Physiological characterization of dunaliella sp. (chlorophyta, volvocales) from çamalti saltworks (izmir-turkey)

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Abstract

Dunaliella (Cyanophyceae) microalgea is a species used for feeding live baits that are used in larval fish production. Dunaliella species are intensively cultivated in algal biotechnology. Because of the nutritional value and chemicals this microalgea contains, it is commonly used in industries such as pharmacy, cosmetics and bait industry. From this point of view, it can be said that this algea species has high economic value. It can be found in areas between ‰ 10 and ‰ 200 salinity content rate. In Turkey this species can be found in salinas near coasts and salty-soft drink lakes. In this research, Dunaliella sp. species which is in Turkey's biggestmarine based saltworks "İzmir Çamaltı Saltworks" ecosystem isolated and cultivation in controlled circumstances determined. As a part of this research, physicochemical parameters such as optimum light, saltiness, density, biomass and pigment determined.

Keywords : Dunaliella sp., saltworks, microalgea, Çamaltı, Izmir, Turkey.

1.INTRODUCTION

Microalgae produce biomass and specific biomass in gredients from solar irradiation at high degrees so that it is possible to produce economically feasible materials by microalgae.

The genus Dunaliella are wall-less eukoryatic algae and found in saline environments. They are flagellate and consist of 23 species. They exhibit ideal growth at various salt concentrations. In those conditions, their colours become orange-red (Massyuk, 1973). Dunaliella belongs to the phylum Chlorophyta and family Polyblepharidaceae. It is halotolerant and green (Avron and Ben-Amotz 1992; Garcia et al., 2007). It can live in aquatic condition between 0,5-5 M NaCI salinities (Shariati & Hadi, 2000, Phadwal & Singh, 2003; Jahnke & White, 2003). Dunaliella species produce some chemicals such as caretonids (Hosseini Tafreshi & Shariati, 2006; Hadi et al., 2008), glycerol (Hadi et al., 2008), vitamins and proteins (Ghoshal et al., 2002) tough conditions (Hosseini Tafreshi & Shariati, 2006; Hadi et al., 2008). The reason how it can adapt in various salt concentrations is that it can change the intracellular concentration of glycerol (Raja et al., 2007). People use glycerol in automotive, leather, pharmaceutical, paint, cosmetic, food, pulp and paper, textile industries and in the manufacture of microbial fermentation or it can be synthesized from petrochemical raw materials. It can also be produced from soap manufacture of fats the amount of glycerol produced in the world is 600,000 t/year (Wang et al., 2001; Taherzadeh et al., 2002).

Dunaliella species are consireded to be the most known microalgae in the autotrophic production of glycerol (Borowitzka and Borowitzka, 1992). Dunaliella sp. are known as the most halotolerant eukoryatic livings and they can adapt even to low salt saturated conditions

such as 0.2%.On the other hand , it is the only eukoryatic photosynthetic organism found in extremely concentrated saline lakes (Ben-Amotz and Avron, 1990).

Dunaliella salina, D. viridisare mostly found microalgae species in salty conditions (Davis, 1990). D. salina accumulates high amounts of β-caroten when there is a lack of nitrogen sources or in high salinities and in high levels of irridance. β-caroten is a pigment and it is added to health food products and is used as a food coloring agent (anti-cancer and antioxidant agent) (Ben-Amotz and Avron, 1990).

2.MATERIALS AND METHODS

Dunaliella spp. were isolated from the Çamaltı solar saltworks and cultivated (Izmir, Turkey). The Çamaltı Saltwork is the largest one in Turkey. It is in Izmir City which experiences marine conditions. Its coordinates are 38°28'N and 26°50'E near the Izmir Bay. The reservoir initial in the saltworks is found approximately in 2-3 inches depth of water. The density of water is about 3 oBe – 5 oBe in November-May. Then, The water is pumped from the sea and the salinity increases by 6-8 oBe. After that the density goes on increasing up to 22–24 oBe. During this process micro algae appear and exhibit different colors. At higher concentrations micro algae collapse. The temperature varied between 6-7°C (December), 4-5°C (February), 20-22 °C (April) to 28-30 °C (June), 38-40 °C (August) throughout the year.

We used single-cell isolation by micropipette.Dunaliella sp. were incubated and stored without any process during two month. Then 1L flaks were incubated and reproduced. And then different salinities determined growth parameters. Dunaliella sp. strain was cultivated at four NaCI concentration (‰40,‰100) in 1L flaks. Laboratory's temperature was 24±1 °C, and lights were 1200 lux. Experiments were observed for 20 days. We used f/2 medium for experiments.

Tablo 1. f/2 Medium (Guillard and Ryther 1962)

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
NaNO3	75 g/L dH2O	1 mL	8.82 x 10-4 M
NaH2PO4 H2O	5 g/L dH2O	1 mL	3.62 x 10-5 M
Na2SiO3 9H2O	30 g/L dH2O	1 mL	1.06 x 10-4 M
trace metal solution		1 mL	…..
vitamin solution		0,5 mL	…..

f/2 Trace Metal Solution

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium

FeCl3 6H2O	....	3.15 g	1.17 x 10-5 M
Na2EDTA 2H2O	….	4.36 g	1.17 x 10-5 M
CuSO4 5H2O	9.8 g/L dH2O	1 mL	3.93 x 10-8 M
Na2MoO4 2H2O	6.3 g/L dH2O	1 mL	2.60 x 10-8 M
ZnSO4 7H2O	22.0 g/L dH2O	1 mL	7.65 x 10-8 M
CoCl2 6H2O	10.0 g/L dH2O	1 mL	4.20 x 10-8 M
MnCl2 4H2O	180.0 g/L dH2O	1 mL	9.10 x 10-7 M

f/2 Vitamin Solution

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
thiamine HCl (vit.B1)	….	200 mg	2.96 x 10-7 M
biotin (vit. H)	0.1 g/L dH2O	10 mL	2.05 x 10-9 M
cyanocobalamin (vit.B12)	1.0 g/L dH2O	1 mL	3.69 x 10-10 M

For the extraction of chlorophlly-a, 5 ml of cultures incubated was taken daily from each flask. Absorbance measurements were made by using a spectrophotometer. Algal growth was monitored by counting number of cells in a counting chamber (Thoma Counting chamber).

3.RESULTS

Growth of Dunaliella sp. Çamaltı strain at different salinities is shown in Fig. 1. Maximum cell density for Dunaliella sp. was obtained in 100 ‰ salinity and the lowest concentration was found in 40 ‰ salinity.

Salinity clearly affected the cell density in Dunaliella sp. The optimum salinity for growth of Dunaliella sp. strain was around 100 ‰ salinity.

A high salinity (100‰) was establisment chlorophlly-a (621,3 pg cell/1), a low salinity (40‰) (347,1pg cell/1). (Fig.2)

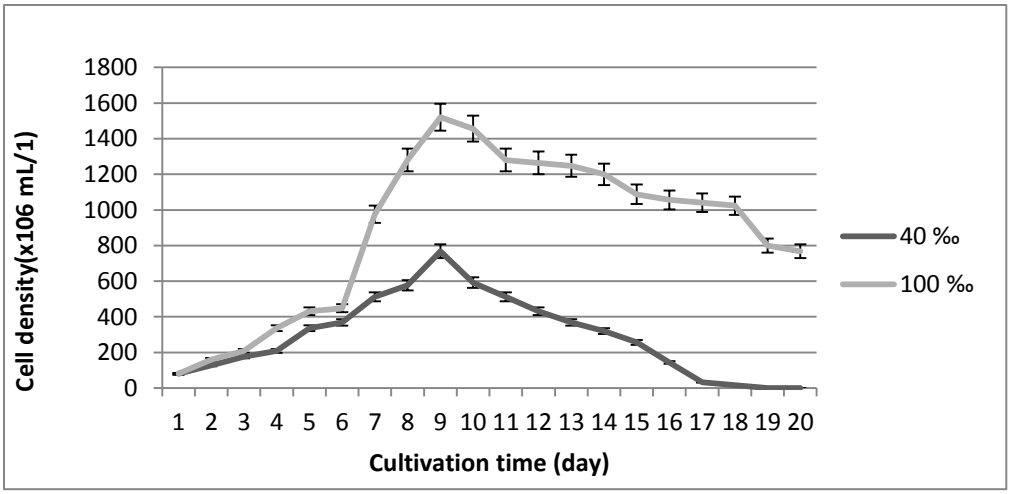

Fig. 1: Increase in cell density under the conditions of different salinities and 25 °C temperatures

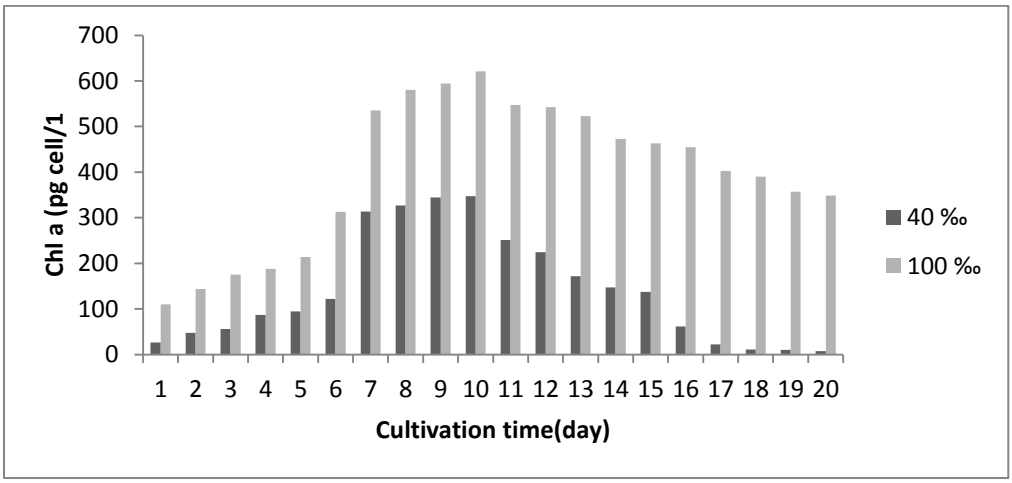

Fig. 2: Maximum chlorophyll-a concentration per cell in Dunaliella sp. grown at different salinities

Optical density was directly proportional the density of the cell. It is shown that a high optical density observed at high salinity. Increasing salinity caused optic density to increase (Fig. 3)

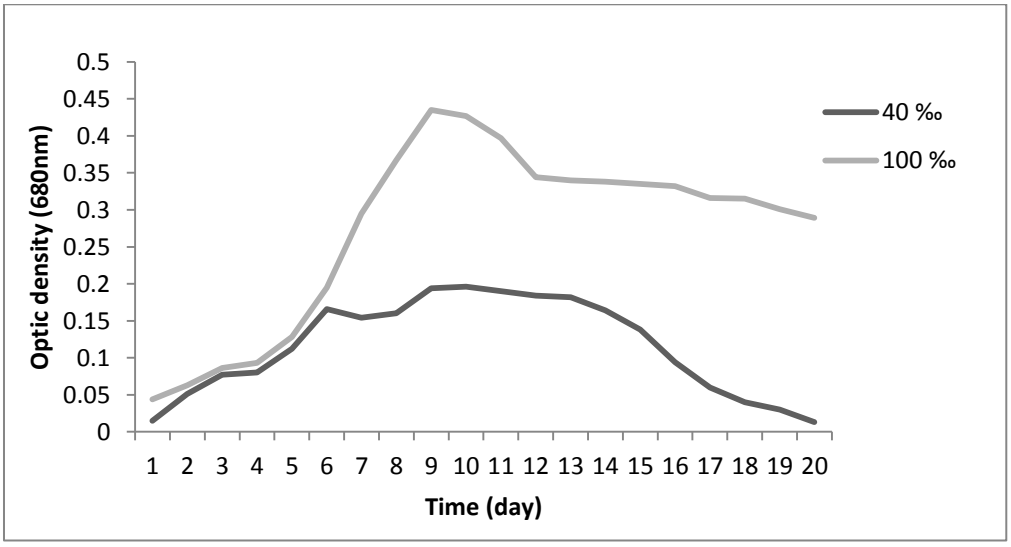

Fig. 3: The optical density of different salinities

At 40 ppt, culture had reached the logarithmic and stationary growth phase on day 3rd and 6th, respectively. On day 20, culture collapsed at 40 ppt. At 100 ppt, culture had reached the logarithmic and stationary growth phase on day 5th and 9th. The highest physiological development in Duneliella sp. was obtained at 100 ppt.

4.DISCUSSION

The Dunaliella species isolated from the solar saltworks on the Çamaltı Izmir differed in their capacity for growth and physiological acclimation to varying culture conditions. In the present study, the effect of salinity intensity, cell intensity, optic density, chlorophlly-a, on growth of Dunaliella sp. Çamaltı strain was determined. It has been observed to grow optimum at salinity around 100 ‰.

Gibor (1956), Jimenez and Niell (1990) reported that the optimum temperature for the growth of Dunaliella viridis was around 30°C. Ak (2008) reported that the highest growth of D. viridis of Çamaltı saltworks was found 25°C. Our study shown that the temperature was 25°C the high salinity the best growing.

InthestudyheldbyDurmazet al in theyear of 2006, theyisolatedDunaliellasalinathecellsfrom Konya Salt Lake bythemethod of dilution. Theymonitoredtheirgrowth in differentsalinities(0.62M, 0.85, 1.25 ve 1.71M). Inthisstudy, themostconvenient salt concentrationwasobservedto be 1.71M NaCI. In1.71M NaCI, morecellnumberandhigher B-carotenevalueswerefoundout.

Inthestudyheldby Dudu et al in 2001 on differentNaCI(10%, 15 %and20%)concentrations, theyannouncedthattheyattainedthemostgrowthin NaCI10 % concentration.

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