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RESEARCH ARTICLE

UTILIZATION OF SEAWATER AS A MEDIUM FOR MASS PRODUCTION OF *SPIRULINA PLATENSIS* – A NOVEL APPROACH

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ABSTRACT

Spirulina is the most important commercial microalga for the production of biomass as health food and animal. It is mainly oriented towards the health food market, utilizing a chemically defined medium. In the present study, four strains of *Spirulina platensis* were isolated from various locations and designated as SP1, SP2, SP3, SP4, and successfully cultivated in different liquid media like Zarrouk's medium, seawater enriched (SEM) with different amount of $NaHCO_3$, $NaNO_3$, K_2HPO_4 medium and seawater medium (SW). In *Spirulina platensis* the biomass concentration, protein, lipid content, chlorophyll and total carotenoids were monitored for 30 days on 10 days interval basis. Among the four strains SP4 recorded the best growth in all types of medium. From the three different media SP4 showed the highest biomass (dry weight) concentration, protein and lipid content in Zarrouk's medium (0.362 mg ml^{-1}, 0.234 mg ml^{-1} & 6.85%) followed by seawater enriched medium (0.334 mg ml^{-1},0.201 mg ml^{-1} & 6.49%) and seawater medium (0.283 mg ml^{-1},0.146 mg ml^{-1} & 5.93%). High level of chlorophyll and total carotenoid were estimated in Zarrouk's medium (31.90 µg ml^{-1}& 6.65 µg ml^{-1}) followed by seawater enriched medium (28.71 µg ml^{-1}& 5.93 µg ml^{-1}) and lowest amount was recorded in seawater medium (25.21 µg ml^{-1} & 4.71 µg ml^{-1}). The results of present investigation revealed the potential use of seawater with some nutrients for commercial cultivation of *Spirulina platensis* at low cost.

INTRODUCTION

Spirulina platensis is an economically important blue-green photoautotrophic and multicellular, filamentous microalga present in aqueous and saline habitats. Due to its richness in protein (Umesh and Sheshagiri, 1984; Cohen *et al*., 1996), essential amino acids, phycocyanin, polysaccharides, carotenoids, minerals, vitamins and essential fatty acids (Cohen *et al*., 1987; Cohen and Vonshak, 1991) *Spirulina* has been regarded as an ideal bio-resource and has drawn increasing attention in recent decades (Moris *et al.,* 2001; Kawata *et al*., 2004; Chen *et al.,* 2006). *Spirulina* sp, has been utilized as human food in Mexico and Africa for a long time (Ciferri and Tiboni, 1985; Henrikson, 1994; Vonshak, 1997). *S. platensis* biomass have 70% of proteins and all essential amino acids are present in proportions recommended by FAO, except methionine (Dillon *et al*., 1995; Ciferri and Tiboni, 1985; Colla *et al.,* 2005), an observation which has been use of *Spirulina* as a food supplement for under nourished people in many parts of the world. They have soft layer of mucopolysaccharide instead of a cellulose cell wall thus facilitating cell lysis, release of protein in large amounts (Vonshak, 1997) and high digestibility (Henrikson, 1994).

The cost of nutrient accounts for about 15-20% of the total costs for the large scale production of *Spirulina* (Vonshak, 1997). Currently, the commercial production of *Spirulina* is oriented mainly towards the health food market, utilizing a chemically defined medium (Belay and Ota 1993). The first synthetic medium formulated for cultivation of *Spirulina* was Zarrouk's medium (Zarrouk, 1966) which is still used as the standard medium. Subsequently, different media have been tried for cultivation of *Spirulina* such as Revised medium (Raoof *et al.,* 2006), CFTIR medium (Venkataraman *et al*., 1995), Bangladesh medium (Khatum *et al*., 1994), Rao's medium and OFERR medium (Singh, 2006). The use of sea water as an alternative medium, after being pre-treated (Faucher *et al.,* 1979) or after being supplemented with specific nutrients/ animal wastes (Devanathan and Ramanathan, 2012) under laboratory conditions (Materassi *et al*., 1984) or in outdoor raceways (Tredici *et al*., 1986; Wu *et al*., 1993) has been reported. Commercial products of seawater *Spirulina* including algal powder, tablet and natural blue colour *i.e.* phycocyanin and highly purified phycobiliproteins have been developed (Wu *et al*., 1993; Xiang *et al*., 1994).

The advantages of *Spirulina* production in seawater medium are: 1) lower fertilizer cost; 2) saving farm land by using waste sea beach; 3) seawater culture is not easily polluted by heavy metals and contaminations (Wu *et al*., 1993). The utilization of seawater media for the cultivation of *A. platensis* will be reduce the production cost considerably (Mary Leema *et al*., 2010). *Spirulina* is produced commercially all over the world and the dried product is utilized as a valuable food supplement. It is rich in proteins (60–70% by weight), vitamins (especially B_{12} and β-carotene) minerals and all essential amino acids and

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also possesses considerable amount of lipid content (Ciferri, 1983; Belay *et al.,* 1993). Basically constituted of polyunsaturated fatty acids like linolenic and linoleic in the proportion of 1.24% and 1.04%, respectively. They are considered as a vital role in form of the medicines, which are responsible for therapeutic properties, and also these compounds have antioxidant abilities such as polyunsaturated fatty acids (gamma-linolenic acid C18:3, ω6, GLA), phycocyanin (Estrada *et al.,* 2001), phenolics (Miranda *et al.,* 1998) and nutritional point of view (Richmond, 1988). Furthermore, *Spirulina* contains a high level of several pigments like carotenoids, xanthophylls, phycobiliproteins and chlorophyll *a* (Richmond, 1988). The self-renewing nature of the sources of chlorophyll has generated much commercial interest in recent years due to its economic value. A major portion of commercial chlorophyll is used in the food industry, pharmaceutical and cosmetic industries. The current commercial output of chlorophyll stands at the estimated figure of 11-108 tonnes year^{-1} (Branen *et al*., 2002).Considering the various applications and production cost of *Spirulina platensis*, the aim of this work was to study the mass production of *Spirulina platensis* by the use of sea water enriched with some nutrients and the chemical composition of the *S. platensis* biomass was also investigated.

MATERIALS AND METHODS

Culture Collection and Maintenance

Spirulina platensis strains were isolated from different locations *viz.,* Chidambaram, Aalampadi, Tiruvanamalai and Puducherry, Tamilnadu, India and designated as SP1, SP2, SP3 and SP4 respectively. The cultures were routinely maintained in modified Zarrouk's medium and pH was adjusted to 9.0 - 9.5. All the reagents used were of analytical grade (AR) and purchased from Hi-Media, Mumbai, India. Growth and maintenance of the culture was done in an illuminated (4500 lux) growth room at 35 ± 2 °C under 12/12 hour light-dark cycles. Manual shaking of cultures was done 3 times daily.

Collection and enrichment of seawater

Natural seawater was freshly collected from the coastal belt area of Samiyarpettai, Cuddalore District, Tamilnadu, India. Fresh seawater was pretreated by modified methods of Faucher *et al.,* (1979) and enriched with some nutrients like $NaHCO_3$, $NaNO_3$ and K_2HPO_4 at different concentrations.

Cultivation of Spirulina platensis in different media

Spirulina platensis were inoculated in three different media *viz*., Zarrouk's medium ($NaHCO_3$ 8.0; $NaNO_3$ 1.25; $CaCl_2$ 0.02; K_2SO_4 0.50; NaCl 0.50; $MgSO_4$ 0.10; FeEDTA 0.004; pH 9.5; g l^{-1}), seawater enrichment medium slight modification of Faucher method (1979) ($NaHCO_3$ 5.0; $NaNO_3$ 1.5; $K_2HPO_4$0.1; gl^{-1} pH 9.2) and seawater medium ($NaHCO_3$ 5.0; pH 9.0). Total 10 flask of 250 ml capacity containing 100 ml of each medium were inoculated with same amount of inoculums. All flasks were kept at temperature 37° C and manual shaking of cultures was done 3-4 times daily.

Dry weight estimation

For dry weight measurement homogenous suspension of known quantity of *Spirulina* samples were filtered through screen-printing paper and oven dried at 75ºC for 2 to 6 hours. The dried filter paper containing *Spirulina* biomass were cooled and weighted. The difference between the initial and final weight was taken as the dry weight of *Spirulina* biomass. The dry weights were expressed in terms of mg.

Estimation of Protein

Protein content was determined according to Lowry *et al*. (1956), using bovine serum albumin (BSA) as a standard. The protein content per ml of the cultures was noted by referring to the standard curve and the results were expressed in mg.

Estimation of Lipid

The total lipids were extracted from the fresh *Spirulina* biomass using a modified method of Bligh and Dyer (1959). The weight of the crude lipid obtained from each sample was measured gravimetrically and the results were expressed in percentage.

Estimation of Chlorophyll

The algal growth from the culture was harvested by centrifuging 10 ml broth at 6000 rpm in a centrifuge for 10 minutes. The algal pellet was washed twice with distilled water and suspended in 10 ml of 95 per cent methanol. The tubes containing algal suspension were kept in a water bath at 60°C for 30 minutes. The tubes mouth was covered by glass beads to minimize the evaporation. Intermittent shaking of the tubes ensured complete extraction of pigment. The tubes were removed from water bath, allowed to cool to room temperature and centrifuged again to remove the cell debris. Clear supernatant containing the pigment was transferred to a volumetric flask and volume was made upto 10 ml by adding methanol. The chlorophyll content in the biomass was calculated from the absorbance at 665nm of the methanolic extract (OD_{665} x 13.9 µg ml^{-1}) and using 95 per cent methanol as a blank (Tandeu and Houmard, 1988).

Determination of total carotenoids

A known volume of homogenized *Spirulina* suspension was centrifuged at 3000 rpm for 5 minutes. The pellet was washed with distilled water 2-3 times to remove traces of adhering salts. To the pellet, 2-3 ml of acetone (85%) was added, which was then subjected to repeated freezing and thawing. The suspension was centrifuged and the supernatant containing pigment was collected. The extraction was repeated till the supernatant became colourless, for complete recovery of carotenoids. The pooled fractions of supernatants were made-up to a final known volume. The absorbance was taken at 450nm using 85% acetone as blank and the total amount of carotenoids was calculated in µg ml^{-1} as follows (Saleh, *et al*., 2011).

$$C = \frac{D \times V F}{2500X100}$$

D = OD at 450nm
V = Volume of the extract,
F = Dilution factor
(Assuming that average extinction coefficient of pigments is 2500)

RESULTS AND DISCUSSION

Lower costs of production by the use of a low-cost medium could be a key factor in developing a competitive process for the production of *Spirulina* as a feed and as a source of added-value products. The annual production of the algae is about 10,000 tons which makes it the largest microalga cultivation industry in the world (Zhang *et al.*, 2005). In the present study *S. platensis* was isolated from different location *viz.*, Chidambaram, Aalambadi, Tiruvanamalai and Puducherry, were designated as SP1, SP2, SP3 and SP4. The colour of culture was shifted from light green to dark green in proportion to the increasing cell mass in Zarrouk's medium, while cultivation of *Spirulina* in both seawater enriched medium and seawater medium, appearance dose not changed as compared to cultivation in Zarrouk's medium. These results are similar to the findings of FAO, (2008) microscopic and visual observation revealed culture was grown healthy and morphology of *Spirulina* filament also maintain its colour and shape. *Spirulina platensis* was collected by filtration using whatman's no. 1 filter paper. Collected cells were washed with distilled water twice and dilute HCL (0.0001 N) to remove any excess salt and dust attached to cell surface.

Table 1 Estimation of *Spirulina platensis* biomass (dry weight) in different medium (mg ml^{-1}) at different period

Type of	*SP1			*SP2			*SP3			*SP4		
Medium	10th	20th	30th	10th	20th	30th	10th	20th	30th	10th	20th	30th
Zarrouk's medium	0.245	0.286	0.217	0.217	0.264	0.303	0.232	0.276	0.321	0.268	0.305	0.362
Seawater enriched medium	0.217	0.278	0.304	0.178	0.232	0.278	0.208	0.255	0.292	0.245	0.287	0.334
Saewater medium	0.167	0.238	0.255	0.123	0.185	0.219	0.151	0.221	0.257	0.188	0.235	0.283
SED	0.010	0.003	0.016	0.011	0.012	0.010	0.009	0.007	0.010	0.007	0.005	0.010
CD (p=0.05)	0.024	0.007	0.035	0.034	0.029	0.022	0.020	0.018	0.025	0.018	0.015	0.024

*SP (*Spirulina platensis*)

In the present study, *Spirulina platensis* strains were grown at different medium like Zarrouk's medium, seawater enriched medium and seawater medium under laboratory condition and the biomass (dry weight) was estimated during different period intervals and the results were showed in Table -1. Among the four strains SP4 showed higher biomass than the other strains in all types of the medium. The genus of *Spirulina*, is the most important commercially cultivated cyanobacterium, due to its high nutritional value, chemical composition and safety of its biomass for human consumption. Standardization of *Spirulina* in different medium was summarized with maximum growth (0.81 gm/ 250ml) noticed in Zarrouk's medium (Jitendra *et al.***,** 2012). Similarly the present investigation reported that after 30 days, the biomass (dry weight) of SP4 was higher amount in Zarrouk's medium (0.362 mg ml^{-1}). Seawater enriched medium (0.334 mg ml^{-1}) showed higher biomass when compared with seawater medium (0.283 mg ml^{-1}). But both seawater media showed lower biomass concentration when compared with Zarrouk's medium (Table, 1). Earlier studies also similarly showed the biomass concentration of *A. platensis* grown in seawater media SW1 and SW3 were lower than the control (Zarrouk's) medium (Mary Leema *et al.*, 2010). Devanathan and Ramanathan, (2012) reported that, *S. platensi*s are grown in seawater medium supplemented with different concentrations of poultry dry manure solution, the highest biomass concentration (dry weight) in Zarrouk's (control) medium (0.623 mg ml^{-1}), seawater based medium(SW3) showed (0.608 mg ml^{-1}), SW2 (0.572 mg ml^{-1}) and the least biomass was recorded in SW1 (0.518 mg ml^{-1}). Warr *et al.* (1985) reported that external sea salt concentration upto 15.0%, seawater also affect the growth and yield of the strain of *A. platensis.* Faucher *et al.* (1979) have also suggested that *A. platensis* recorded higher growth rate in synthetic SOT (standard medium) than seawater supplemented with phosphate and nitrate. *Spirulina* consists of 60-70% protein in dry weight (Zarrouk, 1966). Olguín *et al*., (2001) reported that the anaerobic effluents from digested pig waste were added in a proportion of 2% (v/v) to untreated sea-water diluted 1:4 with fresh water supplemented with 2 g L^{-1} sodium bicarbonate at pH 9.5. The average production during summer 1999 was 14.4 g m^{-2} d^{-1}. This is the highest value reported for a *Spirulina* cultivation system utilizing seawater and the average protein content of the cultures was 48.9% ash-free dry weight. Rafiqul *et al.* (2005), found 58.6% of total protein content when using Zarrouk's medium. Similarly in the present study, the protein content of *S. platensis,* was estimated (Table- 2).

The *S. platensis* (SP 4) strain recorded higher amount of protein content in different types of culture medium after 30 days. The protein content of SP4 in Zarrouk's medium (0.234 mg ml^{-1}) is nearly closer to the values (68.01%) recorded by Olivera *et al.* (1999) and in mineral medium described by Paolettai *et al.* (1985) at 25ºC temperature. Olguin *et al.* (1997) reported that the protein content of *A. platensis* observed in diluted seawater (SW2 65.20 and SW3 66.96%) falls within the range reported, whereas *Spirulina* cultured in seawater enriched medium (65.61%).

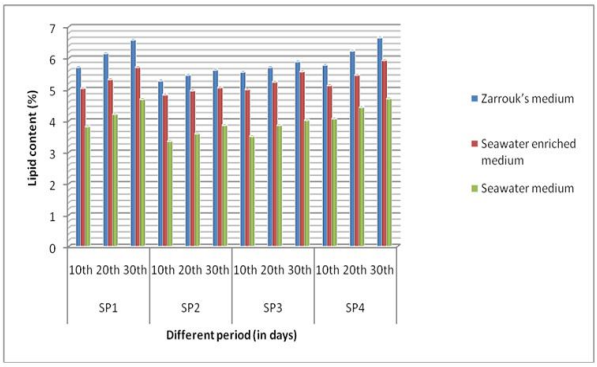

Figure 1 Estimation of lipid content (%) in *Spirulina platensis* in different medium

Table 2 Estimation of protein content in *Spirulina platensis* in different medium (mg ml^{-1}) at different period

Type of	*SP1			*SP2			*SP3			*SP4		
Medium	10th	20th	30th	10th	20th	30th	10th	20th	30th	10th	20th	30th
Zarrouk's medium	0.154	0.181	0.218	0.131	0.161	0.186	0.142	0.171	0.200	0.169	0.195	0.234
(Protein, %)	(62.8)	(63.2)	(63.9)	(60.3)	(60.9)	(61.3)	(61.2)	(61.9)	(62.3)	(63.0)	(63.9)	(64.6)
Seawater enriched medium	0.128	0.164	0.182	0.102	0.134	0.162	0.120	0.148	0.174	0.132	0.170	0.201
(Protein, %)	(58.5)	(58.9)	(59.8)	(57.3)	(57.7)	(58.2)	(57.6)	(58.0)	(59.5)	(58.6)	(59.2)	(60.1)
Seawater medium	0.082	0.118	0.129	0.059	0.090	0.109	0.074	0.108	0.125	0.093	0.119	0.146
(Protein, %)	(49.1)	(49.5)	(50.5)	(47.9)	(48.6)	(49.7)	(49.0)	(49.3)	(50.2)	(49.4)	(50.6)	(51.5)
SED	0.011	0.006	0.012	0.010	0.011	0.008	0.008	0.003	0.011	0.013	0.009	0.012
CD (p=0.05)	0.024	0.016	0.030	0.025	0.023	0.020	0.018	0.011	0.024	0.032	0.020	0.030

*SP (*Spirulina platensis*)

Zeng and Vonshak (1998) reported that cells under stress conditions, including salinity-stress, have a lower protein synthesis capacity. Similarly, in the present study the protein content of *Spirulina* cultured in seawater enriched medium showed 0.201 mg ml^{-1}, while seawater medium recorded 0.146 mg ml^{-1}. Similarly, Wong and Chan (1990) found that the protein content of sewage grown *Spirulina* was 45.6 %. Generally *Spirulina* contains 6-13% lipids (Cohen, 1997). Total lipids are affected by culture conditions like as salinity, N-starvation, light intensity (Tedesco and Duerr, 1989; Abd El-Baky *et al*., 2004). Mani *et al*. (2007) suggested that the yield of lipid extraction mainly depends on the nature of the solvent, lipid size, sample-solvent ratio, temperature and time of extraction. In the present study, the lipid content of *S. platensis* were estimated (Fig. 1) and SP4 strain showed highest lipid content in all types of culture medium after 30 days.

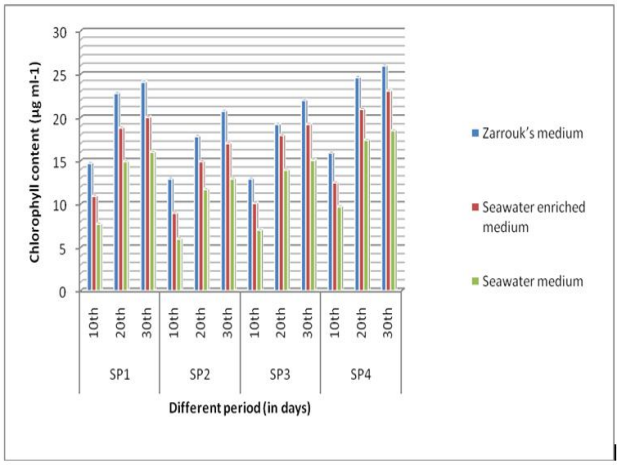

Fig. 2 Estimation of chlorophyll in *Spirulina platensis* in different medium (µg ml^{-1})

The higher amount of lipid content was shown in Zarrouk's medium (6.85%), followed by seawater enriched medium (6.49%), whereas least amount was recorded in seawater medium (5.93%). Similarly Mary Leema *et al*. (2010) reported that the lipid content of *A. platensis* was higher (13.7 % DW) in control (Zarrouk's) medium whereas in seawater (SW2, SW1) medium recorded lower amount 10.61% and 8.04% respectively. *Spirulina* cells have carotenoids, chlorophyll, and phycocyanin (PC), these are the major pigments amounting to 0.4, 1.0 and 14% dry weight, respectively (Belay, 1997). Chlorophyll 'a' is the molecule which makes photosynthesis possible by passing its energized electrons on to molecules which will manufacture sugar. All plants, algae and cyanobacteria which photosynthesize contain chlorophyll-a (Tomaselli, *et al*., 1997). The productions of pigments from microalgae have a number of advantages like cheaper and easy production, easier extraction, higher yields and there is no seasonal variation (Gurpreet Kaur and Khattar, 2009). In the present study, the chlorophyll content of *S. platensis* was estimated (Fig. 2). The SP4 strain showed highest chlorophyll content than other strains in all types of culture medium after 30th day. The chlorophyll content of *S. platensis* (SP4) showed the highest results were obtained in Zarrouk's medium (26.81 µg ml^{-1}) compared with seawater enriched medium (23.98µg ml^{-1}) and the least amount of chlorophyll was recorded in seawater medium (18.92 µg ml^{-1})

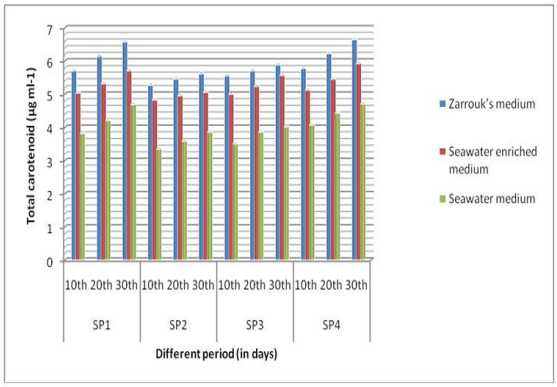

Fig. 3 Estimation of total carotenoid content in *Spirulina platensis* in different medium (µg ml^{-1})

These results were with accordance to the earlier findings on Lamela and Marquer- Rocha (2000) reported that decreased chlorophyll 'a' were recorded when *A. platensis* were cultured in seawater medium. The chlorophyll pigment content was decreased in *A. platensis* when grown in 0.5 M to 1.0M NaCl (Vonshak *et al*., 1996). Mary Leema *et al*. (2010) have also reported the final *chl*-a content of *A. platensis* grown in seawater medium gradually lowers than the control (Zarrouk's medium). Carotenoids, is one of the most important groups of natural pigments found in all higher plants, some animals (Zeb and Mehmood, 2004) and algae (Borowizka, 1988a). These are lipid soluble, yellow–orange–red pigments and have an important role in antioxidants activities, food health, pharmaceutical, cosmetics industries and is thus to be produced potentially in large scale (Borowizka, 1988a).

Similarly the present study revealed that the carotenoid content of *Spirulina platensis* was estimated and the results are presented in Fig.3. Among the four strains, the SP4 showed higher amount of total carotenoid in all types of medium. The highest results were obtained in Zarrouk's medium (6.65 µg ml^{-1}) followed by seawater enriched medium (5.93 µg ml^{-1}). The least amount of total carotenoid content was recorded in Seawater medium (4.71 µg ml^{-1}).

CONCLUSION

In conclusion, the present investigation revealed that the yield of *S. platensis* using seawater enrichment ($NaHCO_3$ 5.0; $NaNO_3$ 1.5; $K_2HPO_4$0.1; gl^{-1}) medium which recorded similar results to the Zarrouk's medium, which is costwise very low expensive. *S. platensis* (SP4) strain showed higher growth activities in all types of medium and this strain is suitable for mass cultivation. The potential use of seawater enriched medium suitable for commercial cultivation of *S. platensis* at very low cost.

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