Immobilized-microalga *Scenedesmus* sp. for Biological Desalination of Red Sea Water: II. Effect on Macronutrients Removal

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Abstract: The objective of this study was to assess the ability of the freshwater green alga *Scenedesmus* sp. to recover nutrients from saline water. The saline water from Red Sea, Ismailia Governorate (about 45g.l^{-1}) was used. Alga was hetero-trophically grown in 10L rough polyethylene bottles. Fresh-saline water mixture was used with ratios ranged from 0.0 to 100%. The duration of the experiment was 24 days in three sequences batches. Physico-chemical analysis of growth media was daily determined. All of nitrogen and phosphorous contents either from original growth medium or saline water were absorbed by the first batch due to their low concentration and high importance. Potassium, calcium and sodium represented their maximum by the first batch, however absorption extended by the following batches due to their higher concentrations. Magnesium represented the lower concentration by the third batch due to the high consumption by the used alga. **[Journal of American Science 2010; 6(9):637-643]. (ISSN: 1545-1003).**

Key word: *Scenedesmus*; saline water; nutrient uptake

1. Introduction:

Photosynthetic microorganisms including microalgae can be used for primary and secondary metabolites production (Scott,1987, Trevan & Mak, 1988 and Park *et al*., 1991); and in the bio-elimination of contaminants from wastewaters (De la Noiie *et al*., 1990 & Proulx & De la Notie, 1988 and El-sayed, 2004). Microalgae biotechnology has become one of the most studied fields in desalination within biological sciences matrix including commercial use for treating wastewater generated by tilapia aquaculture operations (Adey & Loveland, 1998 and El-Sayed *et al*., 2010), agricultural run off (Adey *et al*., 1993); and for N and P removal in tertiary treatment of municipal wastewater (Craggs *et al*., 1996). Experiments on municipal wastewater effluent resulted in 23% reduction in N and 82% reduction in P (Rectenwald and Drenner, 2000). Most filamentous species require abundant Ca^{+2} for growth (Guillard & Lorenzen, 1972), and some calcicola will even bore their way into limestone, to form colonies inside the rock (Whitton & Potts, 1982). The survival value of this activity relates not only to nutrition, but also the fact that excessive solar radiation inhibits growth. On the other hand, the ability of microalgae to assimilate inorganic nitrogen into biomass could be very effective for nitrogen compound detoxification. Florencio and Vega 1983, described the use of nitrate, nitrite, and ammonium by *Chlamydomonas reinhardtii*. Chevalier and De la Nou'e (1985) used immobilized hyper concentrated algae for orthophosphate removal from wastewater. On a laboratory scale, the algae could remove 50 pM phosphate in two hours using 2-3 g algae when they were immobilized in carrageenan. Under certain conditions, different microalgae can consume phosphate at high rates such as *Chlamydomonas* when the cells were exposed to UV light (Scott, 1987) or freely suspended cells of *Catharantus* after periods of phosphate starvation (Nagano *et al*., 1994). *Rodococcus* cells immobilized on saw dust have been used for H,S removal and *Chlamydomonas* has opened new ways of employing microalgae in polymer degradation processes (Maeda *et al*., 1994).

Mallick and Rai (1994) found a higher nutrient removal by *Anabaena doliolum* and *Chlorella vulgaris* immobilized in chitosan compared with cells immobilized in either agar, alginate or carrageenan or their free cells counterparts. Nitrate was depleted by 70% during first 12 hours of treatment by immobilized cells. Blank chitosan beads removed 20% of the nitrate at the end of the experiment (106 h). De la Nou'e and Proulx (1988), reported that 95% nitrogen was removed by immobilized *Phormidium* sp. cells, from medium containing 9.5 mg.l^{-1} of nitrate and 2.5 mg.l^{-1} of nitrite. Lau *et al*. (1998) found a complete consumption of nitrate by immobilized *Chlorella vulgaris* could be performed. Initial nitrate concentration for bio assay was higher (44 mg.l^{-1}) and probably this caused differences in nutrient efficiency removal. Garbayo *et al*. (2000) pointed out a possible inhibition of nitrate uptake by nitrite presence in the culture medium of *Chlamydomonas reinhardtii*. Nitrogen removal by microalgae and cyanobacteria could be limited by several environmental factors, such as light, pH and availability of carbon source (Garbisu *et al*., 1992 and Urrutia *et al*., 1995). Garbisu *et al*. (1992) suggested

that nitrate removal by immobilized *Phormidium laminosum* was strictly dependent on light and CO_2 availability. Chitosan immobilized cells showed a high efficiency in phosphate uptake (90% after 9 hours and 94% after 12 hours). A high phosphate removal 98% of the initial by immobilized cells has been reported by Tam and Wong (2000). Lau *et al*. (1997) mentioned that 38% phosphate uptake by free cells culture of *Chlorella vulgaris* and 94% by alginate immobilized cells after 24 hours. This phenomenon could be attributed mainly to elevated pH values and the release of calcium ions by polymer, causing a chemical precipitation of phosphate ions (Tam and Wong, 2000). Phosphorus is a key nutrient factor for algae growth. Phosphorus removal mechanisms of algae are mainly assimilation absorption and chemical precipitation. Chemical precipitation is mainly caused by the phosphate precipitation. Cation concentrations such as Ca^{2+}, Mg^{2+}, and pH affected the removal efficiency of TP (Li *et al*., 2006). Robinson *et al*. (1985) reported that cellular metabolic activity (indicated by respiratory rate per cell) of immobilized cells of *Chlorella emersonii* decreased as cellular density increased. Therefore, a dense concentration of beads and a high cellular density per beads would reduce the amount of light penetrating the bioreactor and potentially enhance the self-shading effect, which would then limit the growth and metabolic activities of the algal cells. Moreover, it could affect the amount of the necessary aeration required to suspend the beads and provide for the required mixing conditions within the bioreactor. Carpenter and Guillar (1971) indicated that populations of phytoplankton in oligotrophic regions, adapted to low nutrient concentrations, were able to assimilate nitrate and ammonia at a faster rate when higher nitrogen concentrations are available thus, showing a larger half-saturation constant.

The current work was achieved to study the potential of fresh alga *Scenedesmus* sp. in removing of saline nutrients which in turn allows using it for the biological desalination purposes with special refer to macronutrients removal rate.

2. Materials and Methods

2.1. Alga and Treatments

The green alga *Scenedesmus* sp. (El-Sayed, 2004) was used under the current investigation. Cultures were early grown under conditions of BG-II growth medium (Stainer *et al*., 1971). As growth reached the maximum, cultures were harvested and washed three times to remove all surface-accompanied nutrients. Cultures were re-incubated for 12 hours under the same conditions of BG-II to allow the consumption of the given nutrients (El-Sayed, 1999). Filtrated Red Sea Water (RSW) was added to algal cultures in three batches ; for each treatment; at the ratios of 0.0, 25, 50, 75 and 100% Red Sea Water of cultivation volume. macronutrients analysis of the used water is listed in Table 1.

Table 1. Macronutrient contents (ppm) of Red Sea water

T.N	P	K	Ca	Mg	Na
550	8	650	750	260	20304

2.2. Growth conditions

The early growth was performed with continuous illumination 120 µ.e provided from white florescent lamps from one side. Agitation was performed by compressed air. Heterotrophic growth was carried out by acetate addition. Growth containers were formed from the ready made poly ethylene bottles of 10 liters. Treatments were achieved in three replicates for 24 days.

2.3. Sampling and analyses

Algal culture was filtered over membrane filter (0.45µm) and washed with acidified water. The obtained filtrate was used for different nutrients determination according to the adopted methods of Chapman and Pratt 1974. Nitrogen was determined based on micro-kjeldahl method. Phosphorous was spectrophotometrically measured by Vanadate method. Calcium, sodium and potassium were photometrically measured by Flame emission. Magnesium was measured photo-metrically by Atomic absorption. Physical properties including pH and E.C were also periodically measured. As for pigments, the precipitated algal biomass over membrane filters were soaked overnight into DMSO 95%, then after filtered by centrifugation. Chlorophyll absorbance was measured at 666nm. The initial element removal rate is calculated as $Ri = S0 - St / \Delta t$ where Ri represents the substrate removal rate, S0 is the initial substrate concentrations, St is the corresponding substrate concentration at "tt". The slope of time versus effluent concentration at time "tt" gives the initial substrate removal rate.

3. Results and Discussion

3.1. Nitrogen

Nitrogen uptake by the examined alga was found to be associated to the salinity given level. The rise of nitrogen removal rate was closely related to the given salinity level rather than growth rate or biomass accumulation. Control culture which received only urea nitrogen (BG-II) was found to be the lowest, while other treated cultures represented proportional increases on the removal rate. This finding might be

goes back to the initial amount of nitrogen (465 ppm) with respect to the rise of salinity level (550 ppm). By this finding, the rate of nitrogen removal was ranged from 51.7 in control culture to 112.8 ppm. d^{-1} (T4) due to the given nitrogen concentration. No variable results were found among the three employed batches due to the complete removal of nitrogen content from the growth medium following every batch. Also, the complete nitrogen removal might be goes back to many reasons. The first is to insufficient nitrogen content of the original media (465 ppm) even in the full sea water growth media (550 ppm). The second is to the high cell density of alga which allows the competition and fast utilization of nitrogen and other macronutrients. The effect of extra nitrogen concentration on the behavior algal growth was early described in many studies. Most of these studies claimed the ability of different algae species to grow well under high nitrogen concentrations with some differences due to the used strain and nitrogen source.

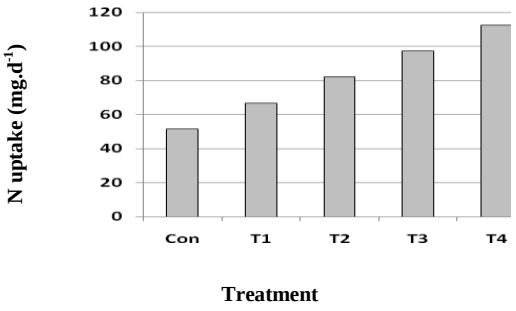

Fig. 1. Nitrogen uptake (mg.d^{-1}) of *Scenedesmus* sp. under different saline water concentrations. Con.= control, T1=25%, T2= 50%, T3=75% and T4=100% of sea water.

Supporting extra nitrogen is required for desalination purposes because of the lack of sea water nitrogen content and to enhance the vegetative growth of the used alga (El-Sayed, 2010). An average of 72% nitrogen and 28% phosphorus removal by *Chlorella vulgaris* from 3–8 mg NH_4-N.l^{-1} containing diluted ethanol and citric acid production effluent was obtained (Valderrama *et al*., 2002).

3.2. Phosphorous

Like nitrogen, all of the added amounts of phosphorous were absorbed by the incubated alga by the first batch of all treatments. The completely absorption of phosphorous ion returns at first to the low concentration of sea water (8 ppm) even in the growth media used which contain 40.77ppm. Actually, the lower P range (8-40.77 ppm) led to minimize the differences on the expected results. Data could be subjected to the rate of absorption by the control cultures that serve as 4.53ppm (on the average). In this context, El-Sayed(1999) found that maximum phosphorous absorption rate by five different algae species was ranged from 30 to 285.1 µg.h^{-1} at the first early 12 hours of incubation. The lowest was for *Scenedesmus obliquus*, while the higher was observed by *Chlorella* sp. The next incubation time represented a sharp decrease on absorption rate.

In this connection, Sicko-Goard and Jensen (1976) reported that maximal accumulation of poly phosphates was observed after 5 to 8 hours of phosphate addition. The level at such time was much higher than in un-starved cells. Phosphorous is required for normal growth of all algae. They vary among themselves for utilization ability of organic phosphate, even though they contain phosphatase and normally these compounds must be hydrolyzed by extra cellular phosphatase (Reichardt *et al*., 1968). Indeed, phosphorous absorption markedly affected by pH, Na, K, Mg and/or various heavy metals concentrations (Kuhl, 1974).

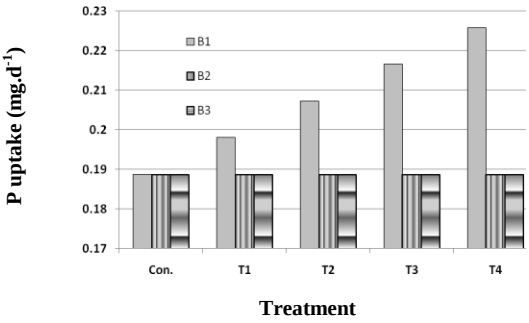

Fig. 2. Phosphorous uptake (mg.d^{-1}) of of *Scenedesmus* sp. under different saline water concentrations of three batches (B1, B2 and B3). Con.= control, T1=25%, T2= 50%, T3=75% and T4=100% of sea water.

Microalga *Chlorella kessleri* was able to uptake only 8–20% phosphorus for PO_4-P concentration of 10 mgl^{-1} (Lee and Lee, 2001). Although Dumas *et al*. (1998) reported the complete phosphorus removal by *Phormidium bohneri*, however the initial concentration was considerably lower (0.05mg PO_4-P l^{-1}). Gonzales *et al*. (1997) obtained 55% phosphorus removal from agroindustrial wastewater with the total phosphorus concentration of 111mgl^{-1} by 216 hours by the batch cultivation of *Chlorella vulgaris* and *Scenedesmus dimorphus*. However, only 15% for PO_4-P concentration of 93 mgl^{-1} was obtained by Aslan and Kapdan (2006). In addition, An *et al*. (2003) investigated the effect of the initial nitrogen and phosphorus concentration on nutrient removal

performance of the green alga *Botrycoccus braunii* from secondary treated piggery wastewater. They also found that culture was able to consume N and P completely up to 510 mgl^{-1} NO_3-N and around 29 mgl^{-1} PO_4-P; respectively within 6 days of batch operation.

In this context, over 97% nitrogen and phosphorus removal was achieved by *Scenedesmus obliquus* for the nutrient concentrations of 27.4 and 11.8 $mg.l^{-1}$, respectively (Martinez *et al*., 2000). Olgu'ın *et al*. (2003) obtained 96% NH_4-N and 87% PO_4-P removal by *Spirulina* in an outdoor raceway treating 2% diluted anaerobic effluents from pig wastewater containing almost the same amount of nitrogen and phosphorus. Concerning the specific removal rate, as mentioned above with nitrogen results, the true differences were only observed with the first batch since the initial concentrations were slightly varied. As the disposal concentration of phosphorous was consumed (8 ppm) by the first batch, cultures during the second and third batches became equal to the initial phosphorous concentration (40.77ppm). No variations were observed concerning either salinity doses or batch number.

High P uptake rates in urban wastewater suggested that bacteria and algae were capable for removing phosphorus from the urban wastewater. The removal by algal and bacterial activity, combined with phosphates precipitation caused by the pH increment (9 – 9.5) ;could be probably; contributed to the decrease of phosphorus levels (Lau *et al*., 1997)

3.3. Potassium

Potassium removal rate was varied due to the given sea water level as well as batch number. Maximum K removal rate was observed in the first batch with respect to salinity levels.

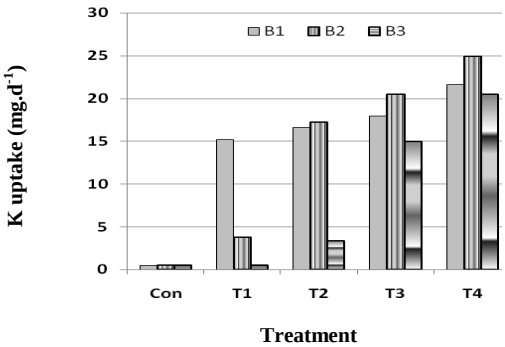

Fig. 3. Potassium uptake ($mg.d^{-1}$) of *Scenedesmus* sp. under different saline water concentrations of three batches (B1, B2 and B3). Con.= control, T1=25%, T2= 50%, T3=75% and T4=100% of sea water.

Such minimum rate was observed compared to control culture that fed only by the initial of BG-II. Extending batches led to the deficiency of nutrients which in turn downed the initial potassium concentration and the removal rate. On the average, the calculated K removal rate was recorded as 0.5, 6.51, 12.4, 17.8 and 22.34 ppm $K.d^{-1}$; respectively for different salinity treatments.

The maximum absorption rate was observed in the second batch. The fifth treatment (full sea water medium) gave the maximum K removal rate (24.9 $ppm.d^{-1}$). In agriculture, the application of seaweeds can be used as soil conditioners, fertilizers and green manure, due to the presence of high amount of potassium salts, micronutrients and growth substances (Thirumaran *et al*., 2009).

3.4. Calcium

In fact, absorption rate must be subjected to population or growth rate. The main hypothesis here is to increase the initial inoculums at zero time to allow the self shading against salinity. No additional quantities of calcium were received from growth media, except those received only from sea water supplementation. As shown in Table 1, full sea water medium contains on the average 750 ppm of Ca ions. Five soft water algae widely varied in concern calcium uptake. The rate was varied (4-25$\mu g.h^{-1}$) due to the examined algae. Extra variation was found as a result of trophic mode (El-Sayed 1999). The rate of removal by survived algae would be expected to rise due to main two reasons (physiological and physical). The first is fast growth (cell division), while the second that meaning the over concentration and osmosis effect.

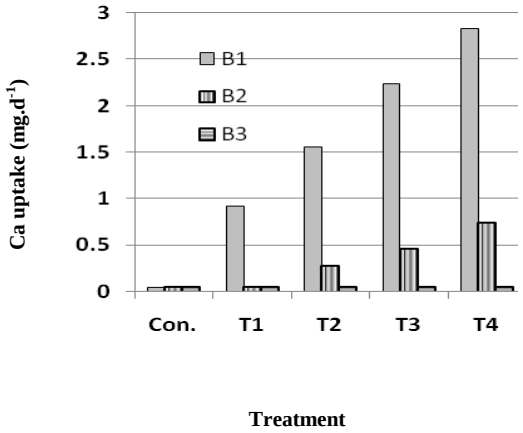

Fig. 4. Calcium uptake ($mg.d^{-1}$) of *Scenedesmus* sp. of different saline water concentrations during three batches (B1, B2 and B3). Con.= control, T1=25%, T2= 50%, T3=75% and T4=100% of sea water.

3.5. Sodium

Sodium was considered as the main cause for the true saline characterizations representing concentration up to 50% of the total salinity. The initial sodium content in full sea water medium was (20304 ppm). Variable results were obtained on sodium removing rate due to batch number and concentration used. Within the sole batch, increasing of sodium concentrations or salinity levels (sea water), led to the reduction on removal rate. Concerning different batches, the removal rate was increased by extending the batch number. Accordingly, the rate was increased within the sole treatment during different batches. The exception is to control culture which represented one pattern due to the minor and fixed initial sodium concentration (0.4 ppm.d^{-1}). Maximum removing rate was observed by the third batch with 50% of sea water level (373 ppm.d^{-1}). The rate however seems higher, returning to the nature of the examined alga to high nutritional doses (El-Sayed *et al*., 2008). The decreasing on removal rate under high sea water ratio (50, 75 and 100%) might be attributed to the induction effect of sodium on growth, nutrient absorption and assimilation rate for every element. This hypothesis could be supported by the results obtained from each batch within the sole treatment, where increasing of removal rate by the advanced batches returning to the dilution effect. At least 5 ppm of sodium is required to meet the normal growth of *Anabeana cylendria* which cannot utilize potassium instead of sodium (Allen and Arnon, 1955),

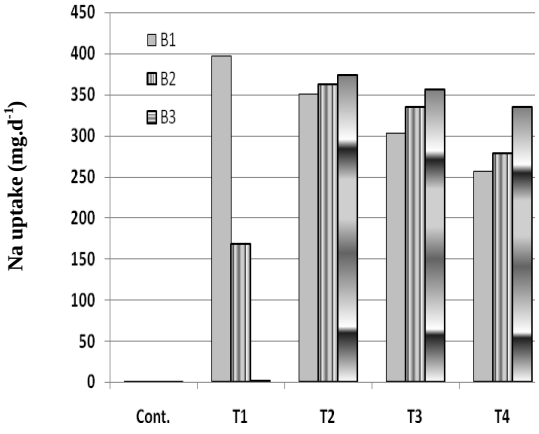

Fig. 5. Sodium uptake (mg.d^{-1}) of *Scenedesmus* sp. of different saline water concentrations of three batches (B1, B2 and B3). Con.= control, T1=25%, T2= 50%, T3=75% and T4=100% of sea water.

3.6. Magnesium

The interruption of CO_2 supply to the algal system can lead algae to flocculate on its own, which is called auto-flocculation. In most cases, this phenomenon was associated with elevated pH due to photosynthetic CO_2 consumption corresponding with precipitation of magnesium, calcium, phosphate, and carbonate salts with algal cells. In case of calcium and phosphate used, excess calcium ions (positive charged) tend to react to algae cells (negative charged) and binds together to provide auto-flocculation process (Sukenik and Shelef 1984). Here, the proportional absorption was found as a result of Mg concentration. This finding could be supported by the data obtained from batch three which represented the lower absorption due to the deficiency of Mg as they absorbed by the ex-batches.

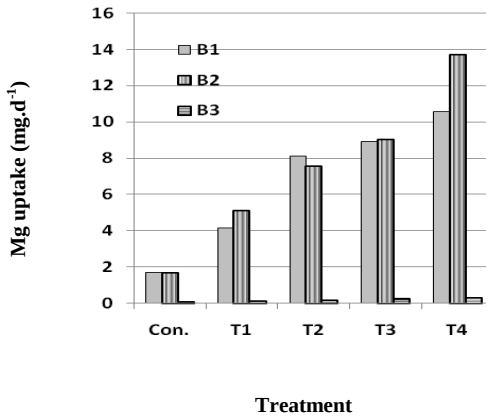

Fig. 6. Magnesium uptake (mg.d^{-1}) of *Scenedesmus* sp. under different saline water concentrations of three batches (B1, B2 and B3). Con.= control, T1=25%, T2= 50%, T3=75% and T4=100% of sea water.

4. Conclusion

The first key of algae surviving against unfavorable growth conditions is their ability to grow under such stress conditions regardless the growth rate. Thus, growth of fresh water algae species under saline water conditions suggested the high osmo-regulatory action against the exo-nutrients effect. Aquatic media; however contains high nutrients played as salting out effect; allowing the mass flow of nutrient to penetrate algal cells even that non desired. The penetration of such nutrients increases their content which in turn regulates the osmotic deviations between endo and exo-content of nutrients. Consequently, algae which able to survive under these stress conditions are able to optimize their growth profile and could be ; at least; used for the artificial desalination of sea water.

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