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Cultivation of *Spirulina (Arthrospira) platensis* in low cost seawater based medium for extraction of value added pigments

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A low cost medium using seawater and prawn hatchery waste water was developed for the cultivation of an economically important cyanobacterium *Spirulina (Arthrospira) platensis*. Quality of the biomass produced was evaluated on the basis of protein and pigment contents (phycocyanin, chlorophyll-a and total carotenoids). A three step process was used for the downstream processing of the biomass produced to obtain a value added pigment phycocyanin. It is evident from the results that there was a negligible effect on growth rate (3-14% decline) in the amended seawater medium as compared to the prescribed growth medium. Phycocyanin content was also comparable (50.9±0.48 mg/g for amended seawater and 50.95±0.47 mg/g for prescribed medium). Purity of phycocyanin (A_{620}/A_{280}) was in the range of 3.08-3.27 which corresponds to superior quality colorant grade phycocyanin. This investigation provides baseline information about utilization of seawater for biomass production of *S.platensis,* and also for further downstream processing of biomass for the recovery of high purity phycocyanin.

[**Keywords**: *Spirulina*, mass cultivation, low cost medium, Phycocyanin]

Introduction

Phycocyanin is used as food colorant, nutraceutical, immuno-diagnostic material , natural dye for food and cosmetics, potential therapeutic agent in oxidative stress induced diseases and as fluorescent markers in biomedical research [1, 2, 3]. It is also used in cosmetics due to its nontoxic and non-carcinogenic properties.[4, 5]

The economics of phycocyanin production mainly depends on the cost of biomass production. Therefore, a low cost medium with least number of expensive synthetic chemicals can reduce the cost of Phycocyanin production. Considering the halophilic nature of *S.platensis*, the salts present in seawater can be successfully used for growing the organism in seawater with few amendments. Other than the salts, nitrate and ammonium are essential for growth of *S. platensis*. Waste water discharged from prawn hatchery is rich in inorganic compounds such as ammonia, nitrate, nitrite, phosphate, potassium etc. These nutrients are required for cultivation of *S. platensis*, therefore, hatchery waste water if mixed in right proportion with sea water can support the growth of algae without or minor supplementation of above compounds which can help in reducing the cost of algal biomass production [6, 7].

Production of *Spirulina* using a low cost medium could be a crucial factor in production of value added products like phycocyanin [8]. Culture of *Spirulina* using seawater as an alternative medium amended with some nutrients was accomplished by some of the researchers [9,10,11,12]. Animal wastes and other waste water are also been used as a nutrient source in the *Spirulina* production [13, 14]. This investigation focused on the evaluation of a seawater based medium amended with hatchery wastewater for the mass cultivation of *Spirulina platensis*, the biomass produced in seawater based medium was subjected downstream processing for the recovery of high value pigments such as phycocyanin, carotenoids and chlorophyll-a .

The observations of present investigation will serve as a baseline for further detail investigations on large scale cultivation of *S. platensis* in seawater based medium. Considering the availability of well established culture technology and a volume of data on applications of the biomass for this species in comparison to marine species of *Spirulina* . In spite of being a freshwater species, *Spirulina platensis* is a potential candidate for biomass production in coastal areas where seawater and hatchery waste water can be used for *Spirulina* cultivation.

Material and Methods

Unialgal culture of cyanobacterium *Spirulina platensis* was obtained from Algal Biology Laboratory of Central Institute of Fisheries Education (CIFE), Mumbai. Pure culture was sub-cultured in modified Nallayam Research Centre medium (Prescribed by Nallayam Research Centre, Chennai; referred as NRC medium in following text) under photoautotrophic conditions. Batch and airlift culture experiments were carried out with an illumination of 3500 ± 100 lux using compact fluorescent lamps (Philips, 23 W). Intensity of light was measured using lux meter (LX-103, Taiwan). Photoperiod was fixed at 12:12 hours light and dark periods. Temperature was maintained at 26 ± 2°C. Medium selected for cultivation of *S. platensis* was further modified. In modified medium urea and phosphoric acid of NRC medium were replaced by Sodium nitrate (2.5g/l) and Di-potassium hydrogen phosphate (0.5g/l) and also the concentration of ferrous sulphate heptahydrate (0.01g/l) was reduced from 0.5g/l.

Comparison of cost of the media is shown in the Table 1. Mother cultures were grown in 250 ml Erlenmeyer flask containing 100 ml of m-NRC medium inoculated with a known quantity of cell suspension of *Spirulina platensis*. Specific growth rate was calculated by measuring change of optical density (OD) every day using double beam spectrophotometer (UV 1 model, Thermospectronic, England) at 750 nm.

The seawater (SW) was collected from Madh Island area, Mumbai during the high tide. Seawater was stored in a settlement tank for 24 hours to remove sand and mud. Then three culture media were prepared after filtration (0.45 µ filter paper, Milipore, USA) seawater. Eight grams of sodium bi carbonate was added per litre of seawater to raise the pH to 9.5. Hatchery waste water (HWW) was collected from the larval rearing section of *Macrobrachium rosenbergii* hatchery at CIFE, Mumbai. This hatchery waste water was used to amend seawater. The different seawater based media are given below (Table 1).

Seawater was treated with $NaHCO_3$ and kept for the Ca and Mg precipitate. Raw seawater was mixed with Hatchery waste water in a proportion 1:1.

Table 1: Composition and approximate cost (for 1000 litre)* of different culture media for *Spirulina platensis*

Sl.NO	PARTICULARS	Quantity of chemicals in Kg/1000 litres	Price in rupees per 1000 litres	Price in USD* per 1000 litres		
				Modified NRC medium	Treated SW: HWW	Treated SW
1	$NaHCO_3$	8	4032	73.3	73.3	73.3
2	NaCl	5	1260	22.9	-	-
3	$NaNO_3$	2.5	1500	27.3	-	-
4	K_2SO_4	0.5	366	6.7	-	-
5	K_2HPO_4	0.5	504	9.2	-	-
6	$MgSO_4.7H_2O$	0.16	87.7	1.6	-	-
7	$FeSO_4.7H_2O$	0.01	7.3	0.13	-	-
8	Total cost	-	7758	141.1	73.3	73.3

*HWW= Hatchery waste water, SW= Seawater (The exchange rate of USD is Rs 55/- as on 15/09/2012).

Table 2: Composition of hatchery waste water used for amendment of seawater

Salinity	20 ppt
Phosphate	2.3 mgL^{-1}
Ammonia	0.14 mgL^{-1}
Nitrate	8.8 mgL^{-1}
Conductivity	8 ms
Calcium	360 mgL^{-1}
Potassium	190 mgL^{-1}

Outdoor cultivation was done in circular FRP tanks of 1000 litre capacity and the volume of the medium was 60 litres. Culture was mixed using air injection tube and the tank was covered with polythene sheet to avoid the dust particle and droppings of the birds or animals and also to prevent the water loss due to evaporation. Perforated glass head was attached at the end of the air injection tube to achieve the uniform distribution of air throughout the medium. Outdoor culture at Mumbai was conducted with two best growing seawater media, which was selected after indoor cultivation. Modified NRC medium was used as control. Parameters like temperature, pH, and light intensity were measured thrice a day at 10 A.M., 2 P.M., and 5 P.M. Growth of the organisms was monitored everyday by measuring turbidity at 750 nm using a Spectrophotometer (Thermo Scietific, UV-I Model, USA).

Samples were collected at daily intervals and the optical density of cell suspension (Turbidity) was measured using a double-beam spectrophotometer at 750 nm. Specific growth rate and generation time were calculated by using the formula given by Guillard[15].

Specific growth rate was measured during exponential growth phase and was calculated using the formula given below,

$$\text{Specific growth rate } (\mu) = \frac{\ln Nt - \ln N_0}{t_t - t_0}$$

Where, N_0 and Nt are the values of absorbance at 750 nm during the exponential phase at time t_0 and t_t respectively.

Generation time (T_2)

The generation time or mean generation time (Days) was calculated using the formula,

$$\text{Generation time} = \frac{\ln (2)}{\mu} = \frac{0.693}{\mu}$$

Major pigments in *Spirulina*, like chlorophyll-a, phycocyanin, and total carotenoids were estimated to assess the quality of the biomass produced in different media.

Chlorophyll was extracted with acetone as a solvent [16]. This procedure was carried out in subdued light to avoid degradation and amber coloured bottles were used for storing the acetone solution. Chlorophyll-a content was estimated by following formula:

$$\text{Chlorophyll-a (mg/l)} = \frac{26.7 \times (A_{664b} - A_{665a}) \times V_1}{V_2 \times L}$$

Subtract the 750nm OD value from the readings before (OD 664nm) and after acidification (OD 665nm).

26.7 = Absorbance correction

Where,

V1 = Volume of extract (ml), V2 = Volume of sample (ml)

L = Path length (cm)

Where, A_{620} and A_{652} are the Optical Density (OD) values at 620 and 652 nm respectively.

The phycocyanin was extracted by Repeated Freezing and Thawing (RFT) of cells in 50 mM sodium phosphate buffer at a pH of 6.8, and estimated by a method [17]. The amount of phycocyanin was calculated as mg of phycocyanin per ml using the following equation,

Phycocyanin (mg/ml) = $[A_{620} - 0.474 \times A_{652}] / 5.34$

Where, A_{620} and A_{652} are the Optical Density (OD) values at 620 and 652 nm respectively.

Purification factor:

The purity of phycocyanin extract was monitored spectrophotometrically by the ratio of O.D values at A_{620} / A_{280} ratio. The flow chart of purification of phycocyanin is given in the Figure 1.

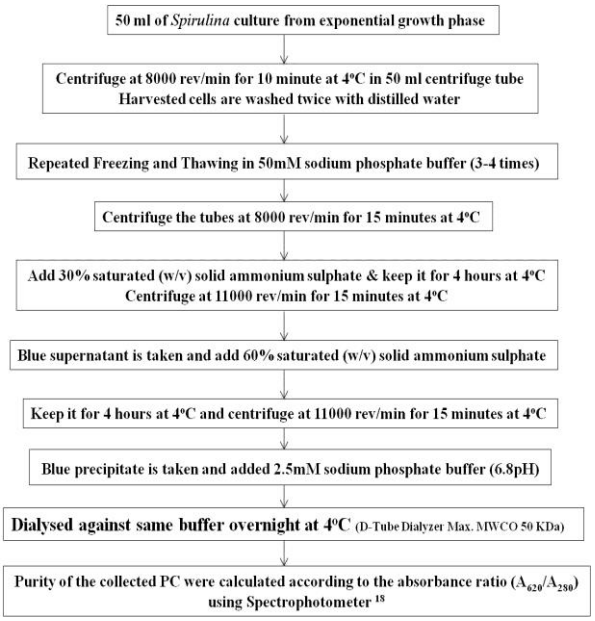

Fig. 1- Flow chart of extraction and purification of phycocyanin

The method developed by Cyanotech [19] was followed to estimate the total Carotenoids in dried *Spirulina.*

Protein content (%) of *S. platensis* biomass obtained from different culture media and from different culture conditions (outdoor and indoor) were estimated by microkjeldal method (MicroKjeldahl unit, Pelican equipments). Freeze dried biomass was used for the estimation [20].

Unless otherwise specified all the reagents and glassware used for the cultivation of *S. platensis* were of general reagent grade procured from Merck, India. De-ionized water was obtained from Milli- Q system (Millipore, France).

Water quality parameters such as temperature, salinity and pH were estimated before and after cultivation of *S. platensis*. The phosphorus content of different media was determined by ascorbic acid method [16]. Ammonia (NH_4^+-N) nitrogen was measured estimated by phenate method [16].

The data were statistically analyzed by statistical package SPSS version 16.0 in which data were subjected to one way ANOVA and Duncan's multiple range tests were used as posthoc tests to determine the significant differences between the means at 5% significant level.

Results

Growth of *Spirulina platensis* was measured in terms of turbidity (Abs 750nm) for seven days in indoor culture system using different seawater based media. Growth and specific growth rate has been compared with the control, m-NRC medium. Highest growth was observed in seawater treated with $NaHCO_3$ (8g/l): Hatchery Waste Water (composition of hatchery waste water was shown in the Table 2). Growth was 80.5% of that of m-NRC medium (Fig. 2) but in the case of Treated Seawater ($NaHCO_3$, 8g/l) growth was 75% of that of m-NRC medium. A mixture of seawater: Hatchery Waste water (1:1) exhibited 58% growth as compared to m-NRC medium. The growth of *S. platensis* in seawater was 52% of the control (m-NRC medium). Two best compositions were selected for outdoor experiments.

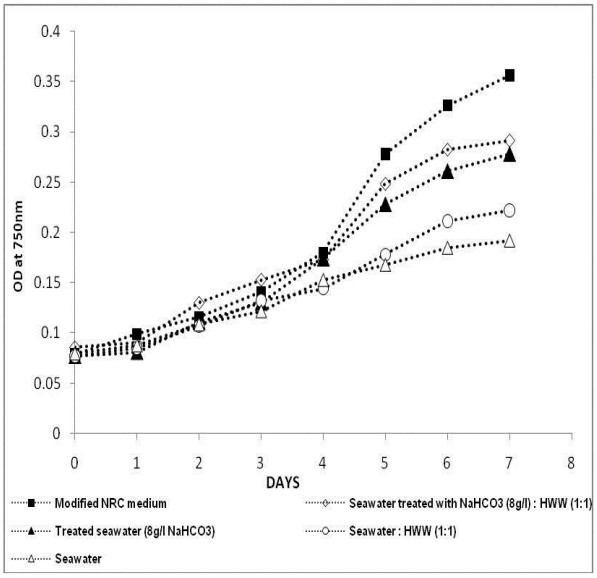

Fig. 2- Comparison of growth curves of *S. platensis* in indoor culture using different seawater based media.

Outdoor cultivation of *S. platensis* was conducted using two different seawater media and control (m-NRC medium) for 7 days in FRP tanks. Growth of *S. platensis* in Treated seawater: Hatchery waste water was comparable to that of m-NRC medium (Fig 3). Only 3% and 14% reduction in growth as compared to the control was recorded for the mixture of bi-carbonate supplemented sea water amended with hatchery wastewater and bi-carbonate supplemented seawater, respectively.

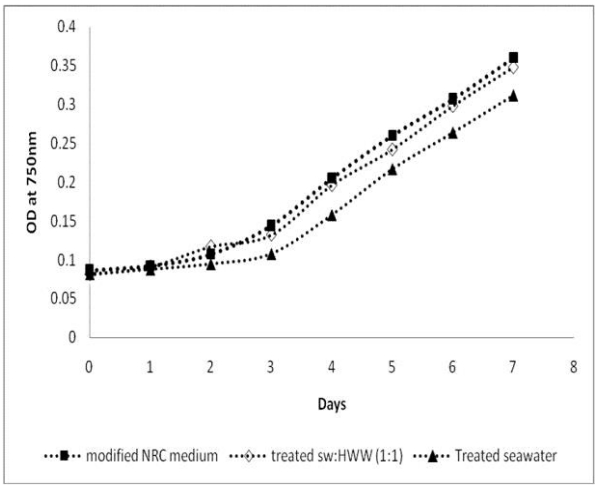

Fig. 3- Comparison of growth curves of *S. platensis* in outdoor culture using seawater and HWW

SGR of treated seawater: hatchery waste water (1:1) shows no significant difference ($p>0.05$) from m-NRC medium. But specific growth rate in TrSW, SW:HWW and SW media are significantly different from that of control. In the case of outdoor cultivation, even though the SGR of TrSW:HWW and TrSW is significantly different ($p<0.05$) from the control, the percentage reduction of SGR is only 11.86% and 4.3% in the above mentioned seawater based media (Figure 4).

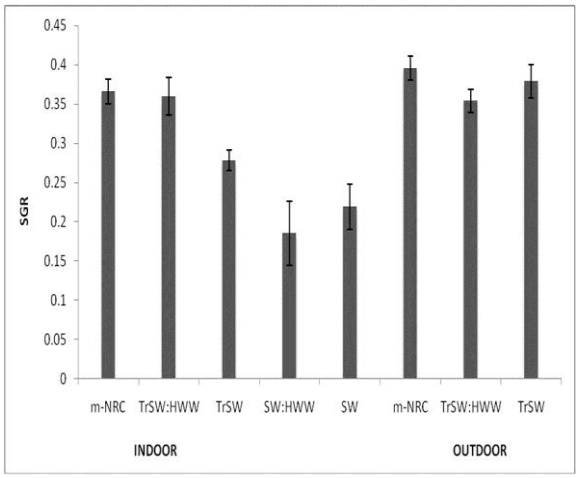

Fig. 4- Specific growth rate (after 7 days of culture) of *S. platensis* from indoor and outdoor culture using seawater based media (values are mean ± SE)

Table 3: Change in pH during culture of *S. platensis* in seawater based media

Media	pH	
	Before culture	**After culture (one week cycle)**
m- NRC	8.50	9.30-9.42
Treated seawater: HWW (1:1)	8.62	9.65-9.71
Treated seawater	8.66	9.38-9.50

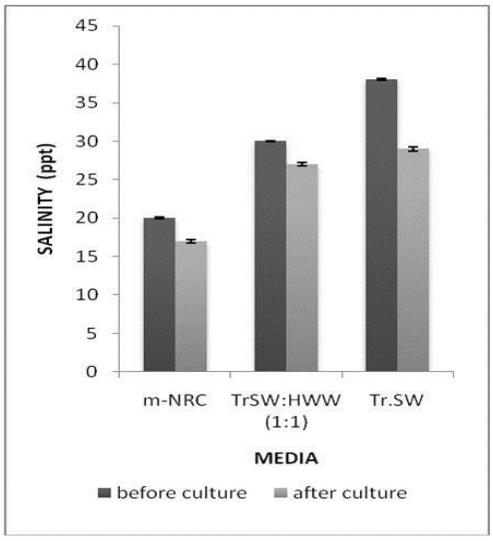

Fig. 5- Changes of salinity in different seawater based media

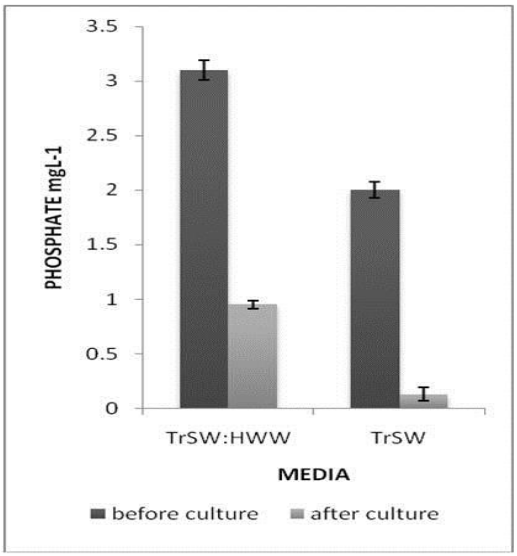

Fig. 6- Changes of phosphate content in different seawater based media

There was an increase in pH of the spent medium after *Spirulina* cultivation (Table 3). However, a decrease in salinity of the medium was noticed after 7 days culture of *S. platensis* (Fig. 5). A pronounced decrease in Phosphate concentration after one culture cycle of seven days was recorded (Fig. 6). Nitrogenous compounds (nitrate and ammonia) also decreased after one week cultivation however; the decrease in concentration was moderate and not as considerable as for Phosphate (Fig. 7 & 8).

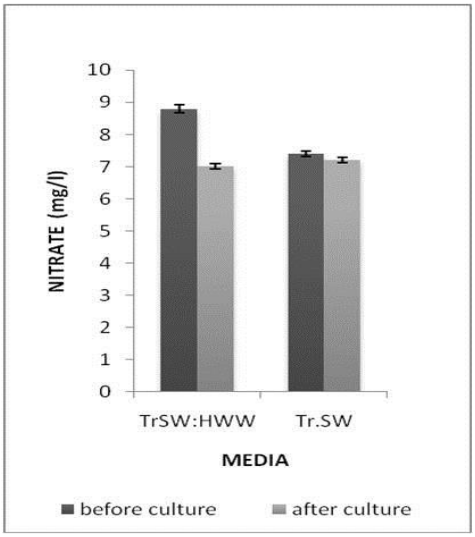

Fig. 7- Changes of nitrate content in different seawater based media.

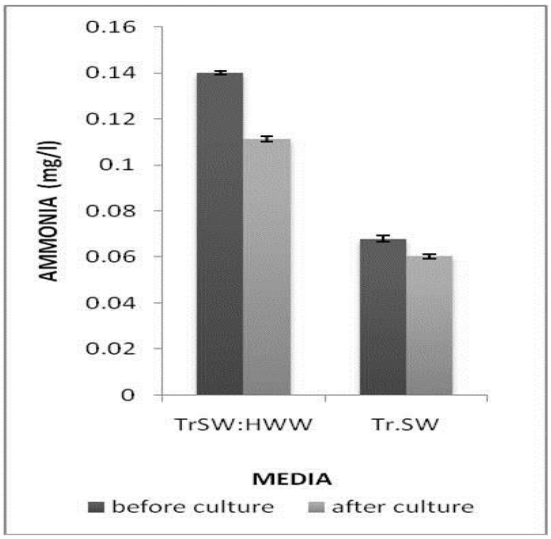

Fig. 8- Changes of ammonia content in different seawater based media

Three major pigments viz. phycocyanin, chlorophyll-a and carotenoids (mg/g dry weight) were estimated in the harvested biomass to check the quality of the biomass in different culture media (Fig 9). There is no significant difference ($p>0.05$) in the phycocyanin content of treated seawater (50.9±0.48 mg/g) and control (50.95±0.47 mg/g). Phycocyanin content in the treated seawater: HWW was 49.82±0.69 mg/g. Chlorophyll-a content in the treated seawater: HWW medium and control (m-NRC medium) were comparable. A 20.3% reduction has been observed in the total carotenoid content in the treated seawater: HWW medium as compared to the control.

The phycocyanin obtained was further subjected to simple three stage purification process to obtain high value pigment. In each stage different purity has been obtained in different culture media (Table 4). Purity increased remarkably from 1st stage to the 3rd stage.

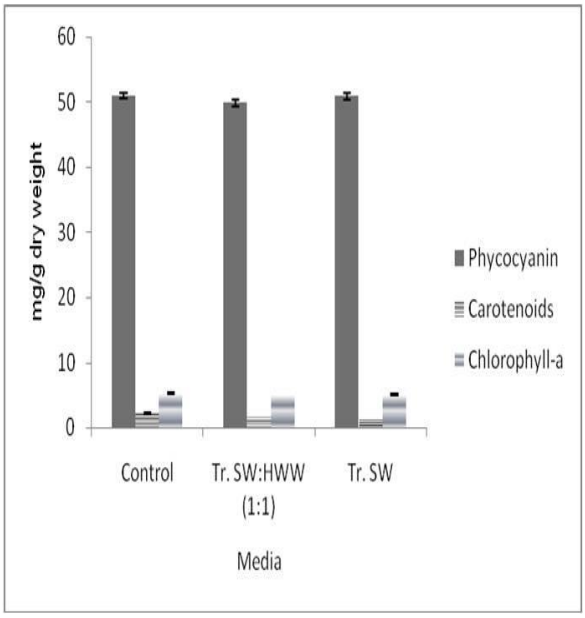

Fig. 9- Pigment composition of *S. platensis* cultured in seawater based media after 7 days (values are mean ± SE)

Table 4: Purity ratio (A_{620}/A_{280}) of 3 stage purification of phycocyanin from outdoor culture of *S. platensis* using seawater based media after 7 days (values are mean ± SE)

Different culture media	Stage 1	Stage 2	Stage 3
Modified NRC media	0.511±0.016[b]	1.273±0.102[a]	3.55±0.075[cb]
Tr SW: HWW (1:1)	0.450±0.006[a]	1.133±0.128[a]	3.08±0.037[a]
Tr SW	0.485±0.003[b]	1.190±0.050[a]	3.27±0.029[b]

*Different alphabets indicate significance ($p<0.05$)

In indoor culture experiments, the protein content obtained in the treated seawater amended with hatchery waste water was 52.5±0.29%, whereas in the outdoor culture it increased to 55.7±0.82%. In both experiments, protein percentage of the control was 59.00±0.43% and 59.93 ± 0.89%, respectively (Fig 10 & 11).

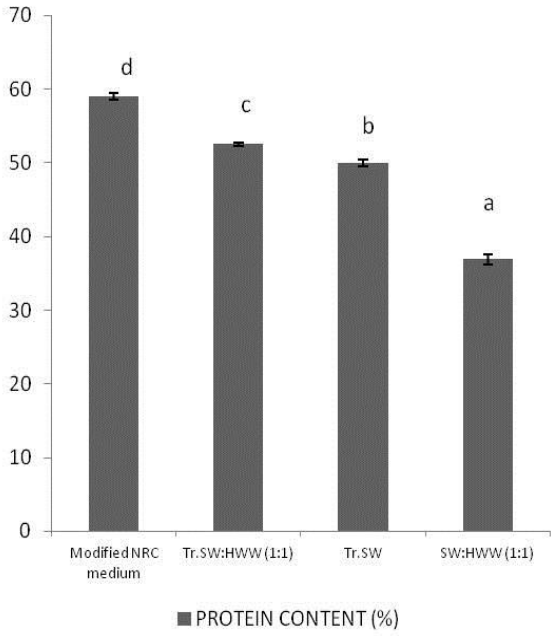

Fig. 10- Protein content (%) of *S. platensis* biomass in indoor culture using seawater based media after 7 days

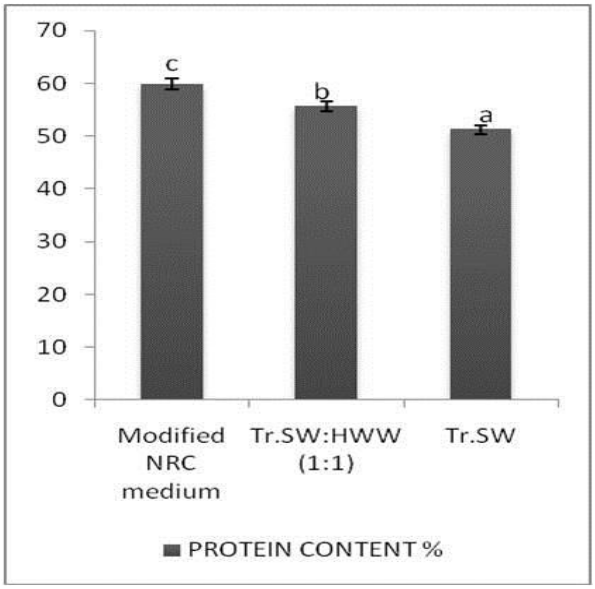

Fig. 11- Protein content (%) of *S. platensis* biomass in outdoor culture using seawater based media after 7 days

Discussion

S. platensis grown in different seawater media and m-NRC medium (control) was studied, and growth was evaluated during seven days of cultivation in indoor as well as outdoor units (Fig.2 & 3). All the seawater media used for the cultivation supported the growth of *S. platensis.* Among the seawater media, seawater treated with $NaHCO_3$ (8g/l): hatchery waste water (1:1) showed best growth in indoor batch culture. There was 19.5% decrease in growth as compared to that in m-NRC medium. In case of treated seawater ($NaHCO_3$, 8g/l) growth was 25% lesser than that of m-NRC medium. From the growth curve (Fig.3), it is evident that the growth of *S. platensis* in treated seawater: hatchery waste water in outdoor culture is comparable with that of m-NRC medium.

The observations indicate that seawater medium amended with sodium-bi-carbonate and hatchery waste water can support equivalent growth as recorded in an earlier study by Leema et al (2010) [21]. However, it is interesting to note that above workers used five chemicals ($NaHCO_3$, K_2HPO_4, $NaNO_3$, $FeSO_4.7H_2O$ and Fe_2EDTA) in seawater based medium whereas, the medium used in the present experiment was supplemented with sodium-bi-carbonate and equal volume of hatchery waste water only. These observations reveal that a proper mixing of hatchery waste water with seawater can reduce the dependence on commercial chemicals to a considerable extent. Therefore, the cost of production can be drastically reduced if the nutrient rich waste water

like hatchery waste water is used for the enrichment of seawater.

It was observed that SGR of treated seawater: hatchery waste water (1:1) was almost equal (98%) to that of modified NRC medium (P>0.05). A reduction of 24% SGR from that of modified NRC medium was observed in bicarbonate treated seawater. Generation Time obtained was 1.6, 1.9 and 2.3 days for control, treated seawater: hatchery waste water (P>0.05) and Treated seawater respectively. In case of outdoor cultivation of *S. platensis* using seawater based media, highest specific growth rate was obtained in treated seawater. Lower generation times are preferred for the mass cultivation, as the generation time increases, the rate of cell duplication declines and make the commercial cultivation uneconomical [22]. The generation time reported here for *S. platensis* cultured in seawater media was very close to the generation time reported for *S. platensis* cultured in Zarrouk's medium [23].

In another study, the growth of *S. platensis* has been evaluated in a complex medium containing seawater supplemented with anaerobic effluents from digested pig waste [24]. Biomass concentration (as dry weight) after 12 days of cultivation in the experimental medium was similar (P>0.05) to the one observed in chemically defined medium (Zarrouk's medium). But the protein content of the biomass in that medium was significantly lower as compared to the Zarrouk's medium.

The protein content obtained in indoor batch culture of *S. platensis* cultured in treated seawater amended with HWW medium showed only 6.5% decrease as compared to m-NRC medium. In case of outdoor culture, it increased slightly (3.6%). Similar observations were reported for *S. maxima* [11, 25]. Protein content in the seawater medium (55.7±0.82%) obtained in this study is comparable to the values reported for *S. maxima* (55.4-59.4%) cultured in seawater [10]. In a study, Ilknur [26]observed no significance difference (P>0.05) in crude protein values of *S.platensis* grown in an organic fertilizer media and prescribed medium. Desired level of protein for feed and food grade *Spirulina* is 50-70% [27]. Therefore, the quality of biomass produced in seawater based media was on par with the prescribed protein content for feed and food grade.

There is no significant difference (P>0.05) between phycocyanin content obtained in indoor batch culture using treated seawater: HWW (1:1) with that obtained from m-NRC medium. This comparison of phycocyanin content of *S. platensis* grown in prescribed (m-NRC) medium, seawater water based media reveals that phycocyanin content of the biomass grown in seawater water based media is comparable to the content of prescribed medium. Therefore, it is evident from above observations that utilization of seawater does not affect the quality of biomass and its vital constituents like phycocyanin. Since, higher phycocyanin content is one of the criteria for the assessment of the quality of biomass; the biomass produced in seawater amended with hatchery waste water media was of as good in quality as the biomass produced in prescribed growth medium where a large number of synthetic chemicals are used.

Extracted phycocyanin from different media subjected to a three stage purification process. Purity ratio (A_{620}/A_{280}) was measured after each stage. A final purity of 3.08-3.25 was obtained in seawater based media, whereas purity ratio obtained in m-NRC medium was 3.55 (Table 4). Hence, Phycocyanin extracted from *S. platensis* cultured in seawater based media is of good quality colourant grade and the purity level was very close to the reagent grade phycocyanin (Purity: A_{620}/A_{280} >4).

There is no significant difference (P>0.05) observed in chlorophyll-a among m-NRC medium (5.44 ± 0.15 mg/g) and treated seawater (4.95 ± 0.10). In pre-treated seawater enriched with certain chemicals, *S. platensis* showed chlorophyll-a content 7.85 ± 0.16 mg/g dry weight [21]. Carotenoid content obtained in treated seawater: HWW (1.81 ± 0.03) was significantly different (P<0.05) from that of m-NRC medium (2.14 ± 0.09). In an earlier investigation Carotenoid content of 1.55 mg/g DW from *S. platensis* at 32^{o}C was reported in the cultures grown in Zarrouk's medium [28].

An increasing trend of pH has been observed in all culture media. Similar observation has been obtained by other workers also [29]. It has been explained that the pH of the culture rises gradually as bicarbonate added to the medium is dissolved to produce CO_2, which releases OH^- during cultivation of *S. platensis.* Maximum growth, biomass, chlorophyll-a and protein content from *S. platensis* has been obtained at a pH range of 8-9 [30]. At the end of the culture period the pH range was 9.06-9.66.

The phosphate level in all culture media decreased at the end of culture period (Fig 5). The *S. platensis* biomass might have utilized phosphate for the growth. Same phenomenon was observed in an earlier study by Lodi et al (2003) [31]. Phosphate removal in the seawater media was more than 60% which is comparable to the values (67% reduction) reported by

Cheunbarn et al 2010[14] who used swine wastewater for cultivation.

10-20% removal of ammonia in seawater based media was observed which is lower than the values reported earlier for media amended with animal waste water[11, 14]. In the case of nitrate concentration, a slight reduction was observed in seawater media after the *S. platensis* cultivation. Though, the extent of nitrogen removal was not so pronounced as compared to earlier reports[11] however further increase in nitrogen removal can be achieved by increasing the culture density in the ponds.

From the table 1 it is clear that the cost of both treatment media could be considerably reduced (percentage reduction in price: 48.1 %) as compared to the prescribed medium. Moreover it is interesting to note that only 3% and 14 % reduction in growth in treated seawater amended with hatchery waste water medium and treated seawater medium, respectively (Fig 3), as compared to the growth in prescribed medium in the outdoor culture. Similarly there was no significant difference observed between control and treatments in the case of phycocyanin content even the prices are significantly reduced.

Conclusion

The data obtained during this investigation strengthens and validates the hypothesis that nutrients present in hatchery waste water and seawater can be utilized for the cultivation of commercially important, salt tolerant organisms like *S. platensis*. On the basis of the observation it can be concluded that in spite of being a fresh water species *S. platensis* is a suitable candidate species for large scale production in coastal areas. There is a vast scope for the technology for the production of value added products like phycocyanin from *Spirulina* biomass which can open new avenues of livelihood for the farmers in coastal areas.

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References

1. Ciferri, O., *Spirulina* an edible microorganism, *Microbiological reviews, American society of microbial.*, 47(4) (1983) 551-578
2. Glazer, A.N., Phycobiliproteins a family of valuable widely used fluorophores, *J. Appl. Phycol*., 6(1994) 105-112
3. Richmond, A., *Handbook of microalgal culture-biotechnology and applied physiology*, (Blackwell publishing, London) 2004, pp. 95-125
4. Henrikson, R., Earth and Food *Spirulina*, edited by Robert, (Ronore Enterprises Inc., Laguna Beach) 1989
5. Romay, C. & Gonzalez, R., Phycocyanin is an antioxidant protector of human erythrocytes against lysis by peroxyl radicals, *J. Pharm. Pharmacol*., 52(4) (2000) 367-368
6. Baldia, S.F., Fukami, K., Nishijima, T. & Hata, Y., Growth responses of *Spirulina platensis* to some physico-chemical factors and the kinetics of phosphorus utilization, *Fish. Sci*., 61 (1995) 331-335
7. Olguin, E.J., Simultaneous high-biomass protein production and nutrient removal using *Spirulina maxima* in sea water supplemented with anaerobic effluents, *World J. Microb. Biotechnol.*, 10(5) (2007) 576-578
8. Raoof, B., Kaushik, B.D. & Prasanna, R., Formulation of a low-cost medium for mass production of *Spirulina*, *Biomass and Bioenergy*, 30(6) (2006) 537-542
9. Faucher, O., Coupal, B. & Leduy, A., Utilization of seawater-urea as a culture medium for *Spirulina maxima*, *Can. J. Microbiol.*, 25(6) (1979) 752-759
10. Materassi, R., Tredici, M. & Balloni, W., *Spirulina* culture in seawater, *Appl. Microb. Biotechnol*., 19(6) (1984) 384-386
11. Olguin, E.J., Galicia, S., Camacho, R., Mercado, G. & Pérez, T., Production of *Spirulina* sp. in seawater supplemented with anaerobic effluents in outdoor race ways under temperate climatic conditions, *Appl. Microb. Biotech*., 48 (1997) 242-247
12. Gami, B., Naik, A. & Patel, B., Cultivation of *Spirulina* species in different liquid media, *J. Algal Biomass Utln*., 2(3) (2011) 15-26
13. Kaushik, R., Prasanna, R. & Joshi, H.C., Utilization of anaerobically digested distillery effluent for the production of *Spirulina platensis* (ARM 730), *Journal of Scientific and Industrial Research*, 63 (2006) 521-525
14. Cheunbarn, S. & Peerapornpisal, Y., Cultivation of *Spirulina platensis* using anaerobically swine wastewater treatment effluent, *Inter. J. Agri. Biol*., 12(4) (2010) 586-590
15. Guillard, R.R.L., Methods for microflagellates and nannoplankton. In: *Hand book of phycological methods*: *culture methods and growth measurements* edited by Stein, J.R., (Cambridge University Press, Cambridge) 1973, pp. 69-85
16. APHA, Standard methods for the examination of water and wastewater, 21st Ed. (American Public Health Association, Washington, DC) 2005
17. Siegelman, H.W. & Kycia, J.H., Algal biliproteins. *Hand book of phycological methods*, (Cambridge University Press, Cambridge) 1978, pp. 72-78
18. Boussiba, S. & Richmond, A.E., Isolation and characterization of phycocyanins from the blue-green algae *Spirulina platensis*, *Arch. Microbiol*., 120 (1979) 155-159
19. Cyanotech., Analysis of Beta-Carotene and Total Carotenoids from *Spirulina* (Spectrophotometric Method), *Spirulina* Pacifica Technical Bulletin #003b, (Cyanotech Corporation) 2002

20. AOAC, Official methods of Analysis, Assoiciation of official Analytical chemists. (Wshington DC, USA) 1984
21. Leema, J.M., Kirubagaran, R., Vinithkumar, N.V., Dheenan, P.S. & Karthikayulu, S., High value pigment production from *Arthrospira (Spirulina) platensis* cultured in seawater, *Bioresour. Technol*., 101(23) (2010) 9221-9227
22. Reinehr, C.O. & Costa, J.A.V., Repeated batch cultivation of the microalga *Spirulina platensis*, *World J. Microb. Biotechnol*., 22 (2006) 937-943
23. Costa, J.A.V., Linde, G.A., Atala, D.I.P., Mibielli, G.M. & Kruger, R.T., Modelling of growth conditions for cyanobacterium *Spirulina platensis* in microcosms, *World J. Microb. Biotech*., 16 (2000) 15-18
24. Olguin, E.J., Galicia, S., Guerrero, O.A. & Hernandez, E., The effect of low light flux and nitrogen deficiency on the chemical composition of *Spirulina* sp. (*Arthrospira*) grown on digested pig waste, *Biores. Technol*., 77 (2001) 19-24
25. Tredici, M.R., Papuzzo, T. & Tomaselli, L., Outdoor mass culture of *Spirulina maxima* in sea-water, *Appl. Microb. Biotechnol*., 24(1) (1986) 47-50
26. Ilknur, A.K., Effect of an organic fertilizer on growth of blue-green alga *Spirulina platensis*, *Aqucult. Int*., 20 (2012) 413-422
27. Vonshak, A., *Spirulina platensis*: *physiology, cell-biology and biotechnology*, (Taylor and Francis Group, LLC) 1986, pp. 79-162
28. Rafiqual, I.M., Hassan, A., Sulebele, G., Orosco, C.A., Roustaian, P. & Jalal, C.A., Salt stress culture of blue green algae *Spirulina fusiformis*. *Pak. J. Bio. Scie*., 6(7) (2003) 646-650
29. Richmond, A., Cell responses to environmental factors In: *Handbook of microalgal culture*, (CRC press, Boca Raton, Florida) 1986, pp. 69-99
30. Pandey, J.P., Pathak, N. & Tiwari, A., Standardization of pH and Light Intensity for the Biomass Production of *Spirulina platensis, J. Algal bio. Utili*., 1(2) (2010) 93-102
31. Lodi, A., Binaghi, L., Solisio, C., Converti, A. & Borghi, M.D., Nitrate and phosphate removal by *Spirulina platensis*, *J. Ind. Microb. Biotechnol*., 30 (2003) 656-660

The antioxidant activity of the cold water extract from food-grade Spirulina platensis was assessed using both chemical and cell-based assays.Â Most of the previous reports on antioxidant activity of Spirulina were based on chemical rather than cell-based assays.Â The 3T3 cells were incubated in medium containing Spirulina extract or vitamin C for 1 h before being added with 20 Î¼M DPPH or 50 Î¼M ABTS and incubated for another 24 h before the determination of apoptotic cell death. The positive control contained cells incubated with vitamin C while the negative control contained medium alone.Â Belay A: The potential application of Spirulina (Arthrospira) as a nutritional and therapeutic supplement in health management. J Am Nutraceut Assoc. 2002, 5 (2): 27-48.