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Production of *Spirulina platensis* in different media

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Abstract: *Spirulina platensis was cultured in various medium as; Standard Zarrouk's Media (SZM), Modified Zarrouk's Media (MZM) and Tap Water Media (TWM). Dehydrated weight and pH was observed for 26 days on every day. In Standard Zarrouk's Media pH was noticed from 9.2 to 10.6, in MZM 9.0 to 10.4 and in TWM 7.40 to 7.45. Gradually increase in dry weight (dw) was observed with length of time of culture, 1.84 dw/L & 1.74 dw/L was attained in SZM and MZM correspondingly. S. platensis inoculated in TWM was live but growth was not increased, achieving higher dehydrated mass of 0.28 dw/L on 17-21 day of cultivation. Tap water prepared with various quantities of basic salts didn't exposed important change on S. platensis growth. Growth was influenced by mutually temperature and light in the experimentation and the effect of temperature was better to light in the experimental time.*

Key Ward: *Spirulina platensis, cultured, SZM, MZM, TWM*

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I. Introduction

The few years ago has noticed substantial activity concerning to the production of blue green algae. A *Spirulina platensis* is a photosynthetic filamentous alga that forms enormous growth in humid and sub-humid water bodies which have high levels of basic salts. About 100 years ago, local peoples have produced *Spirulina platensis* in Africa and Mexico as food [1]. Few data showed that *Spirulina* justify unique attention as a source of Single Cell Protein (SCP) [2] and nutritional properties. *Spirulina* has profitable significance because of its high nutritional values: high protein content (about 65% dehydrated mass), small % of fat, high content vitamin (mainly B_{12}) with vital fatty acid γ-linoleic acid [3]. The pigment of *Spirulina* also makes these algae useful in aquaculture, particularly as feed for poultry [4]. Chlorella *vulgaris*, green algae (single-celled) have the main pigment-chlorophyll as compared to other accessible plant species. Chlorella (*Spirulina platensis)* is measured as an absolute food, because it plays an important part in neutralizing toxins and its more quantity of protein, minerals and vitamins together with carotenoids and enzymes (especially pepsin). It normally exists as tablet form, liquid and granule form [5]. In addition, mechanism of it has been exposed to reduce certain cancer threats, irritation and avoid platelet gathering [6]. *Spirulina platensis* forms bulky colonies in humid and sub-humid water surface having high concentration of basic salts [7, 8]. It requires most favorable pH (8 – 11) and temperature (30 - 35 °C) for its growth [9]. The price rate of nutrients is reflecting on a main factor that affects *Spirulina* bio-mass cultivation. Cost-effective large-scale cultivation of *Spirulina* is easy with conditions of industrial requirements for its high protein production [10]. ZM has been effectively used as standard medium for *S. platensis* culture [11]. Urea may be a capable substitute for low-price nitrogen content for *Spirulina* production. It was noticed that customized organic media and closed system used for enhanced biomass [12]. Accordingly, several customized media have been prepared, using salty water, drainage water and industrial wastewater [13, 14].

II. Material And Method

Spirulina platensis were obtained from Indian Agricultural Research Institute New-Delhi. Obtained strain was earlier maintained on Zarrouk's agar media slants at 4°C. Required culture of *Spirulina* was inoculated in flask having 10 ml germ-free Standard Zarrouk's Medium. All chemicals used were of analytical grade, obtained from the Sigma-Aldrich India. Na_2CO_3 was mixed after autoclaving and pH was maintained to 8.6-9.0. Development and maintenance of the culture society was completed in an illuminated (4500 lux) growth room at 32 ± 2 °C under 12-12 hour light-dark cycles. Physical shaking of cultures was done 5 times in a day.

S. platensis was inoculated in three various media viz; SZM, MZM and TWM (Table no 1). Tap-water was taken from Mewar University, Rajasthan, India. 50ml capacity of 24 flask holding 25 ml of each medium was inoculated with same amount of inoculums. All flask were kept at room temperature under shadowy

circumstance, every day during day time flux and temperature were noted. Physical shaking of cultures was done 5 times a day. Tap-water collected from Mewar University, Rajasthan, India, was supplemented with sodium bicarbonate and sodium nitrate salt. Cells were accumulated by filtration with the help of filter paper (8 mm hole size). Cells were rinsed with neutral buffer solution and then practiced for additional inoculation. After dilution inoculums shaked for preparing homogenized mixture. Culture of each flask earlier than filtration was examined for pH and microscopic. Intended for examination by microscopic, 75µl sample was drained after appropriate trembling by micro pipettes and observed by microscope at 40 X. After completing filtration process and rinsing filter paper was dehydrated in oven about 100°C for 9-10 hr. Kept it in desiccators and cool at 25°C temperature and then measure this weight by weigh balance. Cell mass was dehydrated in at 100°C for 9-10 hrs, placed in desiccators & cool at 25°C temperature and weight the mass. All trials were achieved in triplicate and outcome was articulated as mean value of meticulous factor.

Table no 1: Compositions of Standard Zarrouk's media (SZM), Modified Zarrouk's media (MZM) and Tapwater media (TWM)

Sr. no	**Compositions**	**SZM**	**MZM**	**TWM**
		Amount (g/l)		
1	Natural Tapwater	0	0	1000 ml
2	Distilled water	1000 ml	1000 ml	0
3	NaCl	1.0	1.0	0
4	CaCl2.2H2O	0.04	0.04	0
5	KNO_3	-	2.5	0
6	$NaNO_3$	2.5	-	0
7	$FeSO_4 \cdot 7H_2O$	0.01	0.01	0
8	EDTA (Na)	0.08	0.08	0
9	K_2SO_4	1.0	1.0	0
10	MgSO4・7H2O	0.2	0.2	0
11	$NaHCO_3$	16.8	16.8	0
12	K_2HPO_4	0.5	0.5	0
13	A_5 micronutrient (H_3BO_3, MnCl2.4H2O, ZnSO4.4H_2O Na2MoO4,CuSO4.5H2O)	1.0	-	0

III. Results and Discussion

In culture vessels, *S. platensis* consist its control in giving whole information associated to growth and enlargement of significant chemicals as vitamins, protein, amino acids, polysaccharides and fatty-acids in quantitatively and qualitatively [15,16]. Main work related to promotion of *S. platensis* living at salt lakes in tropical regions has done [17-19]. Physico-chemical characters of *S. platensis* explain relation between growth and some ecological factors particularly irradiance flux, temperature and density [20], which are vital in the progression of algae and cyanobacteria for biomass cultivation, with their common properties. More basicity is compulsory for the promotion of *S. platensis* and bicarbonate is applied to uphold high pH [21-23]. Sources of nutrition are influence the escalation rate of cyanobacteria [24]. The growth of *S. platensis* is maximum at 30-35^oC.

S. platensis was effectively cultured in TWM type from solid media slant. *S. platensis* was cultured in SZM, MZM and TWM for twenty six days. *S. platensis* grows well in both SZM and MZM culture. In SZM and MZM, pH of medium became more basic as culture became older. pH was recorded from 9.0 to 10.5 in SZM, 9.2 to 10.4 in MZM and 7.46 to 7.54 in TWM. Outlook of fresh culture was changed from light green to dark in ratio to grow cell mass, on the basis of daily observation (Table no 3).

Culture of *Spirulina* in flask has its control in providing total information linked to growth, expansion and production of value-added chemicals [16], however it would give initial information for more display or profitable cultivation. Performance of *Spirulina* in TW was found totally different as compared to both media. Tap water as a medium did not show any major enrichment in growth of *Spirulina*, maximum 0.32 g/l dry mass was found. One different observation was noticed, when *Spirulina* cultured in TW-behavior of pH value. The pH value did not much changed from initial 7.40 to 7.45 on 26th day of experiment. Similar to pH, dry weight of biomass was not improved considerably.

Environmental factors particularity irradiance flux and temperature are important evolution of biomass production and their general characterization. *S. platensis* growth is maximum at 30-35°C while high alkalinity is mandatory for growth of *Spirulina* [21]. In present investigation temperature and irradiance lux was found as mentioned in table no 4. Growth was influenced by mutually temperature and light in the experimentation and the effect of temperature was better to light in the experimental time. Growth behavior and dry yield of *S. platensis* cultured in SZM, MZM and TWM clearly indicate that environmental factor as mentioned in table no 3 were supporting growth. Results of *Spirulina* cultivation in similar condition in TWM indicated that tap water

composition is not supportive to growth, but *S. platensis* survived in TW medium indicated that gradually exposure to TWM and further enrichment of TWM would favour the growth of *Spirulina*.

In this experimental data, TWM supplemented with $NaHCO_3$ and $NaNO_3$ were searched only in addition to arrangement at different conc. for *S. platensis* development. *S. platensis* cultivated in modified TWM were noticed for dry mass and microscopy for 26 days. Total three results (o day, 13th day and 26th day) for every combination were considered. It is explained in Table 2, that $NaNO_3$ and $NaHCO_3$ has several manipulates in *Spirulina* agriculture in TWM. Tap water modified with $NaNO_3$ was more suitable comparison to $NaHCO_3$. $NaNO_3$ alone showed good results in *Spirulina* enlargement whereas in grouping with $NaHCO_3$ no important enhancement in *Spirulina* enlargement was noticed. Maximum 0.12 g/l dry weight on 26th day (Table no 2) was accomplished in TWM modified with $NaNO_3$ (0.23 g/l) which was relatively high when evaluated with only TWM as medium (0.12 g/l). Dry mass of *Spirulina* in TWM was not outstanding enough to size it for commercialization but it would believe as research study. During cultivation pH of TWM was not considerably change will indicate very reduced or exploitive growth in particular salt concentration.

Table no 2: Results of pH and dry weight of *Spirulina* platensis cultivation in Tap water prepared with sodium bicarbonate and sodium nitrate

Medium Ingredient	**Amount (g/l)**	**0 Day**		**13th Day**		**26th Day**	
		dw/l	**pH**	**dw/l**	**pH**	**dw/l**	**pH**
Tap water	0	0.07	7.40	0.22	7.46	0.12	7.45
Sodium bicarbonate	1	0.07	8.24	0.12	8.52	0.05	8.60
	2	0.07	8.14	0.16	8.64	0.08	8.76
	4	0.07	8.12	0.20	8.92	0.16	8.98
	8	0.07	7.92	0.22	9.30	0.14	9.39
Sodium nitrate SZM	0.7	0.07	8.49	0.10	8.48	0.08	8.42
	1.4	0.07	8.48	0.16	8.56	0.05	8.39
	2.1	0.07	8.47	0.20	8.25	0.23	8.40
	2.8	0.07	8.45	0.15	8.46	0.08	8.61
Sodium bicarbonate + Sodium nitrate	1 + 0.7	0.07	8.22	0.13	8.52	0.07	8.60
	2 + 1.4	0.07	8.04	0.22	8.84	0.07	8.88
	4 + 2.1	0.07	7.92	0.24	9.22	0.21	9.15
	8 + 2.8	0.07	7.82	0.32	9.42	0.23	9.30

Table no 3: Daily inspection of *S. platensis* cultivation in Synthetic Zarrouk's medium (SZM), Modified Zarrouk's medium (MZM) and Tap water medium (TWM)

Days	**SZM**		**MZM**		**TWM**	
	dw/L	Colour	dw/L	Colour	dw/L	Colour
1	0.04	Culture light green, no contamination	0.04	Culture light green, no contamination	0.04	Culture light green, no contamination
2	0.07		0.06		0.03	
3	0.14		0.12		0.05	Culture light green, some clump were seen on surface,no contamination
4	0.22		0.20		0.05	
5	0.26	Cells free flowing, Culture green, no contamination,	0.28	Culture green, no contamination	0.7	Culture light green, some clump were seen on surface,no contamination
6	0.32		0.44		0.8	
7	0.43		0.58	Culture dark green, thick and clumpy, no contamination	0.10	Culture light green, clumps were formed fade away, no contamination
8	0.52		0.71		0.13	
9	0.54		0.74		0.14	
10	0.61	Culture dark green, thick and few clumps noticed, no contamination	0.84	Culture dark green, thick and few white clumps were noticed, no contamination	0.16	Culture green, clumps become dark green, no contamination
11	0.71		0.96		0.17	
12	0.75		1.04		0.20	
13	0.80		1.12		0.24	
14	0.84		1.13		0.25	
15	0.85		1.14		0.25	
16	0.86	Culture dark green, thick and few clumps were noticed, thin film of cells on flask wall, no contamination	1.15	Culture dark green, thick and clumpy, few clumps were noticed on flask wall, no contamination	0.26	Culture dark green and clumpy, few clumps were noticed at flask bottom, contamination observed(micro algae and
17	0.92		1.16		0.26	
18	0.91		1.19		0.27	
20	0.92		1.20		0.27	
21	0.96		1.31		0.28	

						protozoa)
22	1.21	Culture dark green, thick and few clumps were noticed, thin film of cells on flask wall and part towards surface become light yellowish green, no contamination	1.47	Culture dark green, thick and clumpy, attachment of clumps to flask wall, some clumps were changed to yellowish green, no contamination	0.24	Culture becomes more yellowish with similar contamination
23	1.46		1.52		0.21	
24	1.62		1.58		0.20	
25	1.73		1.60		0.19	Culture becomes more yellowish, contamination percentage relatively high
26	1.84		1.74		0.18	

Table no 4: Temperature and Irradiance lux behavior during experimentation of cultivation of *S. platensis* in different mediums

S.No.	Day times (hr)	K Lux	Temp.℃
1	8	1.3	27.2
2	9	2.3	29.0
3	10	4.4	30.3
4	11	5.1	32.4
5	12	5.2	32.9
6	1	5.1	33.4
7	2	5.3	34.0
8	3	5.1	34.6
9	4	4.5	33.4
10	5	4.4	32.2
11	6	4.0	30.2

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V. Conclusion

This paper has confirmed that temperature, irradiance lux, pH and different medium have a significant manipulate on the production of biomass by *S.* platensis. On the basis of utility, *S. platensis* can be cultured under variable natural, artificial and laboratory conditions. Growth of *S.* platensis was maximum in SZM and minimum in TWM.

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