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ORIGINAL ARTICLE **OPEN ACCESS**

The Laboratory Scale evaluation of multiple pH ranges on *Spirulina platensis* culture in the production of dry biomass, chlorophyll, Phycocyanin, & Carotenoids

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ABSTRACT
Spirulina is a complete food resource of Chlorophyll, Phycocyanin, & Carotenoids. It is also has an application as a natural dye in food industry, cosmetic and pharmaceutical industry. In this study, the effects of a wide range of pH were evaluated on the growth of Spirulina platensis on various media such as, ZM, SWMX2, NRCM, CFTRI, & Wastewater. In addition, the pH modification was also done to evaluate the rates of dry weight, phycocyanin, & chlorophyll production. The Spirulina platensis cultivation was done on ZM, SWMX2, NRCM, CFTRI, & Wastewater media and the extraction of chlorophyll, phycocyanin, and carotenoid were done by ultrasonication, homogenation, & acetone methods, then all products were analyzed by HPLC.In this study, the pH ranges of 8-10.8 were used in the Spirulina platensis cultivation. The dry weight, chlorophyll, and phycocyanin concentration was in ZM medium with pH=9.6, 9, and 9.6 was 2.28 , 12.83 mg/g, & 44.27 mg/g, respectively. The highest concentration of carotenoid was in CFTRI medium with pH= 10.2, as 3.13 mg/g. All the media used in this study had an acceptable growth and production. Although, the pH range of 9-10.2 had the highest efficacy of production and growth on Spirulina platensis.
Keywords: *Spirulina platensis, Dry Weight, Chlorophyll, Phycocyanin, Carotenoid.*

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INTRODUCTION

Since centuries cyanobacteria have been receiving increasing interest due to their potential to produce a diverse range of chemicals and biologically active compounds, such as vitamins, carotenoid pigments, proteins, lipids and polysaccharides [1]. For exploration of these potentials of cyanobacteria it should be cultivated in commercial way. Globally researchers are trying to produce microalgae/cyanobacteria commercially [2, 3]. Yet very little or primary information is available on detailed design criteria, location selection, scaling considerations, or constrains involved in large scale cultivation. Spirulina is a planktonic photosynthetic filamentous cyanobacterium that forms massive populations in tropical and subtropical bodies of water which have high levels of carbonate and bicarbonate and alkaline pH values of up to 11. Spirulina from Chad Lake in Africa and Texcoco Lake in Mexico have been harvested as a source of food [4]. Spirulina has been studied for single cell protein (SPC) [5], vitamins, minerals, proteins and polyunsaturated fatty acids (gamma-linolenic acid) [6], therapeutic properties [2, 3], antioxidant activity [7]. Several cultivation methods like; open ponds [8], tubular photobioreactors (9), inclined glass panels (10) have been tried. Cost and composition of cultivation media along rate of the algae us challenging factors for commercially viable production. Different media have been tried for cultivation of spirulina such as Zarrouk's media [11], SWMX2 media (12), NRCM media (13) CFTIR media, Wastewater media (14). Recently, it has been focused on valuable products such as chlorophyll, phycocyanin, and

carotenoids. This study has been done to evaluate the effects of various pH ranges of different selected media on the production rate of dry biomass weight, chlorophyll, phycocyanin, and carotenoids.

MATERIAL AND METHODS

Isolation & Mass Cultivation of Spirulina

The water sample was inoculated into the Zarrouk medium for isolation of Spirulina and incubated in a growth chamber with light flux of 12:12 h, light intensity of 4Klux, in 30C and pH=9. The purity of the culture was ensured by repeated inoculation, and identification was accomplished by determining cellular morphology observed by using light microscope and

Culture medium:

Five media were included in this study. For this experiment Zarrouk's media (ZM), SWMX2 medium, NRCM, CFTRI medium, Wastewater Medium, were chosen (the composition of all media are described in Table 1, respectively). Prepared 1000ml each medium in 1000ml flask and inoculate 50ml of inoculums in each medium. After 28 days the biomass of *Spirulina platensis* was harvested and the dry weight was determined.

Extraction of chlorophyll:

1g of wet spirulina biomass was taken in a beaker and about 10ml of aqueous acetone solution (Sigma,USA) was added, closed tightly and placed in a dark box overnight. Then sonificated by ultrasonicator for 20 second at the 5 setting. The extract was then brought to a volume of 13 ml with aqueous acetone solution, which was closed tightly and kept again in the dark box overnight in a cold room. The next day the extract was transferred to a centrifuge tube and mixed well. Then the extract was clarified by centrifugation for 20 minutes at 1000 rpm. The pellet was discarded and supernatant was separated which contained the chlorophyll pigments (15).

Phycocyanin extraction:

The C-phycocyanin was extracted from fresh biomass by the following procedure. Fresh biomass was homogenized with 50mM sodium phosphate buffer, the homogenate was subjected to alternate freezing and thawing (3 to 4 cycles) and centrifuged at 5000rpm for 10 minutes. The phycocyanin content was estimated.

Carotenoid extraction:

Total carotenoids were extracted by cell lysis (sonication) and homogenization in 90 percent acetone as described by (11). The final extract was measured at A450 nm against acetone as blank. As for β-carotene, the cells were homogenised using acetone and petroleum ether and the extracts were pooled and measured at A453 nm against petroleum ether as blank (16).

HPLC analysis:

The chemical constituents of the *S. platensis* extracts were identified by HPLC method reported by (17). Dionex Summit IV HPLC system consisted of a Dionex P680 dual gradient pump, an ASI-100 auto-sampler equipped with a 20- mL loop and PDA- 100 photodiode array detector were used. A reversed phase column C18 (250 x 4.6 mm, 5 mm partials) was used. The mobile phase was a mixture of solvent A (methanol/ammonium acetate 0.1 N; 7:3, v/v) and solvent B (pure methanol) at rate of 0.9 ml min–1 as a step gradient, lasting 35 min, which started from 25% B, changing at 50% in 1 min, rising up to 100% B at 10 min. Then, the mobile phase composition was kept constant until the end of the analysis. Total acquisition time was 35 min. The temperature was set at 25ºC. The identification of the peaks was performed, when possible, using standards. When no standards were available, tentative identification was done based on UV-Vis spectra characteristics and compared with that data appearing in the literature.

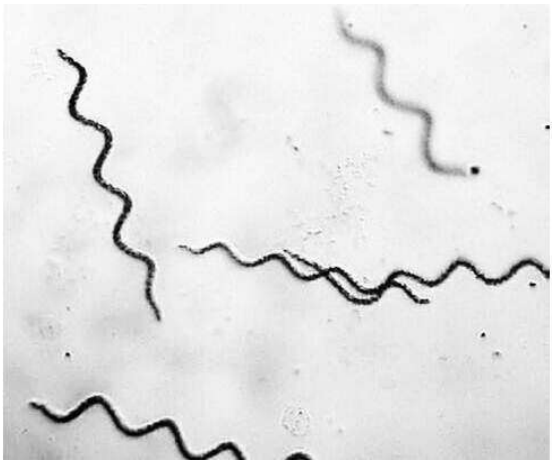
Statistical analyses:

The data recorded in triplicate for the parameters in various strains were subjected to ANOVA (analysis of variance).

Results

The Results of Light Microscopy:

The microscopy result of *Spirulina platensis* cultivation in the media used in this study, which was been seen as a spiral-shaped cyanobacterium in the microscopy field.



The effects of pH on obtaining the highest dry weight could be seen on tables 2,3& 4. The cultivation done on ZM, SWMX2, NRCM, CFTRI, and Wastewater at the pH ranges 9.6, 8.6, 8.8, 9, 9.8 had the highest concentration of 2.28±0.13, 1.16±0.52, 0.95±0.94, 1.75±0.04 g/l, respectively.

Table.1 indicates the contents of all media used in this study. The data are mentioned as g/l.

	ZM	SWMX2	NRCM	CFTRI	Wastewater
NA HCO_3	16/8	0/2	8	40	8
K_2HPO_4	0/5	0/016	0/5	0/5	1
$NaNO_3$	2/5	2	2/5	1/5	1/5
K_2SO_4	1	0/5	1		
NaCL	1	5	1		
$MgSO_4.7H_2O$	0/2	0/16	0/2		
$CaCL.2H_2O$	0/04	0/04			
$FeSO_4.7H_2O$	0/01	0/01	0/01		
EDTA	0/08	0/01			

Tables.2 to.4 indicate The effects of pH on obtaining the highest dry weight could be seen on tables 2,3 & 4. The cultivation done on ZM, SWMX2, NRCM, CFTRI, and Wastewater at the pH ranges 9.6, 8.6, 8.8, 9, 9.8 had the highest concentration of 2.28±0.13, 1.16±0.52, 0.95±0.94, 1.75±0.04 g/l, respectively.

Table 2: Effect of PH on biomass (dry wt) of *S. platensis* in ZM,SWMX2,NRCM,CFTRI,Wastewater media.

Medium	pH				
	8	8.2	8.4	8.6	8.8
ZM	0.86±0.36	0.91±0.62	0.99±0.07	1.2±0.79	1.24±0.02
SWMX2	0.97±0.32	0.99±0.66	0.1±0.96	1.16±0.52	1.05±0.25
NRCM	0.53±0.18	0.61±0.09	0.66±0.78	0.88±0.49	0.95±0.94
CFTRI	0.72±0.38	0.77±0.57	0.87±0.67	1.01±0.97	1.16±0.56
Wastewater	0.61±0.58	0.55±0.62	0.79±0.53	0.82±0.64	0.92±0.67

Mean ± SD in each column with different superscripts indicating significance (P0.05)

Table 3: Effect of PH on biomass (dry wt) of *S.platensis* in ZM, SWMX2, NRCM, CFTRI, Wastewater media.

Medium	PH				
	9	9.2	9.4	9.6	9.8
ZM	1.84 ±0.46	1.92±0.53	2.13±0.49	2.28±0.13	1.97±0.063
SWMX2	0.84±0.15	0.87±0.48	0.91±0.25	0.95±0.61	0.72±0.27
NRCM	1.42±0.057	1.53±0.98	1.65±0.19	1.91±0.041	1.54±0.18
CFTRI	1.75±0.04	1.46±0.57	1.44±0.12	1.26±0.58	1.17±0.01
Wastewater	0.26±0.16	0.28±0.69	0.49±0.76	0.68±0.38	0.94±0.47

Mean ± SD in each column with different superscripts indicating significance (P0.

Table 4: Effect of PH on biomass (dry wt) of *S. platensis* in ZM,SWMX2,NRCM,CFTRI,Wastewater medium.

Medium	PH				
	10	10.2	10.4	10.6	10.8
ZM	1.26±0.12	1.42±0.07	1.11±0.95	0.96±0.66	0.89±0.57
SWMX2	0.73±0.36	0.66±0.38	0.77±0.97	0.64±0.28	0.41±0.32
NRCM	0.55±0.69	0.49±0.67	0.48±0.55	0.33±0.01	0.28±0.96
CFTRI	0.81±0.36	0.79±0.36	0.92±0.65	0.96±0.91	0.84±0.39
Wastewater	0.56±0.25	0.72±0.36	0.76±0.49	0.37±0.95	0.16±0.09

Mean ± SD in each column with different superscripts indicating significance (P0.05)

As it could be seen, in order to obtain the concentrations of chlorophyll, phycocyanin, and carotenoid, the pH is essential in various media, which as shown in graph 1-5, had the highest value in ZM medium. The results for the highest chlorophyll, phycocyanin and carotenoid in this medium at the pH= 9.6-9.6-8.4 are 12.83 mg/g, 44.27 mg/g, and 3mg/g, respectively. The highest concentration of chlorophyll, phycocyanin, and carotenoid was in SWMX2 medium with the pH range of10.4-8.6-9.8, and the results were 2.62 mg/g, 15.23 mg/g, 3.4 mg/g, respectively. The highest concentration of chlorophyll, phycocyanin, and carotenoid was in NRCM medium with the pH =9.2-9.6-10.4, and the results were 12.1 mg/g, 35.99 mg/g, 3.58 mg/g, respectively. The highest concentration of chlorophyll, phycocyanin, and carotenoid was in CFTRI medium with the pH range of 8.8-9-10.2, and the results were 12.31 mg/g, 7.98 mg/g, 3.13 mg/g, respectively. The highest concentration of chlorophyll, phycocyanin, and carotenoid was in Wastewater with the pH range of, 9.2-9.8-10.2 and the results were 2.92 mg/g, 12.27 mg/g, 2.46 mg/g, respectively.

DISCUSSION

Spirulina platensis was grown at various pH ranges (8-10.8) in flask culture and monitored and expressed in term of dry weight (Table 1-3). The maximum bulk density about 2.28 g/1000ml was noticed when the pH of culture medium was, maintained at 9.6 with medium volume 1000 ml flask. The maximum bulk density was attained on 28th day after the inoculation of culture in medium. The increase in the production of *Spirulina platensis* could have been due to the availability of mire space, oxygen and light to the culture flask. Earlier results also demonstrated that optimum pH for maximum growth of *Spirulina platensis* was 9 to 9.5 ranges (18). *Spirulina platensis* is considered to be an alkalophilic organism by nature (19). The Chlorophyll a content is also maximum in pH 9, 12.83 mg/g of dry weight. Similar studies have also been done by other scientists on cyanobacteria. (20, 21). Phycocyanin content is also maximum in pH 9.6, 44.27 mg/g of dry weight. Similar studies have also been done by various workers of cyanobacteria (22). The Carotenoid content is also maximum in pH 10.2, 3.13 mg/g of dry weight. Similar studies have also been done by various workers of cyanobacteria (23).

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