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The Effect of Different Salinities on Density of *Spirulina plaetensis* under Laboratory Conditions

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ABSTRACT: This research conducted with five treatments of salinity of 15, 20, 25, 30 and 35 ppt on culture of *S. plaetensis*. Comparing the average of density of the species under treatments revealed that the least density is at salinity of 35 ppt. A significance difference was at the level of 5% comparing to other treatments. The most density was obtained in treatment of 15 ppt. The most cellular density was for 15, 20 and 25 ppt and salinities of 30 and 35 ppt rated 4th and fifth respectively. Relying on above statements, one may come up with this conclusion that cellular density of *S. plaetensis* is less in lower salinities than that of higher salinities. Upon raise of degree of salinity, growth of the species shall decrease. In other words, in its natural habitation, this species tolerate higher salinity. However, the nature of the species is more compatible with lower salinity.

Key Words: *Spirulina plaetensis*, Cellular Density, Salinity, Nature of Species

INTRODUCTION

Microalgae are the species that are capable of producing valuable metabolites such as pigments, proteins and vitamins for edible additives, medical products and other goals. From among these microalgae, one may name *Spirulina plaetensis* which is one of the most prominent species of alga that has been studied in biotechnology. Thanks to easiness of growth of species, its drying process, and due to its high protein ingredient up to 70%, specific pigment especially the blue one named Phicociyanin, fatty acids such as Linolenic acid, B_{12} vitamin and minerals, the species has received great attention all over the world [3]. Further to success of Spirulina in aquatic farming and poultry feeding, pH and temperature are among the key elements involved in farming the said kind of alga in large scale. The optimized temperature for farming Spirulina is 35-38 degrees centigrade. Further to the required pH for *S.plaetensis* is given as 9.1-9.5 that effectively prevents contamination of most algae while farming. Thus, high quantities of Sodium Bicarbonate must always exist in the medium[3]. The name of Spirulina has been taken from its spiral form. Further to its high nutrients, Spirulina is every effective in response to safety system and protection against radiation [4].

As a foodstuff that provides health, Spirulina plays a prominent role in prevention and improvement of symptoms of diseases. Hormones, antibiotics and other chemicals that have positive effect also enjoy destructive environmental effects, high price and development of resistant bacteria. Thus, tendency toward safety stimulants have promoted. Use of Spirulina instead of antibiotics causes decrease of water contamination, decrease of medical costs and promotion of efficiency of farming systems [8]. Spirulina is introduced as a diet and its effect on growth, survival, safety and different tissues of animals have been studied [2]. Various studies have been conducted in the field of using Spirulina as feed for animals, human and aquatics [1]. For example, using *Cyprinus carpio* as diet, different species of Spirulina has been replaced fish powder as a source of protein.

MATERIALS AND METHODS

The required means consist of 15 bottles of 2 liters per each, colorized water (that has been blown) with salinity of 40 ppt, two florescent lamps, blowing pump, Spirulina Stock, and Conway medium. For producing the required salinities, it is required to mix the drinkable water with salt water with a salinity of 40 ppt, the water of the required salinities (15, 20, 25, 30 and 35 ppt) has been obtained.

Five treatments with three treatments have been considered. Blowing has been done by using an air pump. Light with an intensity of 5000 Lux and temperature of about 28 degrees centigrade has been constant. Spirulina has been supplied in stock form through Alga Culture Laboratory of Bushehr in southern parts of Iran. Conway medium had been prepared before and then, it entered the farming environment after its temperature has equalized to that of the environment for about 1.5 milliliter and then, after being homogenized, Spirulina stock of about 15 milliliters was added to the said environment. One cc of water of the farming environment was put on microscope every day and cellular density of Spirulina has been numerated and increasing or decreasing process of its growth has been studied. The specific slide consists of 1000 equalized cells. Four cells have been selected at random. Number of the cells placed on these four parts of the slide has been numerated and corresponding average has been calculated accordingly. String Spirulina alga consists of some small and large spirals. Each spiral consists of nine cells. Multiplying number of spirals per nine cells, number of cells of the alga is obtained. The following formula was used to obtain number of cellular density in one milliliter of water:

Spirulina in one milliliter of solution = X*9*20*103/4 cellular density

X = number of spirals of Spirulina spiral alga

9 = number of cells of each spiral

20 = Dilution percentage

10^3 = Number of cells of the numerate slide

4 = Number of numerated cells of the slide

RESULTS

The results of numerating cellular density of *S. plaetensis* in salinities of 15, 20, 25 and 35 during a period of 14 days and average of cellular density numerated, are given in Tables 1 and 2 as well as in Fig. 1.

Corresponding results of data analysis for density of the species under different treatments of salinity during a period of 14 days of farming revealed that these treatments had no significant difference in the second day. However, on the 1st, 3rd, 4th, 5th, 7th, 9th, 10th, 11th, 12th and 13th day with a probability of 95% is significant. On the 6th, 8th, and 14th, with a probability of 99%, it is significant as well.

Similar small letters on the right side designates significant indifference (P>0.05) and dissimilar letters designates significant difference in columnar form (P<0.05).

Table 1: Analysis of cellular density of *S. plaetensis* affected by different treatments of salinity.

		Mean Square				
		Days of Culture				
Source of change	df	1	2	3	4	5
Treatment	4	11089.12*	21090 ns	586710*	364627.5*	306360*
Error	10	5835.6	117420	341820	262950	264720
Total	14					
		6	7	8	9	10
Treatment	4	3751080.9**	362275.68 *	1555034.23 **	528530.22*	695267.1*
Error	10	2460419.16	326757.12	729660	30980122	583492.3
Total	14					
		11	12	13	14	
Treatment	4	908405.4*	655635.6*	504447*	1747347.48**	
Error	10	801354	369416.8	106497.12	1354948.56	
Total	14					

No significant difference: ns *Significant difference at level of 5% **Significant difference at level of 1%

Table 2: Comparing the average of cellular density of *S. plaetensis* in different treatments of salinity.

	Cellular Density				
	Days of Culture				
Salinity (ppt)	1	2	3	4	5
15	58.5±5.3 ^{c}	760±12^{a}	1010±21 ^{a}	1100±21^{a}	1070±14^{a}
20	74±4^{c}	670±11^{a}	810±12^{b}	1025±21^{a}	1090±9^{a}
25	89±5.5^{c}	620±10^{a}	1340±13^{a}	950±13^{a}	820±11^{a}
30	106.5±6^{b}	590±9^{a}	360±21^{c}	955±14^{a}	950±12^{a}
35	212±7^{a}	780±11^{a}	290±13^{c}	240±11^{b}	310±13^{b}
	6	7	8	9	10
15	1030±23^{b}	680±20^{b}	1010±23 ^{b}	1150±21^{a}	1200±32^{a}
20	2880±32^{a}	890±12^{b}	540±11^{c}	490±11^{b}	590±21^{b}
25	990±15^{b}	1030±13^{a}	1950±36^{a}	475.33±6.31^{b}	960±12^{ab}
30	220±11^{c}	740±14^{b}	710±14^{c}	320.02±14^{b}	320.02±14^{c}
35	70.02±5^{d}	120.04±15^{c}	0.06±0.01^{d}	0.06±0.01^{c}	0.06±0.01^{d}
	11	12	13	14	
15	1340±24^{a}	980±21^{a}	1020±21^{a}	1870±25 ^{a}	
20	1040±25^{b}	960±31^{b}	960±31 ^{b}	980±23 ^{b}	
25	690±23^{b}	510±15 ^{b}	510±15^{b}	270.02±32^{c}	
30	250.04±15^{c}	130.04±14^{c}	120.04±24^{c}	240.04±31^{c}	
35	0.06±0.01 ^{d}	0.06±^{c}0.01	0.06±0.01 ^{d}	0.06±0.01^{d}	
Numbers by a factor of 1.5*10^{3} is calculated					

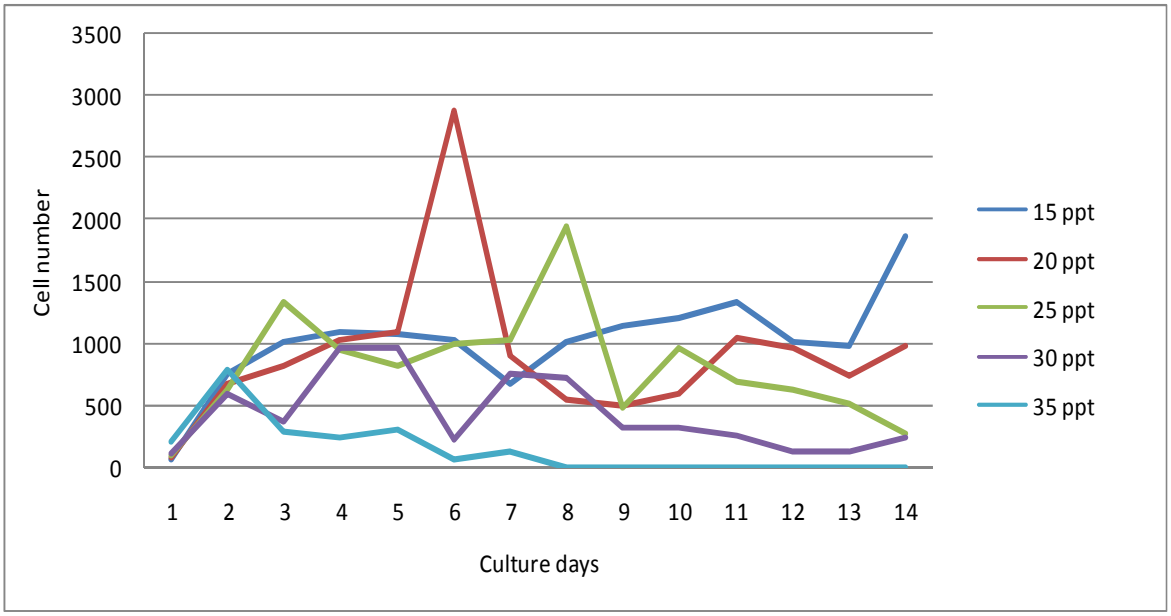

Fig. 1. Growth of Spirulina affected by different salinities during a fourteen-day period of culture.

Comparing the average of cellular density of Spirulina affected by different treatments during a fourteen day period of culture

Table of comparing density of the alga in the first day revealed that the least density of alga was for treatment 15 and 20 ppt. It showed a significant difference at the level of 5% with other treatments. The most density of alga was obtained in treatment 35 ppt. In the second day it showed a significant difference with other treatments at level of 5%. The most density of the alga was in treatment 35 ppt. In the second day, it has been revealed that there was no significant difference either. In other days except for the sixth day, the most density of alga was in treatment 15 ppt and revealed a significant difference with other treatments at levels of 5% and 1% (7^{th} and 14^{th} days) respectively and the least density was for density of alga in treatment 35 ppt and the third day of treatment 25 and the sixth day of treatment 20 ppt showed the most density of alga and a significant difference with other treatments was revealed at level of 1%. The least density of alga was obtained in treatment 35 ppt. Corresponding results to data analysis of cellular density affected by different treatments of salinity revealed that these treatments have a significant difference at level of 5%. Comparing the average of cellular density of *S. plaetensis* affected by different treatments of salinity showed that the least cellular density is in salinity of 35 ppt (144.46+/-21.34), revealing a significant difference with other treatments at level of 5%. The most cellular density was obtained at treatment of 15 ppt (1019.89+/-85.65). On the whole, it may be concluded that cellular density of the Spirulina at lower salinities is more than higher salinities.

Table 3: Analysis of variable of cellular density of *S. plaetensis* affected by different treatments of salinity during culture period.

Resources of changes	df	Mean of squares
Treatment	4	18901.12*
Error	10	5385.6
Total	14	
No significant difference: ns *Significant difference at level of 5% **Significant difference at level of 1%		

Table 4: Comparing cellular density of *S. plaetensis* after applying different treatments of salinity during culture period.

Salinity (ppt)	Cellular Density
15	1019.89 ± 36.05^{a}
20	912.07 ± 25.54^{ab}
25	808.88 ± 41.2^{b}
30	429.4 ± 25.7^{c}
35	144.94 ± 16.8^{d}
Numbers by a factor of $1.5*10^{3}$ is calculated	

DISCUSSION

Investigations carried out on growth process of species of *S. plaetensis* affected by salinity of 15 ppt revealed that cellular density of this species has had an increasing trend from the beginning through the end of process except for the 7^{th} and 12^{th} day during which it showed a slight decrease. However, growth diagram of the said species affected by salinity of 35 ppt revealed that cellular density had a slight increase in the second day and followed a decreasingly trend until the 8^{th} day when it reaches zero.

Thus, one may come up with this conclusion that upon increase of salinity, cellular density of the Spirulina has been decreased and at lower salinities, it shall have an increasing and more proper growth accordingly. Further to physical elements (light, temperature, blowing and the ones), *S. plaetensis* needs a medium for fulfillment of its vital activities. The medium or the nutrition consists of macro-element and micro-element [9] and in this research, for nutrition supply of *S. plaetensis*, Conway medium was used since it had nitrate, phosphate, potassium and ferrous as well as vitamin solutions in its formula.

The results obtained from the study of growth of *S.plaetensis* in four mediums of Conway, Gilard, N_8 and TMRL at salinity of 15 ppt, the most growth was obtained at Conway medium [7]. In this research, since the medium (Conway) was constant, the most growth was obtained at salinity of 15 ppt.

Temperature is one of the most important factors affecting the biochemical compound of the alga. Temperature above 35 degrees centigrade is fatal for some species. However, for others like *S.plaetensis*, the most proper temperature for farming is given as 35-38 degrees centigrade. This problem was mentioned in the research by Goksan [3] which studied the growth of *S.plaetensis* in different culture systems under greenhouse conditions. However, higher temperature than 35 degrees centigrade is really dangerous for Spirulina [5]. Concerning the fact that the said test was conducted in winter and low temperature was one of the main elements that limited growth in winter, temperature higher than 28 degrees centigrade was maintained and this caused proper growth of *S. plaetensis* at desirable salinity. The water used for culture of Spirulina must be clean, filtrated and free from any microbes and contamination and enjoys all necessary low-consumed elements and the required compounds. The drinkable water may be used as well. It enjoys enough quantity of calcium. However, if the quantity of calcium exceeds the said amount, it causes slugging and sedimentation of the same and this shall bring about some problems. The limit of light intensity for culture of microalgae is given as 1000-10000 Lux. Its optimum amount is given as 2500-5000 [6]. The light intensity used in this research is given as 5000 Lux. The light period during 24 hours a day is not recommended because during darkness period, synthesis of protein, respiration and other internal reactions are conducted [5]. Concerning the fact that in this test, only cellular density of Spirulina alga and its decreasing and increasing process of growth was considered, the said test was conducted during a light period of 24 hours of brightness. Culture and harvesting Spirulina alga under laboratory conditions revealed that on the whole from applied treatments of salinity of 1-20% (1, 4, 10, 15 and 20%), upon increase of salt concentration, growth has decreased. In this research which was carried out under similar conditions, growth has decreased at higher degrees of salinity [10]. The most growth was observed at salinity degree of 15 ppt. Based on the results, the most cellular density was at salinity of 15 ppt and then, the salinity of 20 ppt obtained the second place. The salinity of 25 ppt obtained the third place and the salinities of 30 ppt and 35 ppt obtained the 4^{th} and 5^{th} places respectively. Relying on above statements, it may be concluded that upon increase of degree of salinity, cellular growth of Spirulina is decreased.

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