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MASS CULTIVATION AND DETERMINATION OF BIOCHEMICAL COMPOSITION OF *SPIRULINA PLATENSIS* IN THREE DIFFERENT MEDIUM

MANIGANDAN M*

M.Phil scholar, Division of Microbiology, Annamalai University, Annamalai Nagar, Tamilnadu, India.

ABSTRACT

In this work, *Spirulina platensis* was cultivated using three different media *viz.*, Synthetic medium (SM), Fertilizer medium(FM) and Seawater medium(SW) in which growth and proximal concentration of Protein, crude fat, total carbohydrate, total phenol, Chlorophyll content, carotenoid content and the crude phycocyanin content were examined for 5 days interval period upto 25 days of cultivation. Direct microscopic count (DMC), Optical density (OD) at 560 nm and the Dry biomass (DB) were examined from three different media used and it was found that maximum growth was obtained in Synthetic medium (DMC-95 cells/ml, OD-0.442 nm, DB-2.55 mg/l), followed by Fertilizer medium (DMC-89 cells/ml, OD-0.395 nm, DB-2.10 mg/l) and the least growth was obtained in Seawater medium (DMC-55 cells/ml, OD-0.251 nm, DB-0.91 mg/l).

KEYWORDS: Optical density, seawater medium, dry biomass.

MANIGANDAN M*
M.Phil scholar, Division of Microbiology, Annamalai University, Annamalai Nagar, Tamilnadu, India.

***Corresponding author**

INTRODUCTION

Spirulina platensis is a cyanobacterium that has been largely studied due to its commercial importance as a source of protein, vitamins, essential amino acids, and fatty acids[1, 2]. It has been used as human food supplement for over 20 years, because of its high nutrient content, including B complex vitamins, beta-carotene, vitamin E, manganese, zinc, copper, iron, selenium, and gamma linolenic acid[3]. *Spirulina platensis* possesses a high tolerance to alkaline pH, for ease of cultivation; a large size of its cell aggregates for ease of harvest; and an easily digestible cell wall[4]. *Spirulina* has been experimentally proven, *in vivo* and *in vitro* that it is effective to treat certain allergies, anemia, cancer, hepatotoxicity [toxicity of the liver], viral and cardiovascular diseases, hyperglycemia [high blood sugar], hyperlipidemia [high cholesterol and triglycerides], immunodeficiency, and inflammatory processes, among others. Several of these activities are attributed to *Spirulina* itself or to some of its components including fatty acids omega-3, omega-6, beta-carotene, alpha-tocopherols, phycocyanin, phenol compounds and a recently isolated complex, Calcium Spirulan[5]. *Spirulina* contains important carotenoids like β-carotene, zeaxanthin and beta-cryptoxanthin as well as lesser known carotenoids such as myxoxanthophyll and echinenone. β-carotene reduces the size of tumors that were already present in hamsters and slowed new tumor growth, extending the hamsters' survival time[6]. Natural beta-carotene is chemically and physically different from the synthetic form and human body absorbs natural beta-carotene ten times more easily than it absorbs the synthetic form[7]. Smokers with low betacarotene levels in the blood have been connected with the later appearance of lung cancer[8].

Researchers in both Japan and China have examined the potential of *Spirulina's* polysaccharides in cancer therapy. Scientists at Japan's Toyama Medical and Pharmaceutical University founded that lung metastasis in human significantly reduced by Calcium Spirulan by inhibiting tumour invasion of the cell membranes resulted in marked decrease of lung tumour colonization[9]. The Chinese study was done on mice and dogs at the Medical and Pharmaceutical Academy of Yangzhou University and they concluded Polysaccharide extract of *Spirulina platensis* has chemo-protective and radio-protective capability, and may be a potential adjunct to cancer therapy[10]. Gamma linolenic acid is synthesized from linoleic acid, and from gamma linolenic acid the body makes a very important hormone- like substance called prostaglandin E1. Prostaglandin E1 helps to prevent heart attacks and strokes, helps to remove excess fluid, improves circulation, slows down cholesterol production, improves nerve function, and regulates cell division[11]. Prostaglandin E1 is anti-inflammatory: it is vital to maintaining a healthy balance in our joints, helping to prevent inflammation and pain. Groups of arthritis sufferers have shown significant improvement after taking GLA supplements[12]. *Spirulina* has many different types of antioxidants; Phycocyanin is a powerful water soluble antioxidant and the unique nature of phycocyanin makes *Spirulina* a level above other antioxidant foods or formulas. The phycocyanin in *Spirulina* is thought to help protect against renal failure caused by certain drug therapies administered in hospitals. Phycocyanin exhibited a positive effect in removing plaque from the arteries potent free radical scavenger and inhibits microsomal lipid peroxidation[13].

MATERIALS AND METHODS

The cultivation was carried out in medium size plastic basins of 20 litres, using three different nutrient medium *viz*., synthetic medium, fertilizer medium and seawater medium[14]. Feeding of low doses of sea salt helps to prevent mosquito breeding. The cultivation was naturally aerated with manual agitation for three times per day. The experiment runs at a temperature of 25^{o}C±2, pH maintained at 10±1 and photoperiod of 12/12 hours light /dark. Axenic culture of *Spirulina platensis* was collected and maintained on Zarrouk's medium slants at 4°C. After autoclaving, sodium carbonate was added and the pH was adjusted (8.5-9.0). Growth and maintenance of the culture were done in an illuminated

growth room at 30 ± 2°C under 12/12 hour light-dark cycles. Agitation of cultures was done 3 times per day. Loop full of *Spirulina platensis* was inoculated in three different nutrient sources such as Synthetic medium (SM), Fertilizer medium (FM) and seawater (SW) as mention in the Table. Natural seawater was collected freshly from the Bay of Bengal, Parangipettai ($11^0$29'N79^0 46'E/11.49^{0}N79.76^{0}E).

DETERMINATION OF GROWTH PERFORMANCE OF Spirulina platensis (Tolga et al., 2007)[15]

The growth of *Spirulina platensis* which was cultivated in Zarrouk's medium and mass cultivation in three different medium was estimated at 5 days interval for 25 days.

Direct Microscopic Count

A loopful of culture was placed on a slide and a coverslip was placed over the culture. The wet mount was observed and counted under the 10X objective[16].

Growth Measurements

Growth performance of *Spirulina platensis* was monitored by optical density. The optical density was determined in a UV - Spectrophotometer. The absorption was read at 560 nm.

Estimation of Biomass (Venkataraman, 1983)[17]

Twenty five ml sample of *Spirulina platensis* cultures were centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded and sediment was oven dried (50^{o}C - 60^{o}C) and weighed. The difference was compared and the dry weight mass was calculated.

Biomass (mg/l) = Final weight - Initial weight.

Harvesting, Drying and storage of Spirulina platensis

Harvesting of *Spirulina platensis* was carried out at the end of 25th day. Harvesting was done by pouring the algal suspension on cotton cloth filter supported by plastic basins. The harvested slurry was washed with sterile distilled water to remove salts and bring the pH at 7.0 to 8.0. Sun drying (traditional way) was the most popular way to dry *Spirulina.* During the drying process as well as afterwards, the product was protected against contaminations from dust and insects and not be touched by hands. Drying temperature should be limited from 68 to 70^{o}C, and drying time to 6 hours. The dry chips or rods convert to powder by grinding in order to increase their apparent density. The best storage was done in heat sealed, aluminized plastic bags.

Determination of dry weight

After filtration and washing, filter paper was dried in oven at 100°C for 16 hrs, kept in desiccators and cooled to room temperature. *Spirulina* dry biomass were weighed carefully up to 0.0001g level by using weigh the balance. Cells were dried in oven at 100°C for 16 hours, placed in desiccators and cooled to room temperature and the mass was weighed using an analytical balance.

Monitoring experiments

Before filtration, the culture of each flask was monitored for pH and microscopic examination. For microscopic examination, 100µl sample was drawn after proper shaking by micro pipettes and observed under microscope at 40 X. All experiments were performed in triplicate and the results were expressed as mean value of respective parameter.

Protein Estimation by Lowry's Method (Lowry et al., 1951) [18]

One ml of *Spirulina platensis* cell suspension from three different medium harvested and was washed twice and resuspended in sterile water. 0.1N NaOH was added and the cell suspensions were heated at 100˚C to lyses cells. The hydrolysate was cooled to room temperature and 1ml of freshly mixed complex forming reagent (2% Na_2CO_3, 1% $CuSO_4$, and 2% Sodium potassium tartarate) was added. The solution was allowed to stand at room temperature for 10 minutes. One ml of folin reagent was added and vortexed and the mixer were allowed to stand at room temperature for 30-60 minutes. The absorbance was read at 660 nm. Standard curve of absorbance was plotted as a function of initial protein concentration and use it to determine the unknown protein concentration.

DETERMINATION OF PROXIMAL COMPOSITION

Determination of Crude Fat

Crude fat content of *Spirulina platensis* was determined by the air oven method[19].

Determination of Total Carbohydrates Content

Total available carbohydrates of *Spirulina platensis* were determined by using the Anthrone method[20].

Estimation of total Phenol content

The concentration of phenolics in *Spirulina platensis* samples cultivated in three different medium was estimated by the Folin-Ciocalteu procedure[21] and expressing the results in Gallic acid equivalent, naturally occurring phenols.

Determination of chlorophyll content

One gram of *Spirulina platensis* cultivated in three different medium were homogenized in 20 ml acetone (80%) and allowed to stand overnight in dark at 4EC for complete extract followed by centrifugation at 10,000 rpm for 5 minutes. The contents of total chlorophyll (T-Chl), chlorophyll- a (Chl-a) and chlorophyll- b (Chl-b) in the supernatant were determined spectophotometrically according to Lichtenthaler (1987)[22] method.

Determination of carotenoids content

The total carotenoids in *Spirulina platensis* samples will be determined spectrophotometrically at 450 nm according to AOAC standard methods, (1995).

Extraction of crude phycocyanin

An aliquot of twenty five days old culture was used as a source for extracting phycocyanin. Harvested biomass was homogenized in hand homogenizer for 20 minutes in the presence of phosphate buffer at pH 6.8 in 1:3 ratios. The homogenized culture was subjected to freezing and thawing for 3 days. Freeze thawed sample subject to Centrifugation at 5000 rpm for 45 minutes. The supernatant raw phycocyanin was taken in sterile tubes covered with aluminium foil (To prevent light penetration) and stored at 40^0C for further analysis. The crude phycocyanin concentration was calculated spectrophotometrically by measuring the absorbance at 615 nm and 652 nm is using the following calculation[16].

Calculation

Phycocyanin mg/ml = A615-0.047(A652) 5.34

Whereas,

A615- absorbance at 615nm,

A652- absorbance at 652nm.

5.34 – constant factor.

RESULTS AND DISCUSSION

Algal growth

Spirulina platensis grew well in both SM and FM culture**.** Growth performance of *Spirulina platensis* cultivated in three different medium under laboratory conditions were recorded in table 1. Microscopic and visual observation revealed that culture was grown healthy and morphology of *Spirulina platensis* filament also maintained its colour and shape. Appearance of culture also shifted from light green to dark green in proportion to the increasing cell mass. After 25 days, the direct microscopic count of *Spirulina platensis* was high in Synthetic medium (95 cells/ml) followed by Fertilizer medium (89 cells/ml) ant the least was recorded in Seawater medium (55 cells/ml). In early growth periods the filaments of *Spirulina platensis* were found to be straight but after 14 days some of the filaments were turned to slightly spiral shape and blue green in colour. Optical density of *Spirulina platensis* at 560 nm was high in Synthetic medium (0.442) followed by Fertilizer medium (0.395) and the least recorded in Seawater medium (0.251). After 25 days of cultivation the optical density was increased with respect to synthetic and fertilizer medium but observed less in seawater when compared to those media. Dry biomass of *Spirulina platensis* was high in Synthetic medium (2.55 mg/l) followed by Fertilizer medium (2.10 mg/l) and the least amount was recorded in Seawater medium (0.91 mg/l) drying of *Spirulina* was done under direct sunlight which is increased day by day during cultivation in both synthetic medium and fertilizer medium but low amount in seawater medium.

Table 1
Growth Performance and dry weight of Spirulina platensis Cultivated in Synthetic medium, Fertilizer medium and Seawater medium

g	No of days per interval	Direct microscopic count (No of cells/ ml)			Optical density values at 560nm			Dry biomass (mg/l)		
		SM	FM	SW	SM	FM	SW	SM	FM	SW
1.	5th day	22	17	09	0.135	0.122	0.085	1.02	0.92	0.21
2.	10th day	45	31	21	0.213	0.193	0.131	1.52	1.06	0.39
3.	15th day	62	53	35	0.292	0.255	0.171	1.79	1.59	0.57
4.	20th day	79	72	42	0.365	0.331	0.201	2.06	1.87	0.69
5.	25th day	95	89	55	0.442	0.395	0.251	2.55	2.10	0.91

SM- synthetic medium; FM- fertilizer medium; SW- seawater medium

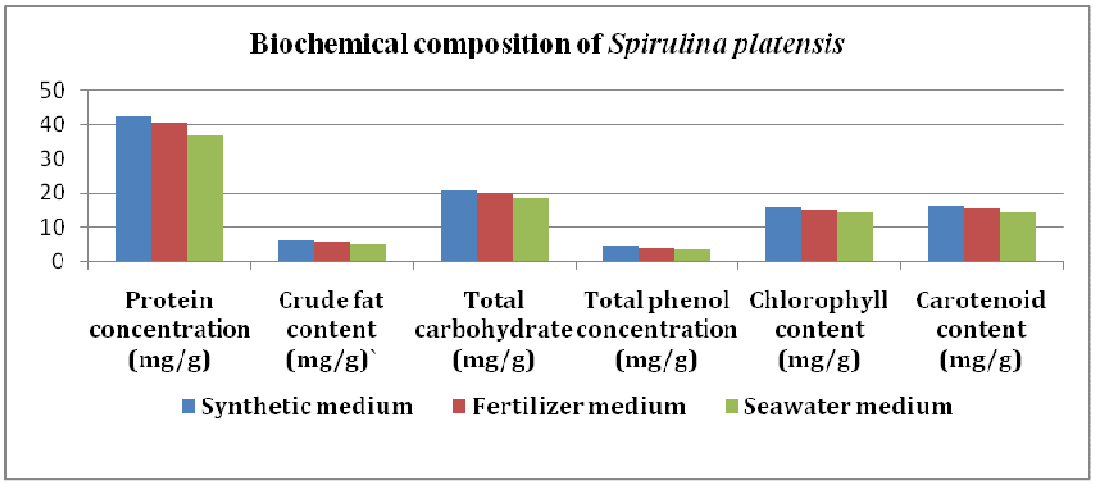
ESTIMATION OF CRUDE BIOCHEMICAL COMPOUNDS FROM Spirulina platensis

Spirulina platensis contains crude biochemical compounds such as protein, crude fat, carbohydrate, chlorophyll, carotenoids, crude phycocyanin and phenolic compounds were estimated and recorded in table 2. The protein content *Spirulina platensis* was high in synthetic medium (42.41 mg/g) followed by fertilizer medium (40.26 mg/g) and least protein content was recorded in SP1 (36.54 mg/g). The crude fat content of *Spirulina platensis* was high in synthetic medium (5.95 mg/g) followed by fertilizer medium (5.65 mg/g) and the least crude fat content were recorded in seawater medium (4.78 mg/g). The total carbohydrates content of *Spirulina platensis* was high in synthetic medium (20.51 mg/g) followed by fertilizer medium (19.97 mg/g) and the least total carbohydrates content was recorded in seawater medium (18.23 mg/g). The total phenol content of *Spirulina platensis* was high in synthetic medium (4.53 mg/g) followed by fertilizer medium (4.01 mg/g) and the least total phenol content was recorded in seawater medium (3.56 mg/g).

Table 2
Estimation of proximal concentration of Spirulina platensis

S.No	Biochemical composition of *Spirulina platensis*	Synthetic medium	Fertilizer medium	Seawater medium
1.	Protein concentration (mg/g)	42.41	40.26	36.54
2.	Crude fat content (mg/g)`	5.95	5.65	4.78
3.	Total carbohydrate (mg/g)	20.51	19.97	18.23
4.	Total phenol concentration (mg/g)	4.53	4.01	3.56
5.	Chlorophyll content (mg/g)	15.68	15.06	14.42
6.	Carotenoid content (mg/g)	16.00	15.53	14.25
7.	Crude Phycocyanin content (mg/ml)	0.289	0.201	0.141

Figure 1
biochemical composition of Spirulina platensis mn



The chlorophyll content of *Spirulina platensis* was high in synthetic medium (15.68 mg/g) followed by fertilizer medium (15.06 mg/g) and the least chlorophyll content was recorded in seawater medium (14.42 mg/g). The carotenoid content of *Spirulina platensis* was high in synthetic medium (16.00 mg/g) followed by fertilizer medium (15.53 mg/g) and the least carotenoid content was recorded in seawater medium (14.25 mg/g). The crude extract of phycocyanin cultivated in synthetic medium, fertilizer medium and seawater medium was estimated spectrophotometrically and calculated by the following calculation. The concentration of crude phycocyanin was high in synthetic medium (0.289 mg/ml) followed by fertilizer medium (0.201 mg/ml) and the least crude phycocyanin concentration was recorded in seawater medium (0.141 mg/ml). The crude extract of phycocyanin which was extracted from the *Spirulina platensis* cultivated in three different medium such as synthetic medium, fertiliser medium and seawater medium was estimated spectrophotometrically. The concentration of crude phycocyanin was high in synthetic medium (0.289 mg/ml) followed by fertiliser medium (0.201 mg/ml) and the least crude phycocyanin concentration was recorded in seawater medium (0.141 mg/ml).

DISCUSSION

Earlier studies show that the new medium was formulated for mass production of *Spirulina* sp by incorporating selected nutrients of the standard Zarrouk's medium. This newly formulated medium contains single super phosphate, sodium nitrate, muriate of potash, sodium chloride, magnesium sulphate, calcium chloride and sodium bicarbonate (commercial grade). Maximum growth rate in terms of dry biomass, chlorophyll and proteins in SM were recorded between 6 and 9 days of growth and values were 0.114, 0,003, and 0.068 as compared to 0.112, 0.003 and 0.069 mg/ml significant differences were observed in the protein profiles of *Spirulina* sp. grown in both the media[23]. Silveira *et al.* (2007)[24] extracted C-phycocyanin from cyanobacteria S*pirulina platensis* was optimized using factorial design and response surface techniques. The effects of temperature and biomass-solvent ratio on phycocyanin concentration and extract purity were evaluated to determine the optimum conditions for phycocyanin extractions. The optimum conditions for the extraction of phycocyanin from S*pirulina platensis* were the highest biomass-solvent ratio, 0.08g/ml/l, and 25^0C. Under these conditions it's possible to obtain an extract of phycocyanin with a concentration of 3.68mg/ml and purity ratio (A615, A280) of 0.46. Bohra (2009)[25] investigated the growth pattern of *Spirulina platensis* in standard and modified media based on seawater-chemicals and seawater fertilizers. During the cultivation, the cell concentrations were analyzed at 540nm along with protein and chlorophyll-A estimation. Growth patterns of different species and strains were monitored for 25 days and specific growth rate, mean daily division rate and doubling time were calculated. *Spirulina platensis* was observed to have different specific growth characteristics in different media at same environmental parameters. Even though, *Spirulina platensis* in standard media exhibited better growth patterns, biomass, protein content and chlorophyll content than other seawater based media, the experiment discusses the feasibility of seawater based media. Recently Shweta and Samuel (2013)[26] used the cooling tower water as a supplementation with CFTRI medium at 30% dilution gave the best results in terms of dry biomass and nutritional value of *Spirulina platensis*. It showed that cooling tower water is a promising wastewater medium for cultivation of *Spirulina platensis.*

CONCLUSION

In the present study, it was concluded that *Spirulina platensis* cultivated on three different medium under various concentration of nutrients, synthetic medium yielded maximum growth and high biochemical composition.

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