Int. J.MAr.Sci.Eng., 4(2), 107-111, Summer & Autumn 2014
ISSN 2251-6743
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Effect of pH and salinity on growth and bloom of *Dunaliella* sp. algae

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Received 10 June 2014; Rivised 15 July 2014; Accepted 20 August 2014

ABSTRACT: In this study, effects of pH and salinity on growth and bloom of *Dunaliella* sp. algae were investigated. The algae were reared in different pH (6.0, 6.8, 7.0, 7.3, 7.6, 8.0, 8.6, 9.0, 9.7 and 11.0) and salinities (20.0, 31.6, 50.1, 79.4, 117.1, 125.9 and 200.0 ppt) at Inland Aquaculture Research Institute Phycolab during 2011. After 96 h, the algae reared in 50ppt salinity and below that had negative growth, however, beyond the 50 ppt, it started to grow and reached the growth peak in 117.1 ppt. In 125 and 200 ppt, the algae growth decreased compared to 117.1 ppt. Exceptsalinityof31.62and50.11descendant of the other groups was significant ($P < 0.05$). At the different pH, after 96 h the lowest growth was observed in pH 11, whereas, the highest growth was in pH 7.6 while increasing pH above 9.0 led to negative growth. These differences were significant ($P <0.05$).

Keywords: *pH; salinity; growth; Dunaliella sp.*

INTRODUCTION

Like plants that are base of life on the earth, algae are foundation of life in aquatic environment. Generally, more algae are known as phytoplankton or suspended aquatic plants. In all aquatic ecosystems, algae are important because of organic matter production and being at the base of energy pyramid. Other organisms, beside their relationships in food chains, are directly or indirectly dependent to phytoplankton. Accordingly, knowledge about the phytoplankton of each water resource is very important (Davies, 1998).

In an aquatic environment, all physicochemical and biological factors affect phytoplankton diversity and biomass (Naz and Turkman, 2005). A variety of mechanisms such as temperature limitation, light intensity, nutrients, sedimentation and zooplankton grazing affect genus diversity and domination (Ortega, 2003). In addition to the above, antagonistic relationships with aquatic plants change in climate and water hydrology, total hardness, water depth and other factors affect diversity and distribution of phytoplanktonic communications (Onyema, 2008). So overall physical, chemical and biological factors are important aspects affecting the phytoplankton communications during different seasons.These factors control phytoplankton population via change in biomass composition and production patterns (Harris, 1986; Boney, 1989; King, 2002).

Green algae are a widely extended and diverse group. Members of this group are very diverse in the case of morphology, reproduction ways, life cycle and habitat. Green algae, due to the presence of green pigments, chlorophyll a and b, are often looked in green. These algae contain alpha and beta carotene and xanthophylls such as

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lutein andviolaxanthinand a small amount of neoxanthin.

Dunaliella sp. are prokaryotic green algae belonged to the family Polyblepharidaceae, phylum Chlorophyta, order Vovocales, which have no cell wall and tolerate high light intensity. In this study, effect of different salinities and pH was investigated on the growth and bloom of *Dunaliella* sp.

MATERIALS AND METHODS

Johnson medium was used to culture *Dunaliella*. To investigate the effect of different salinities on the algae growth, culture medium salinity was adjusted to 6 levels plus a control (the original salinity of the medium 117.1 ppt), by logarithmical calculations (20.0, 31.6, 50.1, 79.4, 117.1, 125.9 and 200.0ppt). First, 250 ml of culture medium was poured into a 500 ml Erlenmeyer flask and then, desired salinities were adjusted. One mg/l algae sample was inoculated to each flask. Then, 2 ml of sample was taken from each flask for initial counting. The photoperiod was 12:12 (light: dark).After 96 h, 2 ml of each treatment was taken number of algae cells was counted and the best salinity for algae growth was determined by calculation of growth percentage in proportion to the initial cell number. Cell counting was performed using a hemocytometer. 10 pH levels (6.0, 6.8, 7.0, 7.3, 7.6, 8.0, 8.6, 9.0, 9.7 and 11.0) were chosen to investigate the effect of pH on the algae growth under photoperiods 12:12. Cell culture, cell counting and other procedures were similar to the salinity trial. Finally, the optimum pH and salinity for maximum algae growth was determined.

Culture was conducted in the Culture Room equipped with ultraviolet (UV) lamp and laminar box apparatus. To sterilize the room, UV lamps were turned on for 45 min, and then, 1 mg algae sample was pipette into each flask under laminar box. Prior to transfer of flasks to culture shelves, 2 ml of each flask were taken and was fixed using 4 % formalin for cell counting. Then pipettes were sealed on the flasks' opening and flasks were transferred to the culture shelves equipped with florescent lamps. The algae were allowed to rear under temperature of 25 ± 2 ºC, light intensity of 3500 ± 350 lux and photoperiod 12:12 dark: light (Piri and Ordog, 1997). Pipettes were connected to air blower and algae growth was determined after 96 h culture.

Data were analyzed by statistical software SPSS v.18 and Microsoft office Excel 2007. Data were subjected by one way ANOVA and Duncan's test to find significant difference between different salinities and different pH concentration during sampling. $P<0.05$ considered to besignificantly different.

RESULTS AND DISCUSSION

The obtained results showed that, after 96 h, the algae reared in the salinities 50 and below 50 ppt had negative growth; however, increasing salinity resulted in the ascending trend of growth (Fig. 1).

The peak point of algae growth was at 117.1 ppt; nevertheless, higher salinities (125 and 200 ppt) led to decrease in the algae growth. The lowest growth was in 20 ppt. (Fig. 1). Except salinities of 31.6 and 50.1 the differences of the other groups was significant ($P < 0.05$) (Table 1).

At the different pH, after 96 h the lowest growth was observed in pH 11, whereas the highest growth was in pH 7.6 while increasing pH above 9 led to negative growth (Fig. 2).

These differences were significant ($P< 0.05$) but differences between pH 9.0vs 9.7; 9.0vs 6.0 and between 6.0 vs 6.7 and 8.6 were not significant ($P> 0.05$) (Table 2).

CONCLUSION

There were significant differences in the*Dunaliella* sp. growth under different salinities and pH ($P <0.05$). The obtained results revealed that *Dunaliella* growth started in the salinities higher than 50 ppt. The maximum growth was observed in 117.1 ppt salinity, whereas in 50 ppt salinity, as well as, the salinities below 50 ppt, the algae showed negative growth ($P < 0.05$).

Huiand Yong-Min (2011) reported negligible changes in cell shape and volume under different salinities in *Dunaliella*, however, a significant change in the shape and volume of *Dunaliellasalina* was observed, when subjected to abrupt salinity change. They also found that an increase in salinity from 2 to 4 and 5 M resulted in increasing the number of fat vacuoles in *D. salina*. When *D. salina* was cultured under different salinities, glycerol concentration was

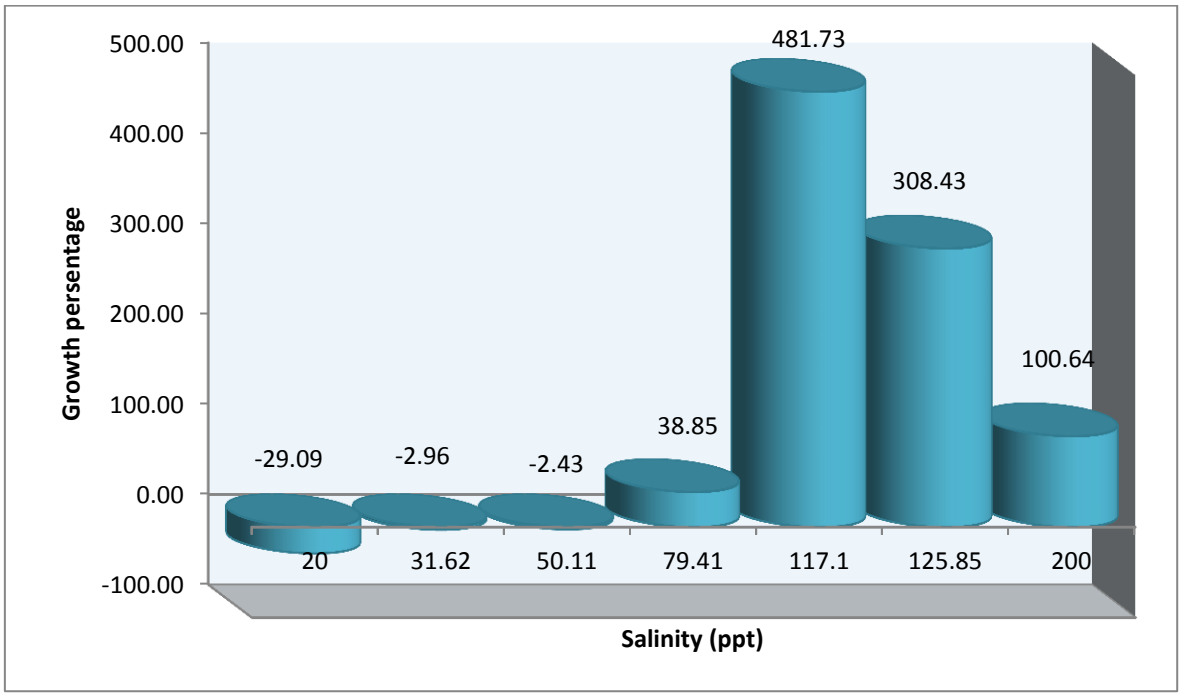

Fig. 1: Fluctuations in the algae growth percentage under different salinities

Table 1: Algae growth percentage under different salinities

Salinity (ppt)	20.0	31.6	50.1	79.4	117.1	125.8	200.0
Maen±SD	-29.09±2.86^{f}	-2.96±0.47^{e}	-2.43±1.29^{e}	38.85±1.72^{d}	481.73±27.67^{a}	308.43±16.85^{b}	100.64±2.33^{c}

-Data are represented as Mean±SD

-Means with the various letters are significantly different (P<0.05)

observed along with the increase of medium salinity.Rad and Aksoz (2011) reported that maximization of medium salinity resulted in the bloom of *Dunaliella*sp., originated from Urmia Lake, northwest of Iran. Abu Sara and Emeish (2011) found that the best growth of *Dunaliella* occurred in high salinities (150 PSU). Aksoy and Koru (2012) investigated the physiological characteristics of *Dunaliella*sp. Originated from salt extraction site and found that the best growth of the algae was at salinity 10-200 ppt.

Tawfiq et al. (2010) reported that the best salinity for *Dunaliella*sp. is higher than 45 PSU. All aforementioned studies are in agreement with the present results. Ben-Amotz (1991), Oren (2005) and Dolapsakis (2011) observed that the best growth of *Dunaliella*sp**.** happened in coastal regions or enclosed salt marsh with the high salt concentrations. Leach and Oliveira (1998) concluded that it is possible to reach cell density of 0.8×10^{6}if *Dunaliella*sp. is cultured in salinity 185 pptand pH8.5.

Jimenez and Niell (1991) showed that *D. viridis* had an optimum growth in 1 M (5.8 %) salinity. This species showed inferior growth in salinities lower that 1 M. Shariati and Madakarhaghji (2007) stated that *Dunaliella* sp. nano-algae is the most tolerant eukaryotic organism to high water salinity and is capable to tolerate saturated sodium chloride concentration. They stated that this species is found in many salty environments such as salty lakes and marshes as well as saltwater holes, which can easily respond to osmotic changes and is able to tolerate the environments containing 0.5-30 % sodium chloride.

In the present study, considering the medium pH, there was no significant difference among different photoperiods, in the case of the algae growth (P >0.05). The results showed that an increase in the medium pH from 6.0 resulted in increasing the algae growth with the peak point at pH 7.6; however, beyond this point, the algae growth showed decreasing trend as lowest

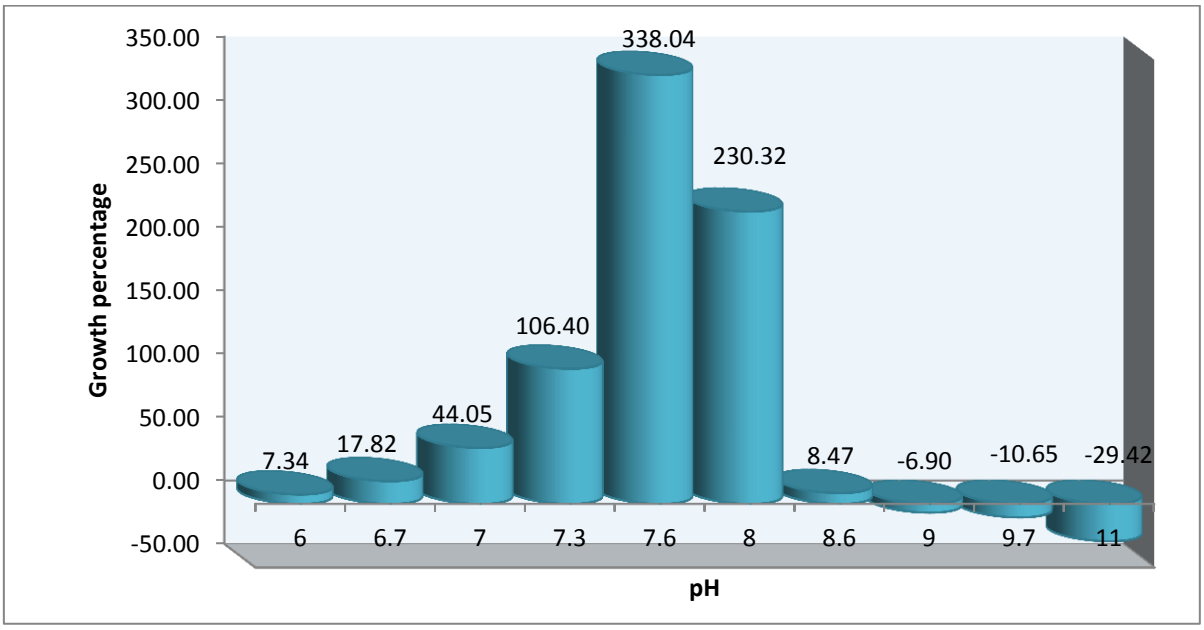

Fig. 2: Fluctuations in the algae growth percentage under different pH

Table 2:The growth percentage of *Dunaliella* sp. under different pH

pH	Mean ± SD
6.0	7.34±0.30^{f}
6.7	17.82±0.73^{e}
7.0	44.05±1.08^{d}
7.3	106. 40±4.54^{c}
7.6	338.04±10.62^{a}
8.0	230.32±9.53^{b}
8.6	8.47±0.84^{f}
9.0	-6.90±1.38^{g}
9.7	-10.65±1.51^{g}
11.0	-29.42±6.31^{h}

-Data are represented as Mean±SD
-Means with the various letters are significantly different (P<0.05)

growth was in pH 11. These differences were significant (P< 0.05).

Johnson culture medium, normally, has pH 7.0; however, the present results showed that pH 7.6 is the best one. The differences between pH 7.0 and 7.6 was significant (P< 0.05).The optimum pH for *Dunaliella*sp. culture was reported 7.0 (Ben-Amotz, 1991). Although in the present study low pH was not resulted in growth inhibition, increase in pH up to 7.6 caused growth promotion significantly (P< 0.05). This study showed that the best growth was at pH 7.0 -7.6.

Tawfiq and Suad (2010) studied on *D. salina* from BubianIsland, Kuwait and found that the best pH and salinity for appropriate growth of the algae was 9.8 and >45 PSU, respectively. They found that growth of the algae increased with the increase in salinity from 25 to 45 PSU.

ACKNOWLEDGMENT

This research was supported by Inland Water Aquaculture Institute, north of Iran. We would sincerely thank all technicians and experts helped us in laboratory

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How to cite this article: (Harvard style)
Alipour, M.; Fallahi, M.; Valinassab, T., (2014). Effect of pH and salinity on growth and bloom of Dunaliella sp. algae. Int. J. Mar. Sci. Eng., 4 (2), 107-111.