Immobilized Microalga *Scenedesmus* sp. for Biological Desalination of Red Sea Water: I. Effect on Growth

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Abstract: The green alga *Scenedesmus* sp. was grown hetero-tropically during indoor stage to evaluate their growth potential under both recommended and drastic salinity conditions. Recommended growth was performed with the routine nutritional regime of NPK, while growth under four different stress regimes was operated. The given ratios of fresh and saline water were ranged from 0.0 to 100% of sea water (Red Sea, Ismailia Governorate) on three sequences batches. Saline media were supported by N and P under free K conditions to allow the completely consumption of Na on the expense of K. The evaluated growth measurements were dry weight and pigments. Growth containers of 10L from rough polyethylene bottles were used for immobilization purposes. Growth was developed with all treatments due to the high initial biomass used. Dry weight was enhanced by the first treatment of 25% sea water and no chlorophyll decomposition was observed. Increasing of salinity levels led to chlorophyll decomposition with carotenoids accumulation increments. The rate of decomposition was markedly decreased by the extended batches. Chlorophyll /carotenoids ratio was decreased by the salinity treatments within the sole batch; however, it was increased among the three batches. [Nature and Science 2010;8(9):69-76]. (ISSN: 1545-0740).

Key words: Red Sea Water; Immobilized; Desalination; Dry weight; Chlorophyll; Carotenoids
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1. Introduction

Many halophytes plants were recognized for the indirect desalination of sea water. Unlike higher plants, algae able to complete their life cycle within a wide range of salinity even within the sole taxa. Microalgae have been used for many years in tertiary sewage treatment to eliminate N and P compounds after reducing the BOD and COD (Oswald, 1988 and Laliberte *et al*., 1994). Many microalgae, such as *Scenedesmus obliquus* able to use organic compounds under illumination and mixotrophic conditions (Martınez *et al*., 2000), and use in an alternative secondary treatment designed to reduce the content in organic matter and eliminate nutrients (Tam and Wong, 1996).

The main limitation in using these systems is related to the composition of the wastewater and the possible presence of high concentrations of urea, ammonium, organic acids, phenolic compounds, and pesticides frequent in wastewaters from the agricultural industry, or other compounds that can inhibit algal growth. Hodaifa *et al*. (2008) found that the composition of lipid fraction of the biomass depended on the percentage of wastewater used as the nutrient medium.

Both freshwater and marine cyanobacteria can accumulate organic compounds as osmo-regulatory solutes in response to salt stress (Reed *et al*., 1984 and Mackay *et al*., 1984). Sucrose and trehalose are the most common osmotic solutes in cyanobacteria. They appear to form a carbohydrate glass to protect membranes under the desiccative circumstance (Page-Sharp *et al*., 1999 and Potts, 1999).

Immobilized-microalgae systems prevent biomass from being washed out and grazed by herbivores, and allow hyper-concentrated cultures. A constant metabolic activity over long time periods for immobilized microalgae has also been reported (Chen, 2001). In addition to wastewater treatment, immobilized algae systems have several applications, which include metal removal, stock culture management and, production of useful chemicals (Cohen, 2001 and Robinson *et al*., 1986). The immobilization matrix could be a synthetic polymer (*e.g*; acrylamide, polyurethane), or a natural polymer (alginate, Carrageenan, agar, collagen). However, it must fulfill various requirements such as photo-transparency, no toxicity, retention of cellular viability, and stability in the culture medium (Mallick, 2002). Higher macro-nutrient removal efficiencies by immobilized cells were found to be higher in comparison with free cell microalgae (Kaya *et al*., 1995 and Lau *et al*., 1997).

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 carragenan or their free cells counter parts. De la Nou'e and Proulx, 1988, reported that 95% of 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*. Garbayo *et al*. (2000) showed that entrapped *Chlamydomonas* *reinhardtii* cells did not perform nitrate consumption at concentrations lower than 0.14 mM. Nitrogen removal by microalgae and cyanobacteria is limited by several environmental factors, such as light, pH and availability of a carbon source (Garbisu *et al*., 1992 and Urrutia *et al*., 1995). Garbisu *et al*. (1992) suggested that nitrate removal by immobilized *Phormidium laminosum* is strictly dependent on light and CO_2 availability.

Regardless the drastic changes in pH involved in the chitosan immobilization method, a high number of viable entrapped cells of three *Scenedesmus* sp. were obtained. Chitosan bead immobilized algae system with *Scenedesmus* sp. was more efficient in removing phosphate and nitrate from water than the conventional free cell system. In addition, there were satisfactory results on viability, growth and nutrients uptake by chitosan-entrapped Scenedesmus cells.

The current work was achieved to study the fresh algal growth potential under different sea water levels which in turn allow using it for the biological desalination purpose.

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. 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. Filtrated Red Sea Water (RSW) was added to algal cultures in three batches at the ratios ranged of 0.0, 25, 50, 75 and 100% of cultivation volume. Chemical analysis of the used (RSW) was listed in Table 1.

Table 1. Some Analyses of Red Sea Water (ppm).

pH	E.C	N	P	K	Ca
8.91	41.1	550	8	650	750
Mg	Na	SO_4	Cl	CO_3	HCO_3
260	20304	770.1	28368	19.2	161

2.2. Growth conditions

The early alga growth was performed with continuous illumination (120µ.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 rough bottles of 10 liters. Treatments were achieved in three batches for 27 days. At the end of every batch, the upper water layer was removed and alga was eliminated. The removed water was re-used by new algal inoculums.

2.3. Sampling and analyses

Sampling was done from zero time of saline water addition and repeated every day. Ten ml were filtered over membrane filter (0.45um) to achieve dry weight ($g.l^{-1}$). As for pigments, the precipitated algal biomass over membrane filters were soaked overnight into 10ml DMSO 95%, and then centrifuged. Chlorophyll absorbance was measured at 666nm and concentration was calculated according to Seely *et al*. (1972). Carotenoids were extracted after chlorophyll decomposition and absorbance was measured at 468nm. Carotenoids concentration was calculated according to Davies (1976).

3. RESULTS AND DISCUSSION

3.1. Growth dry weight

Growth of the incubated alga was varied due to the high initial biomass at zero time of cultivation. This action allows alga to meet vegetative growth under high salinity level even full sea water medium. In addition, supplying growth media by super optimal concentrations of urea as N source and phosphoric acid as P source enhanced vegetative growth. This might be led to increase some nutrient solubility or decrease pH values to near acidic reaction. Here, urea also acts as a complementary source of carbon.

Enhancing growth dry weight was observed with lower and moderate salinized cultures which received 25 and 50% of saline water and possessed extra enhancement on dry weight accumulation as compared with control cultures that received only the recommended NPK. The enhancing effect could be ascribed to the extra N and P supplementation corresponding to the high initial biomass (Fig.1). Cultures received more saline doses; *i.e;* 75 and 100% of sea water resulted in slow growth rate at the early growth period followed by a slight increase on dry weight. The results in general could be attributed to the high potentials of such alga against high salinity levels due to their original habitat (El-Sayed. 2004), increasing of the initial biomass at zero time of cultivation which allows more self shading against osmosis with the high levels of nitrogen on alga growth medium. Otherwise, growth was varied among the different batches of desalination, where fresh alga of the second and third batches represented extra growth enhancement at the early growth phase as compared with the first batch. This could ascribe to the dilution effect by nutrients consumption by the ex-batch. The slightly decline on growth as dry weight at the middle growth curve could be attributed to the deficient N concentration as compared with the high initial biomass at zero time with the first batch. Under control condition, competition could be expected as macronutrients became deficient due to high initial biomass. Earliest hypothesis suggested that under non-favorable growth conditions including salinity as well as certain nutrients depletion, the general biochemical profile of algal cells underwent complicated changes mainly protein and chlorophyll decomposition which in turn affect the net dry weight obtained.

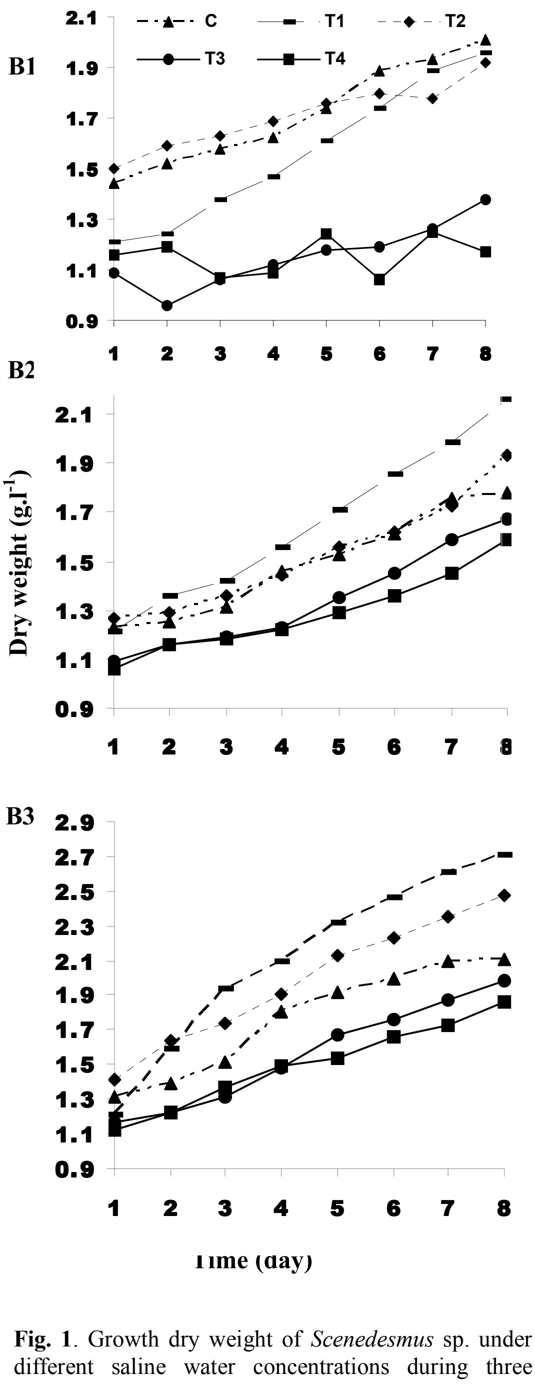

Fig. 1. Growth dry weight of *Scenedesmus* sp. under different saline water concentrations during three batches (B1, B2 and B3). C= control, T1=25%, T2= 50%, T3=75% and T4=100% of the sea water.

As the bio-filter (alga) was re-newed, the used media became more diluted as compared with the initial at zero time. Here, growth expresses more negative response with all treatments used. Control cultures exhibited the afore-obtained result of the first batch. Other examined ratio including 25 and 50% surpasses the control cultures, while cultures of 75 and 100% sea water resulted in the neighbor result of control culture as compared with the first batch.

Comparing the third batch with both first and second batches showed the extra growth development including dry weight, growth rate and the percentage increases. The obtained data indicated the high potential of *Scenedesmus* sp. to grow under these conditions due to success nutrients removal. It was accompanied by a massive accumulation of starch or lipids due to genetic aspects. To overcome such effect, algae tended to accumulate secondary carotenoids to complete their photosynthetic performance via acetate assimilation or other organic carbon source. For instance, *Haematococcus pluvials*; the fresh water green alga; is able to grow well under drastic nutrients starvation. On the other hands, such alga able to complete their life cycle at 2% of NaCl (Boussiba *et al*., 1992). Also, the green alga *Scenedesmus* sp. was well grown under 5 folds of the recommended growth medium (El-Sayed *et al*., 2008). Otherwise, all the given nutrients penetrate algal cells by the mass of action and osmosis even the toxic nutrients or compounds.

3.2. Growth rate (μ)

Comparing the absolute values of dry weight with those calculated as growth rate (μ) could explain the variation of growth among different treatments. However, growth dry weight was surpasses with the first treatment against control culture, the maximum growth rate was observed with control culture at the midst of cultivation period.

The following decline or leakage of growth rate with control culture could be ascribed to the nutrient deficiencies and algal competition as they cultivated at high biomass. Other treated cultures presented the neighbor pattern due to their ability to utilize the saline salts mainly Na and HCO_3 (Fig.2).

Growth rate (μ) was also varied by the second batch of incubation due to salinity levels. Growth rate increasing was observed at the early time of incubation with both control and 25% of sea water treated cultures suggesting the suitability of growth media. Growth dry weight was increased also at 50% sea water enriched cultures. Growth rate was enhanced at the end of cultivation with the concentrations up to 25% of sea water revealing the long adaptation time of such alga to grow successfully under this stream conditions. In addition, the negative μ values were obtained only by the first batch even with control treatment due to algal competition.

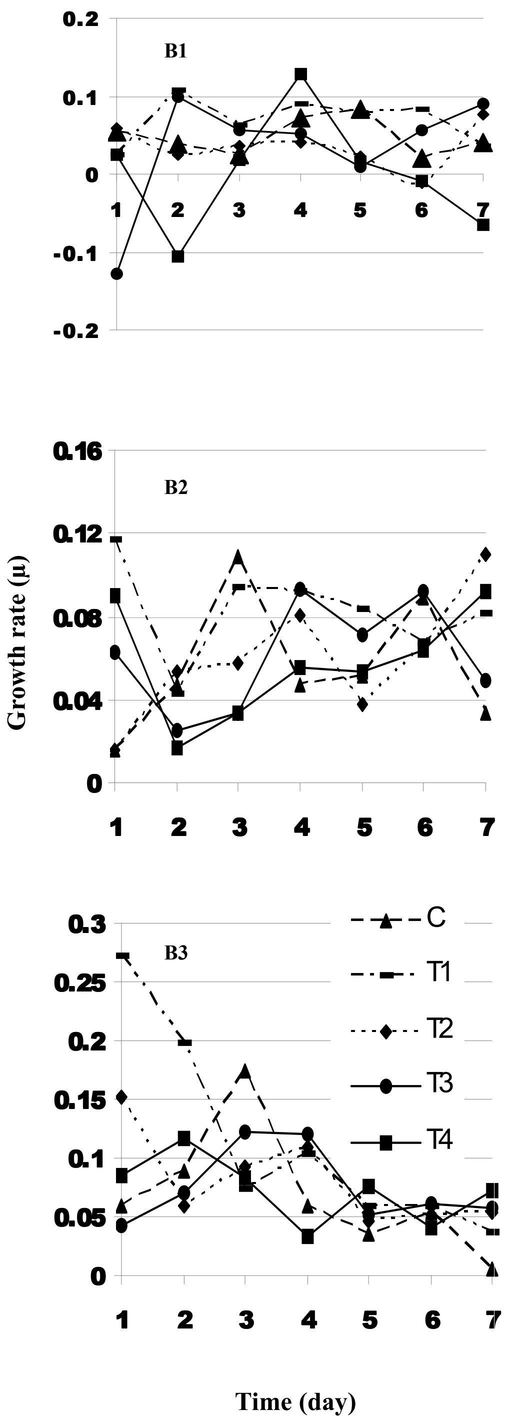

Fig. 2. Growth rate (μ) of *Scenedesmus* sp. under different saline water concentrations during three batches (B1, B2 and B3). C= control, T1=25%, T2= 50%, T3=75% and T4=100% of the sea water.

3.3. Percentage increase

Percentage increases of dry weight suggested the true response of alga against salt treatments. As the initial biomass was varied, the net yield was compared with the initial. Maximum percentage increase was observed with the first treatment of all three batches, followed by the control cultures (Fig.3).

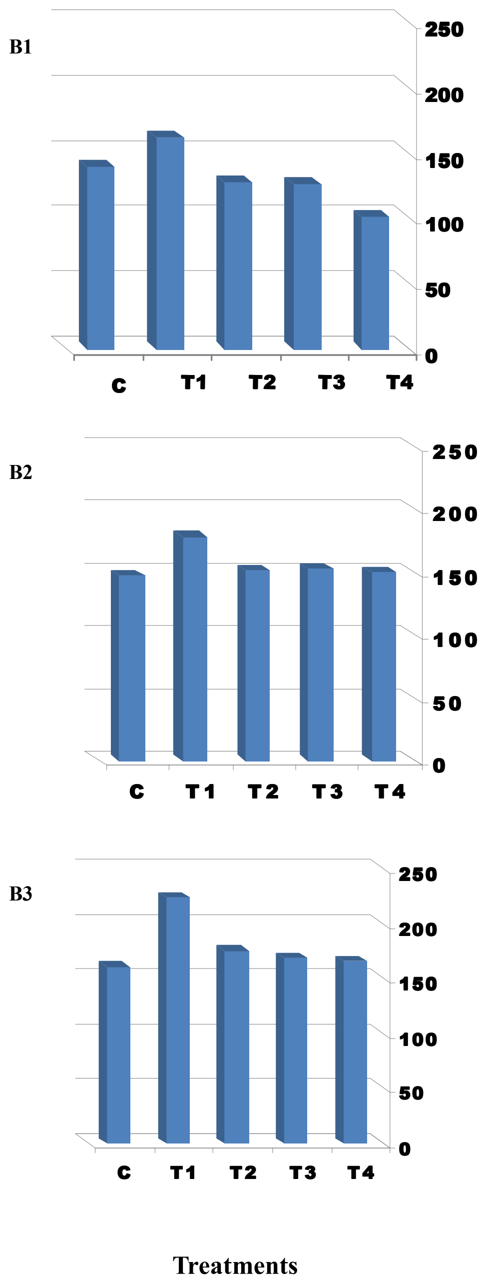

Fig. 3. Dry weight percentage increase of *Scenedesmus* sp. under different saline water concentrations during three batches (B1, B2 and B3). C= control, T1=25%, T2= 50%, T3=75% and T4=100% of the sea water.

As mentioned before, the increasing of biomass obtained under the lower salinity level mainly return to the presence of supplementary doses of nitrogen and phosphorous. Also, the rising of such biomass by the following batches could be ascribed to salinity removing with the accompanied dilution effect, where percentage increase was showed extra increments due to nutrients removal by the first batch and the extreme sea water treatments exhibited slightly result as compared with control cultures.

3.4. Chlorophyll and carotenoids

Algal pigments content including total chlorophyll and carotenoids were more affected rather than dry weight. Chlorophyll decomposition was linearly observed with two main actions. The first one was due to the level of salinity except cultures received 25% of sea water, where the rate of decomposition was increased as salinity level was increased up to 25% of sea water enrichment. The absent effect of salinity under such level (25%) might be attributed to the ability of alga to grow under this condition and to utilize such saline nutrients to complete their life cycle. In addition, the high initial biomass and extra supplementations of nitrogen and phosphorous might be enhance their growth against the given salinity dose (25% of sea water).

As salinity levels were increased up to 25% of sea water, chlorophyll content decreased in a linear correlation with the ambient doses. The complete biodegradation was observed with extremely super optimal concentrations (*i.e*; 75 and 100% of saline water). The effect of salinity on chlorophyll decomposition was downed by the next batches (B2 and B3) due to dilution effect of saline nutrients.

The second action was due to the batch number, where with advancing time the rate of chlorophyll decomposition was decreased as a result of nutrient consumption by the ex-batch which turns growth media to be more diluted (Fig. 4). A completely opposite pattern was observed when data was subjected as total carotenoids; however the green color was not fully disappeared. The masking action of chlorophyll against carotenoids was failed, which might be attributed to the high initial biomass.

On the other hand, an opposite manner was observed with carotenoids content. First batch represented different increases in carotenoids content, however some cultures kept in green color (25 and 50% of sea water). Increasing of salinity up to 50% of sea water breakdown the dominance of green color of chlorophyll and explain the extra carotenoids accumulation. Extension of growing batches (B2 and B3) resulted in a vice profile, where the rate of carotenoids accumulation was decreased proportionally to the batch number.

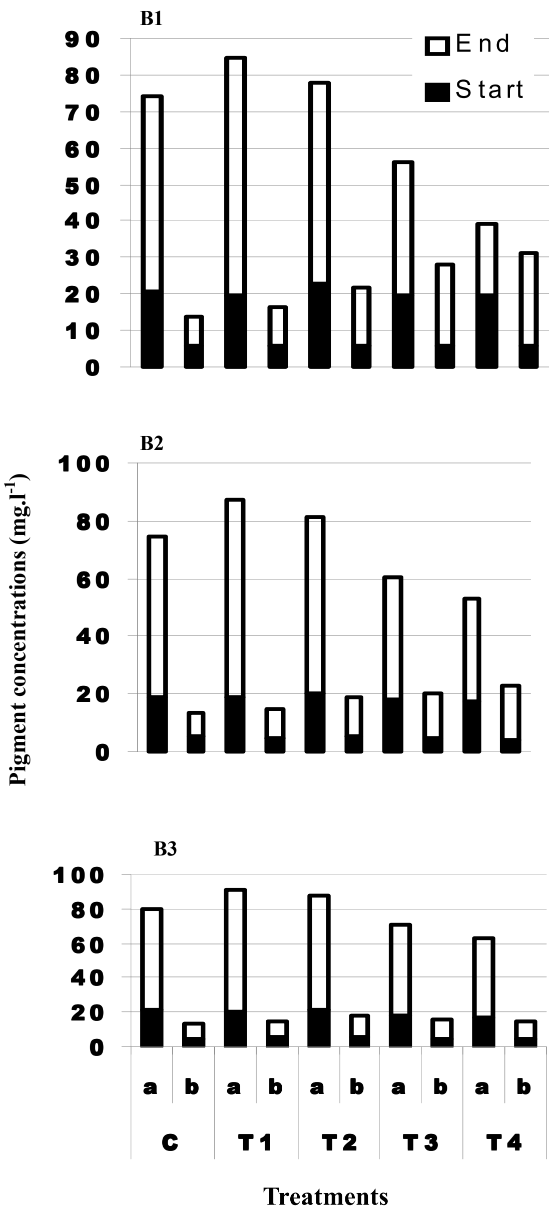

Fig. 4. a) Total chlorophyll and b) total carotenoids ($mg.l^{-1}$) of *Scenedesmus* sp. under different saline water concentrations during three batches (B1, B2 and B3). C= control, T1=25%, T2= 50%, T3=75% and T4=100% of the sea water.

3.5. Chlorophyll/carotenoids ratio

A fixed relationship was observed among salinity, total chlorophyll and carotenoids. Addition of 25% sea water to growth medium enhanced chlorophyll accumulation with slight increase in total carotenoids. Other treated cultures (*i.e*; 75 and 100% of sea water) resulted in chlorophyll decomposition with different rates. Under such concentrations, an opposite manner was observed on carotenoids accumulation, where increasing of salinity levels led to obvious carotenoids increases. With advanced batches, the rate of chlorophyll decomposition against carotenoids accumulation was greatly decreased due to nutrient consumptions and dilution effect. The afore-mentioned hypothesis could be supported by the data that obtained from the calculated chlorophyll and carotenoids ratio (Table 2).

Table 2. Chlorophyll carotenoids ratio as affected by salinity levels at the end of every batch.

Treatments	control	25%	50%	75%	100%
	chlorophyll-carotenoids ratio				
Batch1	6.8	6.0	3.5	1.6	0.8
Batch2	6.8	6.7	4.5	2.7	1.9
Batch3	6.8	7.6	5.3	4.7	4.5

Comparing the effect of salinity on chlorophyll/carotenoids ratio within the sole batch showed the decreasing of such ratio in association with the rising of salinity level due to carotenoids increasing or/and chlorophyll decreasing. As shown in Table 2, the ratio was downed from 6.8 at control treatment to 0.8 in full sea water media. A vice observation was found when the comparison was subjected to the batch number, where such ratio was found to be 4.5 at the end of experiment (batch 3) as compared with the zero time of batch one or control culture that grown in fresh water media. Also, under full saline media (100% sea water) the ratio was rised from 0.8 at the end of the first batch to be 4.5 by such time of the third batch. So, such correlation could be serving as the early monitor to detect the salinity effect on the tested alga.

On the contrast with the common response of algal pigments against salinity such as chlorophyll decomposition and carotenoids accumulation; with all treatments; chlorophyll content was increased and no acceleratory effect was observed as growth was expressed as total carotenoids.

In most algal categories, the obvious behavior of algal growth parameters against salinity is to dry weight failure due to protein and chlorophyll decomposition. The following action is to accumulate a large amount of lipids by shifting photosynthesis operation toward carotenoids. So, accumulation of such pigments took place. Thus, the main concept of this action is to meet the dry weight accumulation under non-favorable conditions

Particularly under changing salinities, the intracellular osmotic adjustment has to follow and compensate the external fluctuations in salt concentration. Under hyper-saline conditions, the accumulation of osmotically active substances by uptake or biosynthesis can be observed, in contrast to excretion or degradation under hypo-saline conditions (Kirst, 1990). The major inorganic osmolytes in most algae include potassium, sodium and chloride (Karsten and Kirst, 1989; Mostaert *et al*., 1995). Cellular concentrations of these inorganic osmolytes are adjusted easily and quickly with low metabolic energy costs, especially compared to the cost of organic osmolyte biosynthesis or degradation. The biosynthesis and accumulation of organic osmolytes in the cytoplasm permits the generation of low water potentials without incurring metabolic damage (Yancey, 2005). Therefore, the organic osmolytes are termed "compatible solutes" to indicate that their presence, even at high concentrations, does not interfere with metabolic activity (Brown and Simpson, 1972; Karsten *et al*., 1996). Exposing algae to hypo- or hyper-saline stresses can affect many sites in their photosynthetic apparatus resulting in significant effects on the photosynthetic performance (Sudhir and Murthy, 2004). In the red alga *Porphyra perforata*, hyperosmotic salinities inhibited photoactivation of electron flow on the reducing side of PSI and electron flow on the oxidizing side of PSII (Satoh *et al*., 1983). High salinity increased carotenoids content in the cyanobacterium *Synechocystis* sp., but decreased the content of *phycocyanine* (Schubert *et al*., 1993). Hypo-osmotic stress in the green alga *Dunaliella tertiolecta* and hyper-osmotic stress in *Chlamydomonas reinhardtii* induced decreased PSII activity (Gilmour *et al*., 1984; Endo *et al*., 1995). However, depending on the stress level, all these effects not necessarily lead to photoinhibition. Instead, all responses can be described as regulatory mechanisms to avoid photo-damage by increasing non-radiative energy dissipation and non-assimilatory electron transport. Photo-inhibition can be prevented as long as these mechanisms work effectively and the balance between energy conversion and energy consumption is kept. Thus, adverse salinity effects leading to an up-regulation of energy dissipation are not necessarily mirrored in a decreased maximum quantum efficiency of PSII.

4. CONCLUSION

Certain algae species even under fully salinity level are able to grow well and complete their life cycle. Under such conditions, removing of at least 25% of total salts per batch was observed. Thus, recycling of such growth medium might be suggesting the fully desalination. Chlorophyll /carotenoids ratio could be serving as the early monitor for desalination progress rather than alga dry weight development.

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