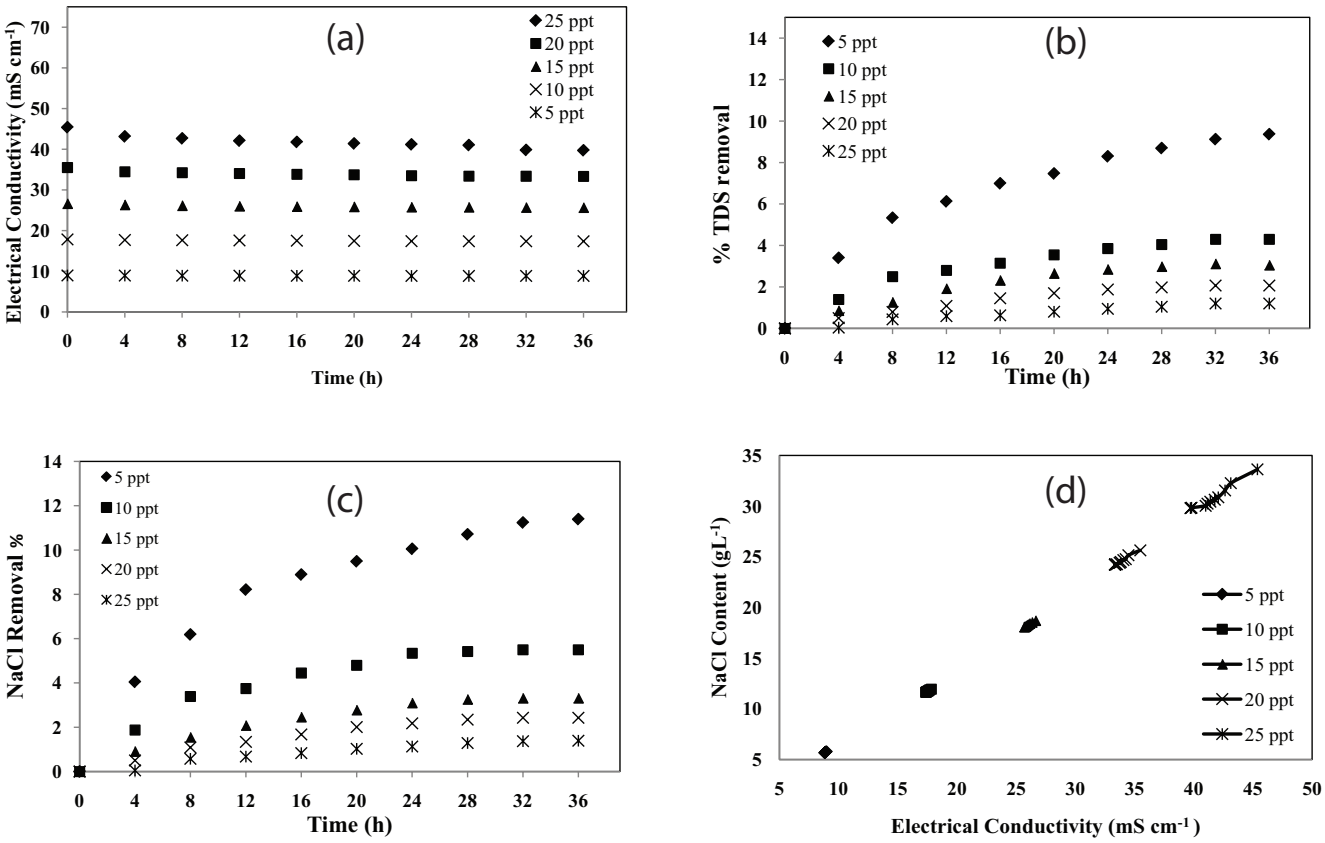

Fig. 2. (a) Electrical conductivity change, (b) TDS removal percentage, and (c) NaCl removal percentage for 10% algae ratio in different TDS concentrations, and (d) relationship between electrical conductivity and NaCl content for 10% alge.

to be 15%. Figs. 3(a)–(c) elucidate the change in electrical conductivity and percentage removal rates of TDS and NaCl concentration for optimization of immobilized cells of algae. A linear dependant relationship is experienced between the electrical conductivity change and NaCl content while using immobilized cells of algae on seawater. This proves that the adsorption of sodium chloride content occurs over the immobilized cells along with the desalination process.

After optimization studies, the optimum algae ratio in immobilized form is utilized in desalination studies of different saline concentrations such as 5, 10, 15, 20, and 25 ppt. The NaCl removal efficiencies for 15, 20, and 25 ppt saline concentrations were 17.43%, 34.71%, and 44.33%, respectively. Negative efficiency is observed in hypo-saline concentrations (5 and 10 ppt). It is seen that under changing saline concentrations, intracellular adjustments follow and compensate the external fluctuation in salt concentration. Accumulation of osmotically active substances either by biosynthesis or uptake can be seen in hypersaline concentration whereas excretion or degradation occurs under hyper-saline conditions [33]. It was observed that under hypersaline conditions, algal activity is high leading to desalination of seawater. In hypo-saline condition, due to the disintegration of cytoplasm and other components of algae, an increase in NaCl concentration was found in seawater. Therefore, a negative response was seen. The response may be due to the osmolytic difference between the immobilized algae and saline water concentration concerning ion concentration, whereas, the complete information is unspecified. Cellular levels of inorganic osmolytes such as potassium, sodium, and chloride are adjusted easily and quickly with low metabolic energy costs [34,35].

3.4. Growth rate of free and immobilized cells

In order to justify the act of desalination process and the retention time of free and immobilised cells in saline water, analysis of chlorophyll content, the number of cells, and growth absorbance at 680 nm had been carried out periodically. Until the completion of the experimentation time, the above parameters were determined from the initial addition of cells at zero time and for every hour. For the first batch, that is, 0 to 4 h of 10% free cells in 25 ppt saline concentration, the initial absorbance and the number of cells were found to be 0.6532 and 1.67 e^{+06} cells mL^{-1}; increase in absorbance was observed during the first 2 h and at the end of 3rd h a decrease in trend was seen and at the 4th h the complete disintegration of cytoplasm and other soluble proteins led to the death of algae. The same pattern was observed for the consecutive eight batches until the completion of the experimental period of 36 h. The rate of increase in chlorophyll content was approximately equal to 0.012 μg mL^{-1} h^{-1} in all the batches for the first 2 h. Thus, the change in absorbance is proportional to chlorophyll content and number of cells.

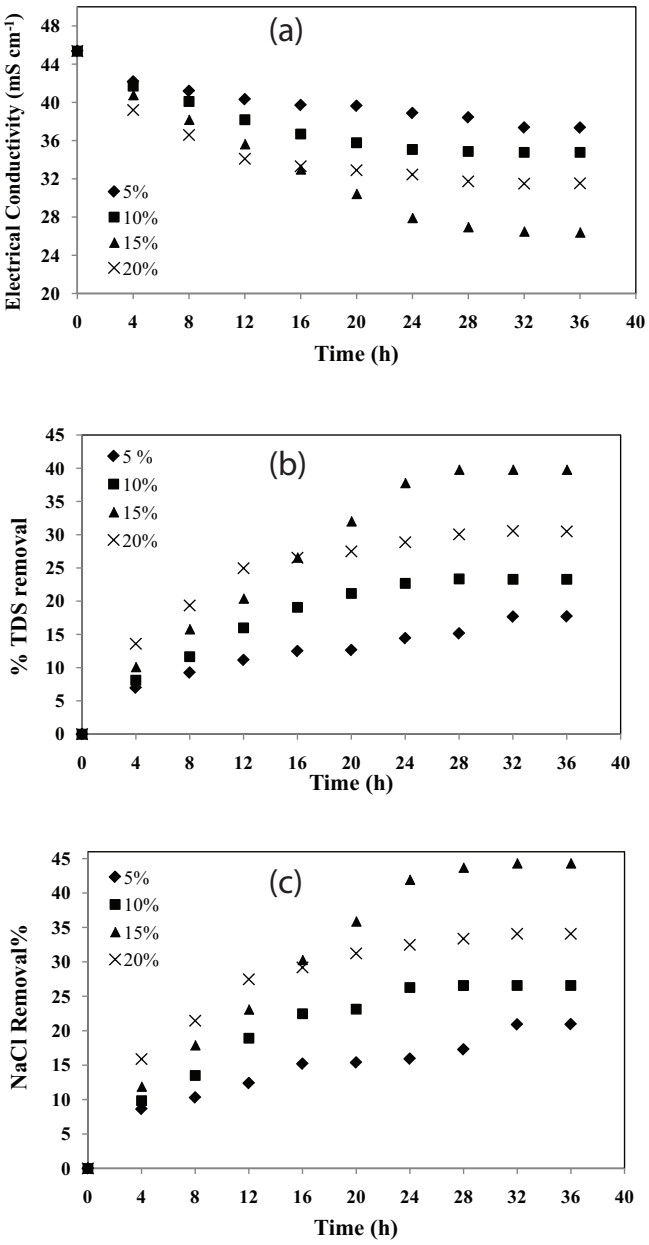

Fig. 3. (a) Electrical conductivity change, (b) TDS removal percentage, and (c) NaCl removal percentage for immobilized cells at 25 ppt saline concentration.

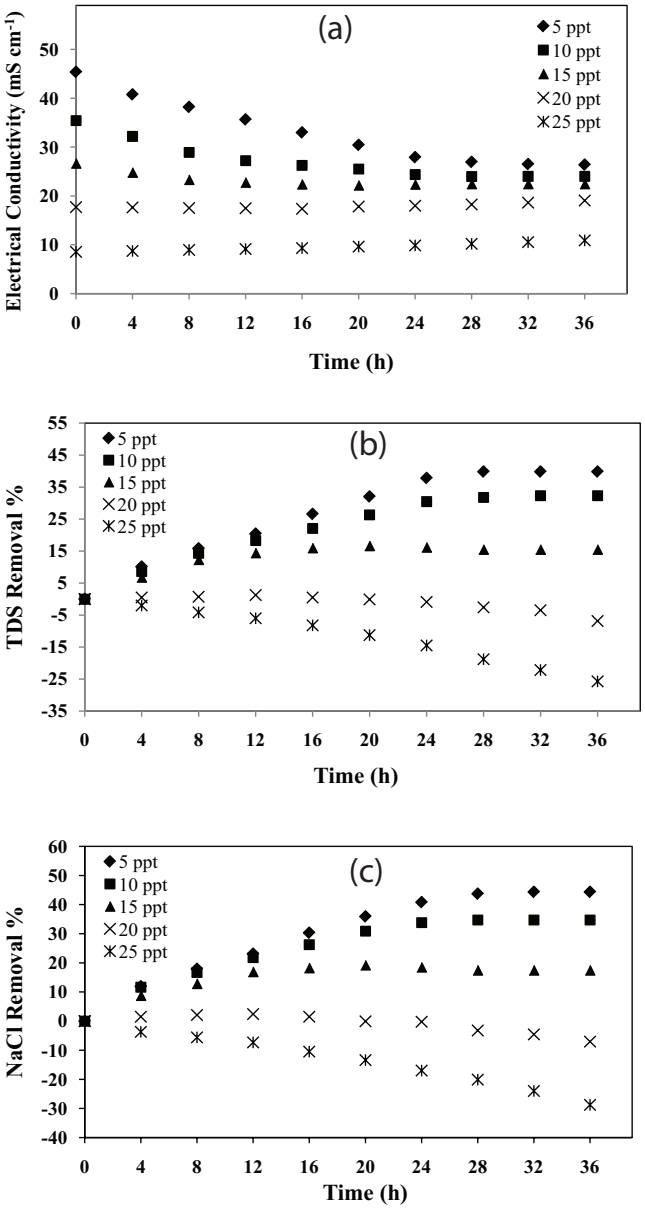

Fig. 4. (a) Electrical conductivity change, (b) TDS removal percentage, and (c) NaCl removal percentage for 15% algae ratio at different TDS concentrations.

For 15% immobilized cells in 25 ppt saline concentration, the same characteristic feature of free cells of algae was seen. Due to the immobilization process, the growth of cells was quite more when compared with free algae leading to higher desalination process. The rate of increase in chlorophyll content was approximately equal to 0.147 μg mL^{-1} h^{-1} for the first 2 h. The absorbance after the 4th h did not show any characteristic difference in both the free and immobilized cells in all the studies, thus, leading to the stabilization of desalination process. Estimation of chlorophyll content is an indicator of the indirect method of cell growth. Loss in chlorophyll content in microalgae is associated with the external environmental stress. The change in chlorophyll a content follows the same pattern as that of the growth rate of 10% free cells of microalgae when introduced in 25 ppt of saline water. The average rate of increase in chlorophyll content was approximately equal to 0.146 μg mL^{-1} for 10% free cells of algae and 0.66 μg mL^{-1} for 15% immobilized cells of algae for the first 2 h. The absorbance after the 4th h did not show any characteristic difference in both the free and immobilized cells in all the studies and also led to the stabilization of desalination process. Fig. 5 depicts the change in chlorophyll a content for 10% free cells of algae and 15% immobilized cells of algae in 25 ppt TDS concentration.

Likewise, the absorbance and the number of cells were calculated for different concentrations of free and immobilized cells in optimization study and also for different

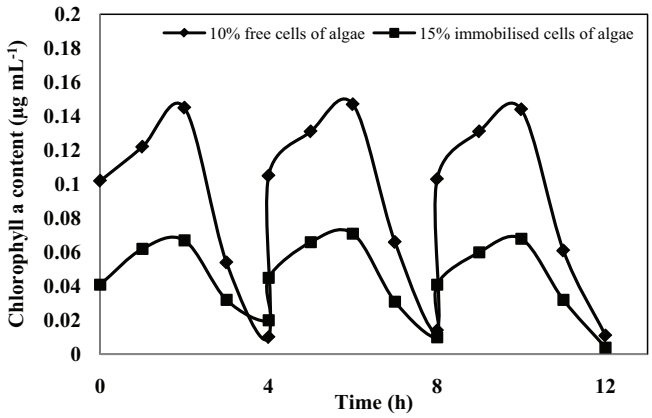

Fig. 5. Change in chlorophyll a content for 10% free cells of algae and 15% immobilized cells of algae in 25 ppt TDS concentration.

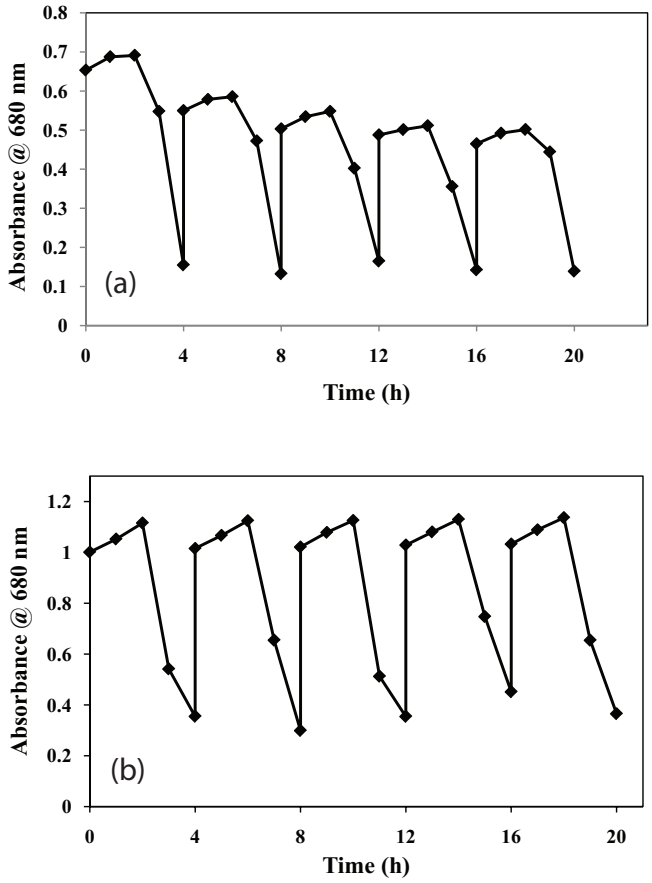

Fig. 6. Absorbance change (growth rate difference) at (a) 10% concentration for free cells and (b) 15% concentration for immobilized cells.

concentrations of saline water. Figs. 6(a) and (b) represent the initial and final absorbance for the optimized concentration of free and immobilized algae for 20 h. Hence, from the above it was concluded that the replenishment of culture to be performed was fixed for 4 h. With continuous replenishment of free cells and replacement in immobilized cells, the entire experiment was carried out for 36 h.

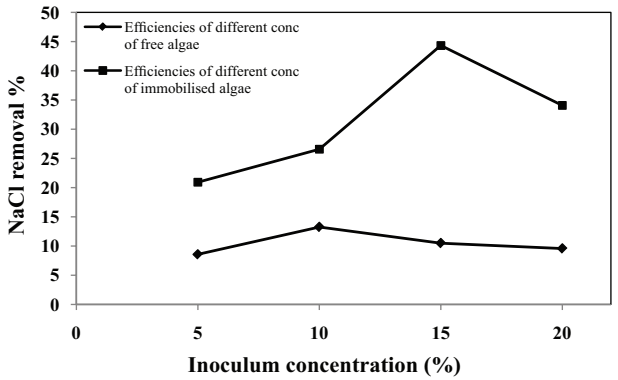

Fig. 7. Comparison of desalination performance for free and immobilized cells of algae in saline water containing 25 ppt TDS concentration.

3.5. Comparability of free and immobilized cells activity in desalination performance

Fig. 7 represents the desalination activity of 10% free algae cells and 15% immobilized cells of algae. The results obtained showed that the desalination performance of the immobilized cells of algae worked significantly faster when compared with free cells as well as the concerning desalination performance. At the end of 36 h, almost more than 40% efficiency was attained by immobilized cells. El-Sayed et al. [25] also proved that certain alga species in immobilized form remove 25% of total salt per batch. Separation of algae cells from saline water can be performed with ease. No additional processes are required to remove the cells. Hence, immobilized cells are preferred for the desalination of saline water.

4. Conclusion

This paper examines the utilization of *S. abundans* in the form of free and immobilized cells for the bio-desalination process. It is also validated that *Scenedesmus* species can easily be adapted to the saline environment as it uptakes salt and uses it in metabolism process. Due to the natural laboratory environment condition and other unknown factors, the algae in free and immobilized cells complete their cycle in shorter period leading to the removal of NaCl ions in 36 h of experimental time with the replenishment of optimized culture percentage every 4 h. The efficient functioning of the system was also affected due to the inlet of TDS concentration. Thus, bio-desalination using immobilized cells of algae can be considered as a pre-treatment to reverse osmosis process and also for microbial desalination cell.

Further advancement in bio-desalination is based on genetically modified species to be incorporated in the process. But, it is essential to overcome the multiple challenges from the viewpoint of social and regulatory acceptance. Another important aspect is the application of effective procedures to separate the cells from the water. Therefore, in this study, immobilized cells have been tried for desalination to overcome the difficulties in separation. Further research is required to analyze the impacts of environmental conditions such as temperature, pH, and nutrient flow through the cells on salt transport in *Scenedesmus* sp. Hence, complete knowledge and research on bio-desalination process utilizing phytoplankton are required to increase the chances of social acceptance.

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