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Isolation, Identification and Purification of Unicellular Green Algae Dunaliella of Maharloo Lake and the effect of salinity on its growth and physiological parameters

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ABSTRACT

Unicellular green algae Dunaliella is of the branches of green algae (Chlorophyta) and a genus of Volvocales that is recently placed in a Chlamydomonadaceae family. In this study, micro-algae, isolated from Maharloo Lake, Shiraz, Iran, was used. Purified algal suspension was cultured in different concentrations of salt (0.17, 0.6, 1,2,3 and 4 M of NaCl) and the number of cells was counted, at different times from zero to 14 days after the date of culturing, every two days and finally the growth curves of purified algae were plotted based on the number of cells per unit of time for different concentrations of NaCl.

KEYWORDS: *Isolation, characterization, species Dunaliella algae, lake Maharloo, salinity on growth, physiological parameters*.

INTRODUCTION

Unicellular green algae Dunaliella is of the branches of green algae (Chlorophyta) and a genus of Volvocales that is recently placed in a Chlamydomonadaceae family. Dunaliella is similar to Chlamydomonadaceae but without cell walls. It has two equal flagella and a cup-shaped chloroplast. It is of salt-loving species and is able to grow in the wide ranges of the concentrations of salt. To date, 29 species of it have been identified (Ben-Amotz & Avron, 1992).

The shape of Dunaliella cells is seen oval-shaped, spherical, cylindrical, pear-shaped and even spindle and sometimes it changes by changing environmental conditions. Dunaliella algae is the most important commercial source of natural beta-carotene. This substance is used as a coloring agent in food and cosmetic industries and pharmaceutical products and also it is the provision of vitamin A in the human and animal feed. It has been proven that the properties of Beta-carotene prevents a variety of tumors and cancers and heart diseases effectively. In addition, it produces the compounds such as retinal, apocarotenoids, ketones, aldehydes and epoxides which can repel the singlet oxygen and absorb free radicals due to its specific chemical structure and biochemical methods. Many studies have proven the anti-cancer and anti-oxidant properties of these compounds (Moghadasi et al., 2011). In some species of Dunaliella such as D. salina and D. parva, the cells are seen orange-red instead of green due to the high concentration of beta-carotene (Hadi et al., 2008). Dunaliella algae, in coping with salinity stress, usually produces glycerol, so, it is important to produce beta-carotene and glycerol in terms of biotechnology (Shariati & Hadi, 2000). Dunaliella algae can be used to remove the heavy metals due to tolerating the high concentration of heavy metals (Imani et al., 2011). Given to the many uses of this algae, producing it in Iran is very affordable, because the accessibility to the cheap sources of salt water, which is the requirements of the industrial production of desired products, is easy. On the other hand, a lot of salt water which is not suitable for the cultivation of any plants, can be used in this way optimally. According to the progress of biotechnology science and the benefits of it, we decided to study the unicellular green algae Dunaliella, which have many uses in the pharmaceutical, health, food and cosmetic industries, by biotechnology science. Given that Iran has many salt lakes and marshes and suitable climate for growing Dunaliella algae and also extensive sunlight to produce the beta-carotene, it seems that some species of this algae can be found in Maharloo Lake in Shiraz City which have the greatest production of beta-carotene. The first description of unicellular red algae with two flagella in concentrated salt water was reported by Dunal in 1838. He found it in Ontpellier salt mine on the Mediterranean coast of France and named it as “Haematococcus salinus” and today, it is known as Dunaliella salina. During the nineteenth century, Dunal red flagellated algae had been observed in salt lakes and other places saturated with salt in Lorraine, Algeria and Crimea, in France and Romania and also reported by other biologists. This organism was given different names by each researcher (Aharon, 2005).

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Testing method:

Gathering unicellular green algae Dunaliella:

In this study, micro-algae, isolated from Maharloo Lake, Shiraz, Iran, was used. Solid medium was prepared by adding 1.5gr of agar to 100ml of modified Johnson's medium (Table3-2) and poured into several petri dishes and after hardening, 100ml of the water of the lake was poured in one of them rotationally by glass spreader. In this way, a medium was prepared in which a cell can grow and create colonies. Due to the specificity of the medium, only the desired sample will grow in it. After 14 days, a single clone was cultured in a liquid medium and then the sample was processed for identification.

Gathering Dunaliella from Maharloo Lake

To gather different species of unicellular green alga Dunaliella, about 200-300 ml of the water of Maharloo Lake was sampled from 3 sites spaced 15 km by suction (due to the shallow water pond).In addition, since some species of algae Dunaliella such as D.salina, D.pseudosolin and D.parva produce a very large amount of beta-carotene in high salinity (hypersaline of NaCl) and change color from green to orange or red, the water of ponds in which the water color is red or orange, was sampled in addition to the ponds in which the water color is green.

Salt shock in Dunaliella:

Some (1000ml) of the algal suspension of the target species of Dunaliella, in a concentration which is appropriate for the growth of the algae (1 M of NaCl), was picked and centrifuged for 5 minutes at 1500-1000 rpm by centrifuge (Hettich universal model) and the supernatant solution was discarded to remove the existing glycerol. 50 ml of fresh medium with the same NaCl concentration was added to the reached algal deposits and to avoid tearing up the cells, it was shaken gently to precipitate the deposit of Dunaliella algae again.

20ml of the algal suspension was picked as a control sample and then 20 ml of it was picked as a treatment of salt shock and the given amount of solid salt (NaCl) was added to it that the final concentration of NaCl in the treatment of salt shock became 3M. Then, the amount of glycerol of control sample and treatment was measured at the times of zero, 60 and 120 minutes after salt shock by spectrophotometry (Wegman, 1989). Meanwhile, the chlorophyll content of the alga suspension was measured by spectrophotometry (Frank & Wegmann, 1974) and the amount of glycerol was calculated in chlorophyll (μ mol.mg chlorophyll-1).

The growth curve of algae Dunaliella in various concentrations of NaCl:

Purified algal suspension was cultured in different concentrations of salt (0.17, 0.6, 1,2,3 and 4 M of NaCl) and the number of cells was counted, at different times from zero to 14 days after the date of culturing, every two days and finally the growth curves of purified algae were plotted based on the number of cells per unit of time for different concentrations of NaCl.

RESULTS

Algal suspension of Dunaliella from the step of purification was identified and examined individually by considering several important identification factors, including optimum growth, size, color and volume of algae in optimal and unfavorable growing conditions (at the concentration of 3M of NaCl) and using identification key of different species of Dunaliliella. In total, four species of D.salina, D. pseudosalina, D. viridis were identified and finally, for additional identification, the amount of glycerol in different concentrations of salt and also their responses to salt shock was determined.

Unicellular green alga Dunaliella produces glycerol to adjust the intracellular osmotic potential with the environment and since the cell membrane has less permeability than the glycerol, its amount increases in the cell and when the osmotic potential of the cell surroundings (by increasing the amount of NaCl of medium) becomes more negative, the production and synthesis of glycerol to adjust the intracellular potential with the environment will be greater. This is one of the characteristics of the Dunaliella cells that can be effective in their additional identification. So, the amount of glycerol in the species of Dunaliella grown in various concentrations of salt (0.17, 0.5, 1, 2, 3 M of NaCl) was measured by the described method (section 2-8-2) and was drawn in a form of linear charts (figures 3-18, 3-19, 3-20) in terms of the number of cells and also chlorophyll with different concentrations of NaCl. The charts show that the amount of glycerol increases linearly by increasing the amount of NaCl in the medium so that if the concentration of NaCl is doubled, the amount of glycerol is also almost doubled.

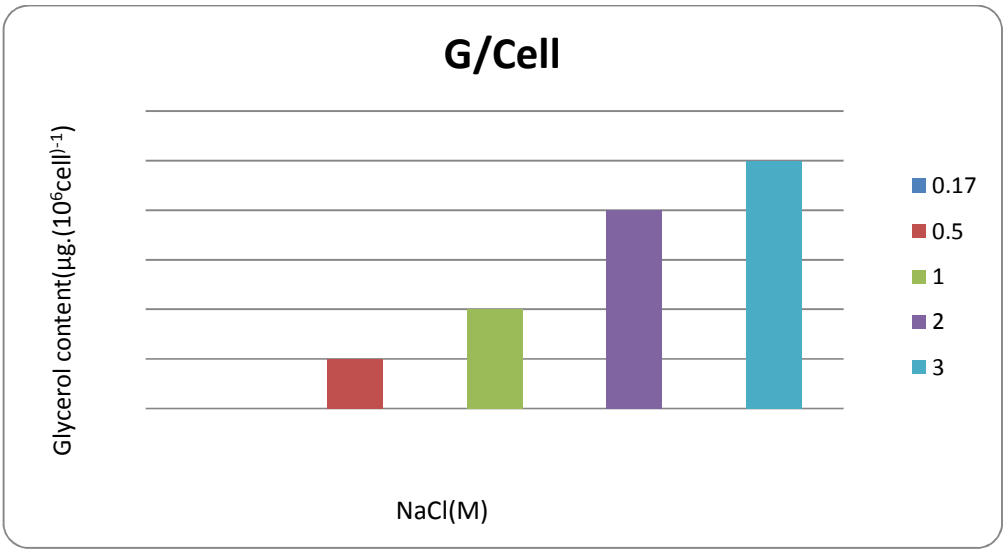

Figure 1. The glycerol amount of D. psedosalina grown in different concentrations of NaCl

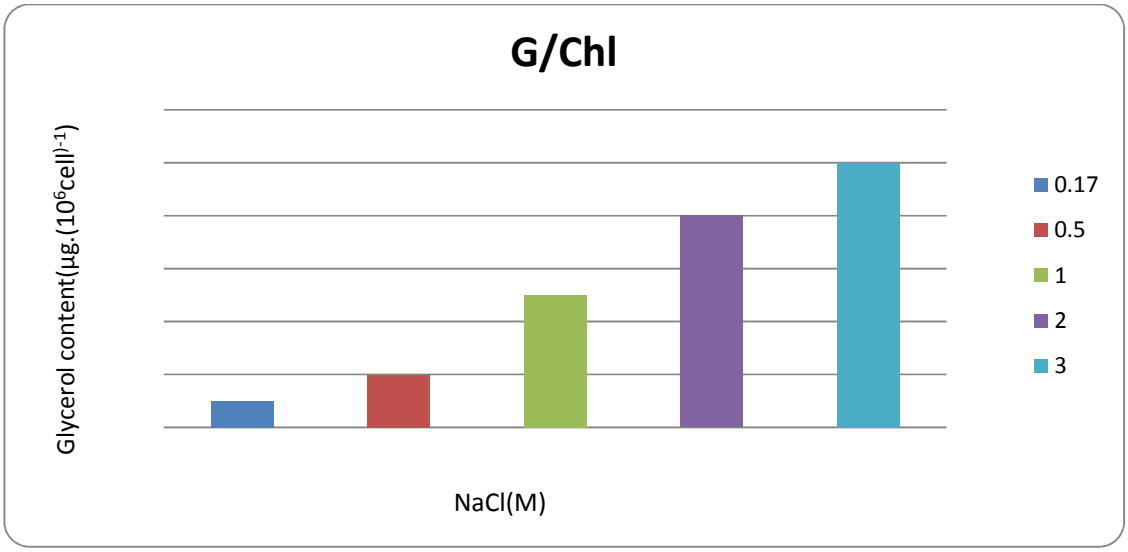

Figure 2. The glycerol amount of D. psedosalina grown in different concentrations of NaCl

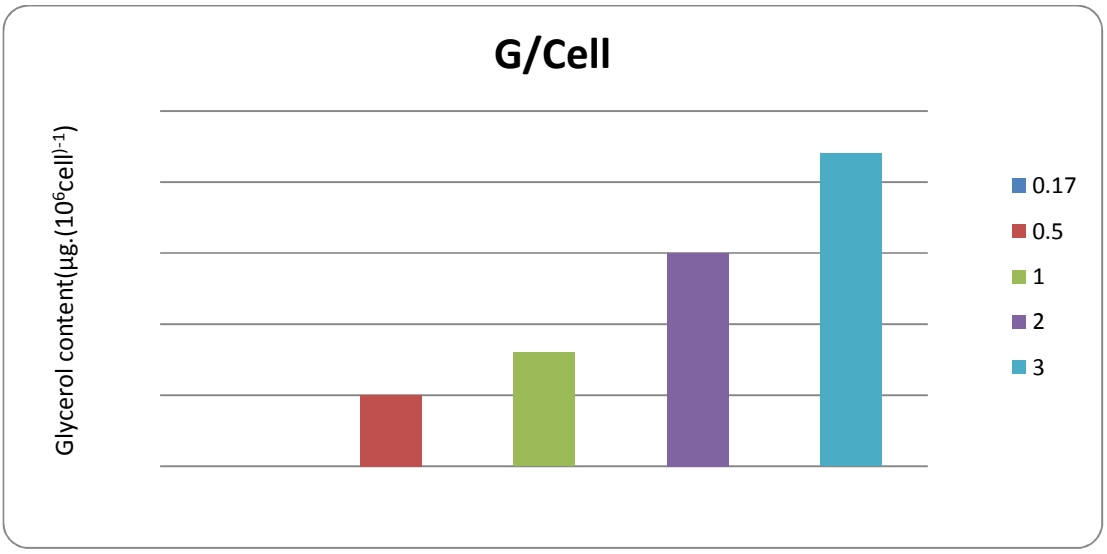

Figure 3. The glycerol amount of D.salina grown in different concentrations of NaCl

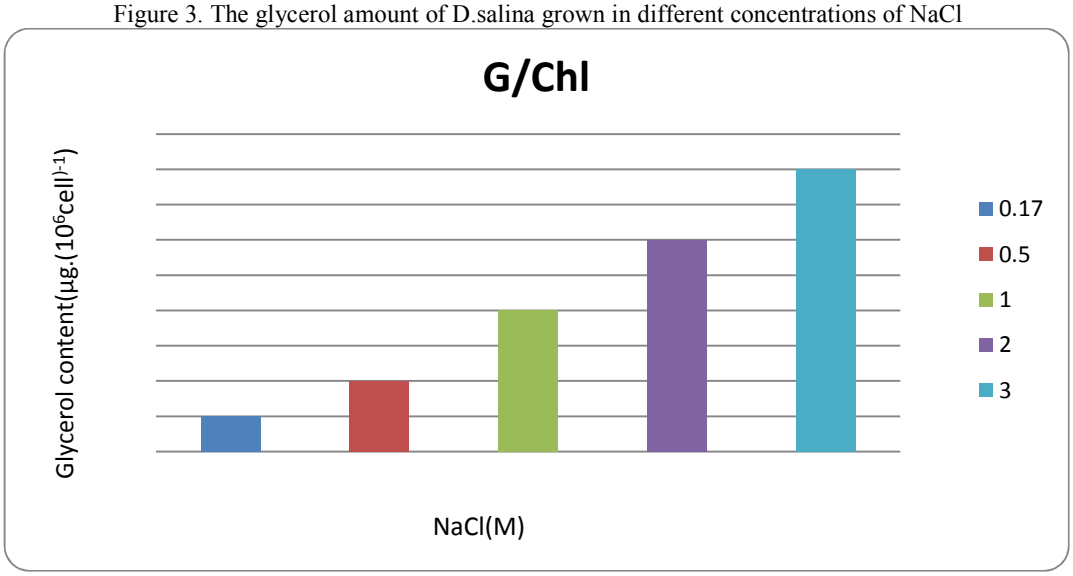

Figure 4. The glycerol amount of D.salina grown in different concentrations of NaCl

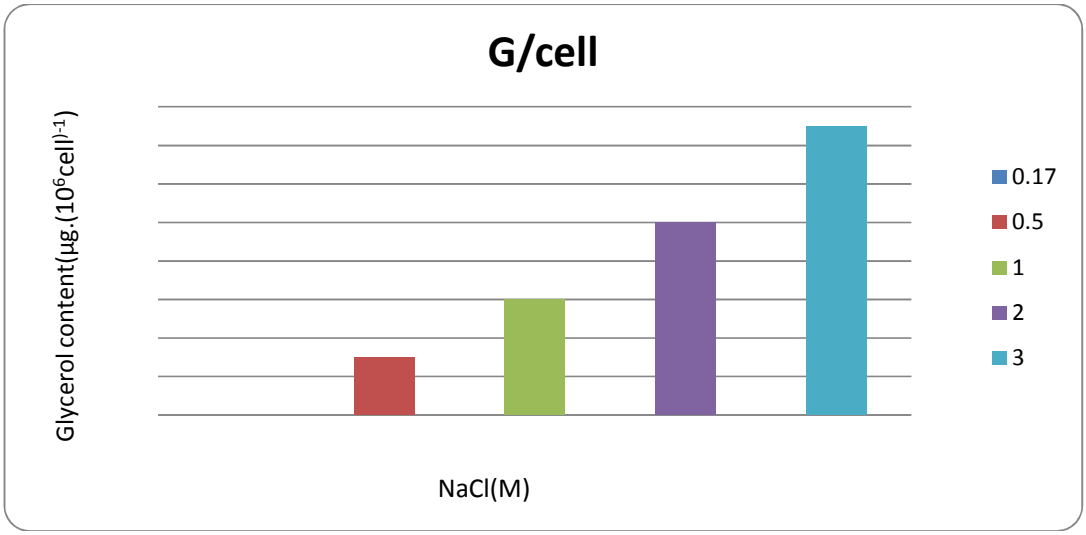

Figure 5. The glycerol amount of D.viridis grown in different concentrations of NaCl

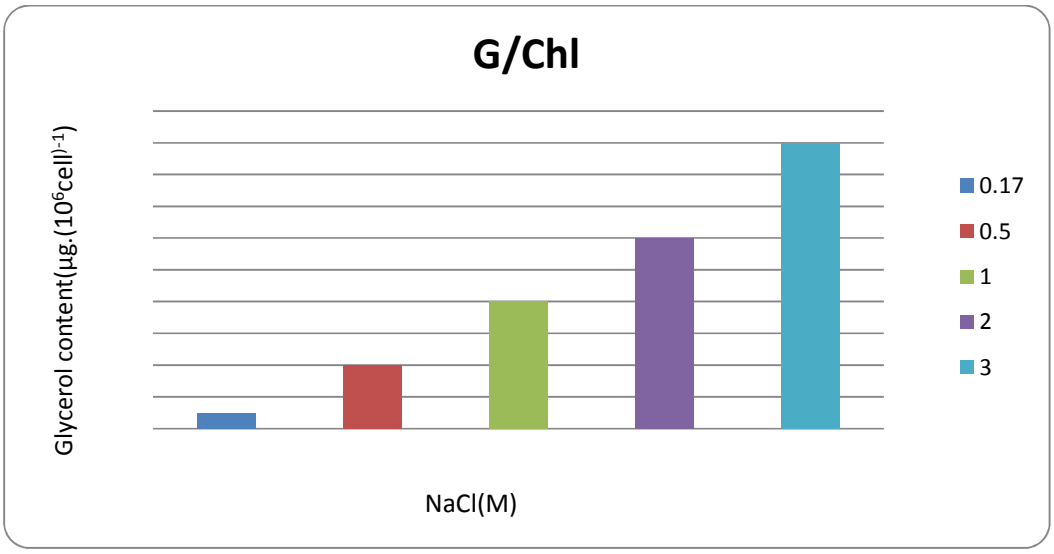

Figure 6. The glycerol amount of D.viridis grown in different concentrations of NaCl

These results correspond with the amount of glycerol in Dunaliella grown in different concentrations of NaCl which reported by Avron in 1989 (figure 1-2) (according to Avron& Ben-Amotz, 1992).

In addition, three species of Dunaliella were placed under the salt shock of 1 to 3 M of NaCl by described method and the glycerol of control and treatment samples were measured at the times of zero, 60 and 120 minute after applying salt shock by the described method and their charts were drawn in terms of time (minute) (figures 1, 2 and 3). These charts show that at the time of 60 minute after applying salt shock, the amount of glycerol in the treatment of salt shock increased significantly compared to the control sample's one and also it increased at the time of 120 minute after applying the salt shock. These results correspond with the results reported in 1989 by Chitlaru and Pick. They applied the salt shock of 1 to 2.5 M of NaCl on the species of Dunaliella. Also, the results showed that the mentioned species follows the response method of Dunaliella species to salinity (NaCl).

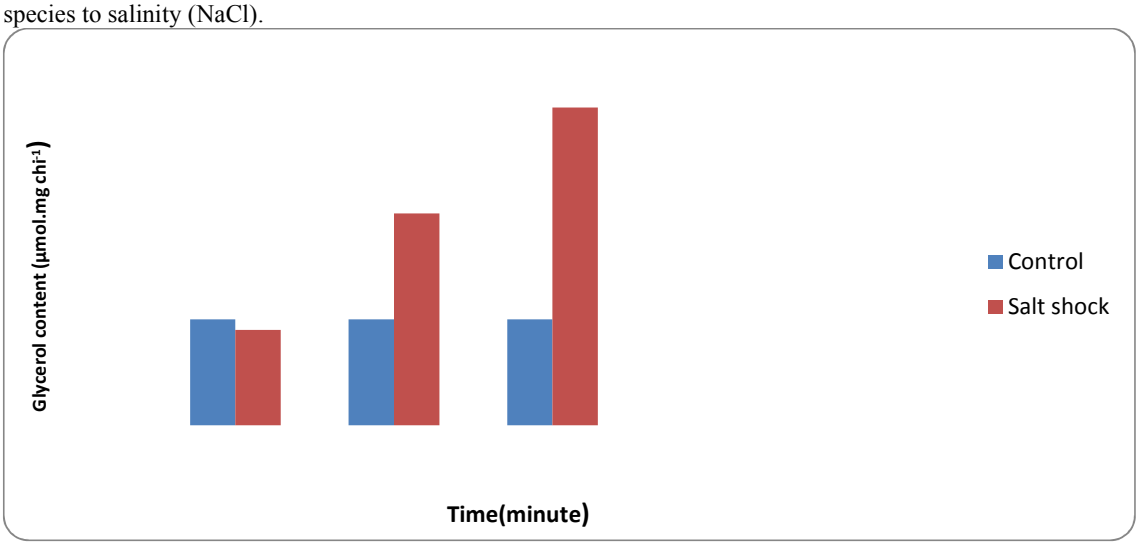

Figure 7. The glycerol amount of D.psedosalina at different times after applying salt shock from 1 to 3 M of NaCL compared with control sample.

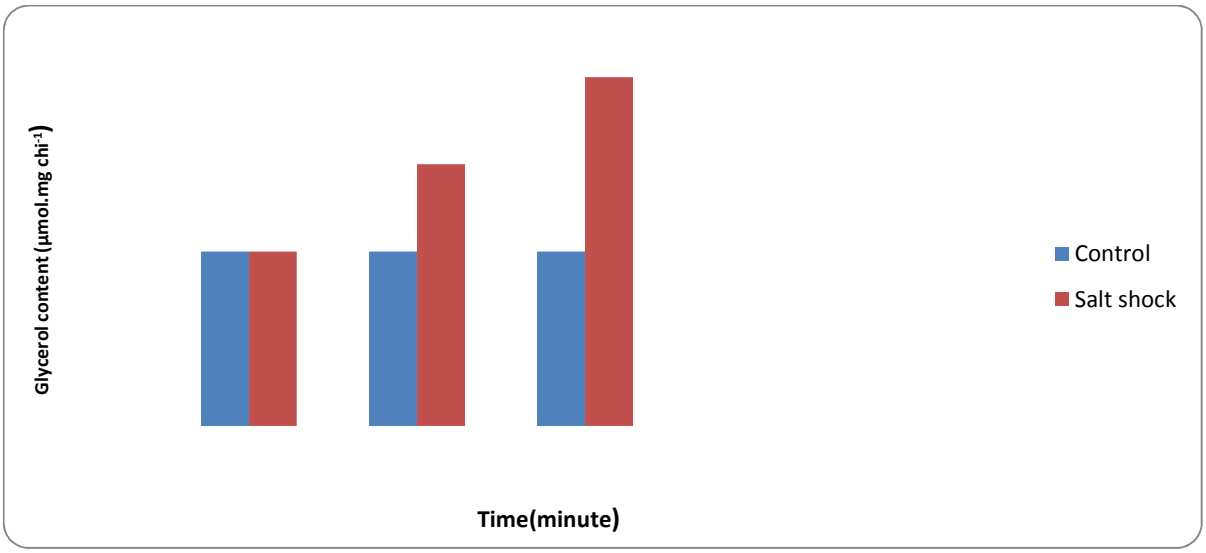

Figure 8. The glycerol amount of D.viridisat different times after applying salt shock from 1 to 3 M of NaCL compared with control sample.

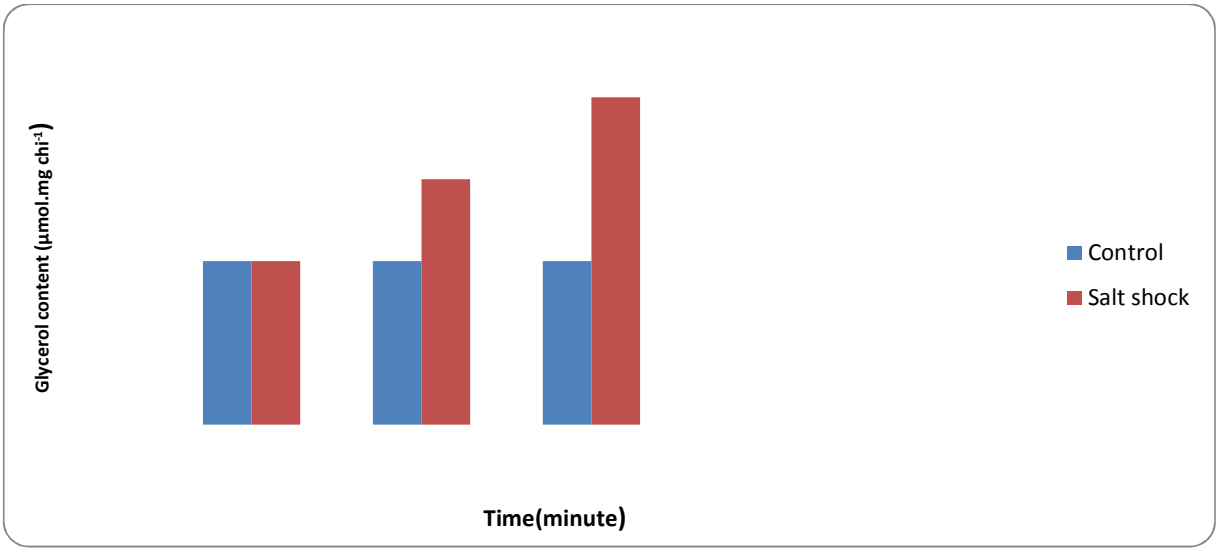

Figure 3-23. The glycerol amount of D.salina at different times after applying salt shock from 1 to 3 M of NaCL compared with control sample.

DISCUSSION AND CONCLUSION

The results of the stress effects on the microalgae of Dunaliella salina show that: if the species of D.salina is subjected to the stress, it will produces beta-carotene. Beta-carotene acts as a protective barrier against stress and protects the sample.

Okotoam et al. (2000), in their study on the responses of algae to heavy metals, reported that depending on the type of stress, the production of two types of carotenoids in Gonyaulax polyedra is different.

Behsem Yatmes (2005), in his research on the physiological responses of D. tertiolecta and D. Salina, reported that the amount of carotenoids in both species will increase by increasing the concentrations of copper chloride and it is reported that in all concentrations, the amount of carotenoids in D. salina is 3 to 5 times more than one in D. Tertiolecta (Nikookar., 2005).

Given that the D. salina algae can grow and propagate and also produce the beta-carotene in difficult conditions and the beta-carotene is a valuable material in terms of economic, commercial and cosmetic aspects and more than 80% of it is produced chemically, if the purpose is to produce the beta-carotene on an industrial scale, because the amount of beta-carotene produced by this micro-algae is increasing in difficult conditions, , it is suggested that at first, the algae of D. salina is put in good conditions for growing and then the conditions

become difficult so that the production of beta-carotene will be more. It is hoped that the production will be more by cellular and molecular research.

Finally, with regard to the production of glycerol in responding to salinity stress in Dunaliella cells is an important factor in their identification, so, the amount of glycerol grown in different concentrations of NaCl was measured and it was found that the amount of glycerol is increasing by increasing the amount of NaCl in medium. In addition, the cells produced the glycerol in responding to salinity stress that was used to identify them further.

finally, it seems that studying the other species of Dunaliella in other parts of Maharloo Lake, Shiraz and other salt water of Iran such as a salt Lake in Qom and Urmia Lake and studying the effective factors of light, temperature and photoperiod in the production of beta-carotene and glycerol in the species extracted from Gavkhouni marsh and comparing their characteristics with the pure species in other countries can provide the optimum use of the salt water.

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