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Potential of enzymatically hydorlysed seaweed products as cost-effective nutrient media for growth and lipid enhancement in the marine microalga *Nannochloropsis salina*

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ABSTRACT

Enzymatically hydrolysed seaweed products (EHSPs) prepared from the red seaweed *Gracilaria corticata* (EHSP-1) and brown seaweed *Stoechospermum marginatum* (EHSP-2) contained abundant levels of macro, micro and trace elements necessary to fulfil the nutritional requirement of the microalga *Nannochloropsis salina*. EHSP-1 had higher levels of N, P, K and Fe in comparison with EHSP-2. The growth performance of *N. salina* suggests that EHSP-1 was more efficient than EHSP-2. The growth rate of *N. salina* in EHSP-1 was 1.07 times higher than the control whereas in EHSP-2 the growth rate was 0.58 times lower than the control. The maximum biomass concentration of *N. salina* was achieved in EHSP-1 and EHSP-2 were 0.37 and 0.21 g l^{-1} with a biomass productivity of 0.027 and 0.0152 g l^{-1} d^{-1} respectively. The results of biochemical analyses also suggest that protein, pigment and lipid yield of *N. salina* was positively stimulated by EHSP. The findings suggest that the best concentration of EHSP-1 and EHSP-2 to achieve maximum biomass and lipid production of the marine microalga *N. salina* were 8 and 4% respectively. EHSP-1 enhanced the biomass and lipid production without affecting the nutritional properties of *N. salina* suggesting its potential applicability in aquaculture, biofuel and other related industrial sectors.

Keywords: Cellulase enzyme, Indigenous seaweed resources, *Nannochloropsis salina*, Nutrient rich media

Introduction

Microalgae hold enormous nutritional properties and their lipid content has drawn much attention for potential application in biodiesel production (Griffiths and Harrison, 2009; Carioca, 2010). They contain abundant levels of proteins, pigments and lipids, which make them play a major role in aquaculture as a food source mainly in larval rearing sector (Cho *et al*., 1999). Biomass production and lipid yield in microalgal cultivation mainly depends on the growth media used *i. e.* the energy source and nutrients present (Chen *et al*., 2011; Benavente-Valdes *et al*., 2016). Hence the culture media used for cultivation should be selected in such a way that it should satisfy the nutritional requirement thereby resulting in higher biomass production. The main constraint in microalgal cultivation is the high-cost of production which is mainly attributed to the price of commercial culture media (Acien *et al*., 2012). Hence nowadays attempts are being made to improve microalgal production methods which will reduce the cost of production and simultaneously increase the biomass yield (Nayak *et al*., 2013). Till date there are many findings on the effective utilisation of seaweeds as fertilisers for crops around the globe. The extracts of seaweeds contain enormous macro, micro and trace nutrients. In addition, they have growth promoting hormones which make them suitable to be used as fertilizers (Spinelli *et al*., 2010). An added advantage is that, they form organic and sustainable sources that can be obtained from nature (Akila and Jeyadoss, 2010). They also contain antioxidants and bioactive substances that play a major role in enhancing the growth rate in plants (Pacholczak *et al*., 2016). With this backdrop, the present study attempted to develop a cost effective culture medium for the marine microalga *Nannochloropsis salina*, incorporating enzymatically hydrolysed seaweed products from two different indigenous seaweed resources *viz*., the red seaweed *Gracilaria corticata* and the brown seaweed *Stoechospermum marginatum*.

Materials and methods

Marine microalga cultivation

Pure culture of the green microalga *Nannochloropsis salina* isolated from Gulf of Mannar region, Tamil Nadu, was obtained from the Phycology Laboratory, Fisheries College and Research Institute, Thoothukudi, Tamil Nadu, India. The pure strain was sub-cultured in F/2 medium and maintained under the following environmental conditions: Temperature: 18±2^{0}C, Photoperiod: light:dark=18:6 h and light intensity: 3000 lux.

Preparation of enzymatically hydrolysed seaweed products (EHSPs)

G. corticata (red seaweed) and *S. marginatum* (brown seaweed) were collected from the Hare Island shore region of Gulf of Mannar, Tamil Nadu, India. The seaweeds were washed thoroughly to remove salt, dirt and other attached organisms. The cleaned seaweeds were dried separately in hot air oven at 60^{0}C for 8 h and powdered. EHSPs were prepared as per the method of Uchida and Murata (2002) with slight modifications. Dried seaweed powder (1 kg each) was taken and mixed with sterile 3% NaCl in the ratio 1:9, to which 10 g of cellulase enzyme (Enzyme Bioscience Pvt. Ltd.) was added and mixed thoroughly. The mixture was kept in an air tight container to maintain anaerobic condition which was stirred daily to boost the fermentation process. After 20th day of enzymatic hydrolysis of seaweed powder, the mixture was filtered and subjected to centrifugation at 5000 rpm for 20 min to obtain a clear supernatant which was used as culture medium for *N. salina*.

Nutrient composition of EHSPs

The major, minor and trace elements of the EHSPs prepared were analysed on the 5th, 10th, 15th and 20th day of the fermentation process. The level of ammoniacal nitrogen and phosphorous present were estimated by Nessler method (Crosby, 1968) and Amino Naphthol Sulphonic method (Allen, 1940) respectively. Sodium and potassium levels were analysed using flame photometer (Hald, 1947). The level of other trace elements like iron, zinc, calcium, magnesium, manganese, cobalt, copper and molybdenum were estimated using Atomic Absorption Spectroscopy (AAS) (AA-6880, Shimadzu Corporation, Japan). Nutrient levels of the hydrolysed products were also analysed

Experimental design

All the experiments were carried out in triplicates with different concentrations of the EHSPs *viz*., 1, 2, 4, 6, 8 and 10% as treatments and F/2 media as control. Algal inoculum of *N. salina* at 5% was added in all the treatments as well as control.

Algal cell count and growth rate estimation

Algal samples of one ml each were drawn from all the treatments as well as control and counted using haemocytometer (improved Neubauer Chamber) under light microscope (Nikon Eclipse, TS 100). The specific growth rate (μ) of the microalga was estimated using the formula given by Levasseur *et al.* (1993):

$$\mu = \ln (x_2) - \ln (x_1)/ t_2-t_1$$

where, μ = Growth rate per unit amount of cell concentration; x_1 and x_2 = Cell concentration at time 1 (t_1) and time 2 (t_2), respectively.

From the specific growth rate, the doubling time (hours) of microalgal cells was calculated using the equation (Fogg and Thake, 1987):

$$td = \ln 2/\mu$$

Determination of biomass dry weight

A 5 ml algal culture was filtered to obtain the wet biomass, to which 10 ml of ammonium bicarbonate was added to remove the adhered salts. This was filtered using membrane filter of pore size 0.7 μm. The filtered biomass along with filter paper was dried at 80^{o}C in hot air oven for 12 h and then in desiccator and the dried filter paper with biomass was weighed to obtain the algal biomass dry weight (Rizwan *et al.,* 2017).

Pigment analysis

To analyse the pigment content, 5 ml of algal sample was centrifuged (Remi C-24 plus refrigerated centrifuge) at 5000 rpm for 10 min. To the algal pellet, 5 ml of N, N, dimethylformamide was added and kept for 24 h. The same centrifugation step was repeated and the supernatant was collected. The OD value of the supernatant was measured using a spectrophotometer (PerkinElmer, Lambda 25) at 461, 647 and 664 nm.

The pigments *viz.,* chlorophyll-*a* and chlorophyll-*b* (Chamovitz *et al.,* 1993) and carotenoid (Moran, 1982) present in the microalgae were calculated as follows:

$$\text{Chlorophyll-}a\ (\mu g\ ml^{-1}) = OD664 \times 11.92$$

$$\text{Chlorophyll-}b\ (\mu g\ ml^{-1}) = -5.6 \times OD664 + 23.26 \times OD647$$

$$\text{Carotenoid}\ (\mu g\ ml^{-1}) = [OD461 - (0.046 \times OD664)] \times 4$$

Protein analysis

To analyse the protein content, 1 ml of algal sample was taken to which 4.5 and 0.5 ml of reagent I and II were added. The mixture was kept undisturbed for 30 min and the absorbance was measured at 660 nm using spectrophotometer (PerkinElmer, Lambda 25). Reagent I was prepared by adding three different reagents A

(2% Na_2CO_3 in 0.1 N NaOH), B (1% NaK tartrate in H_2O) and C (0.5% $CuSO_4.5H_2O$ in H_2O) in the ratio of 48:1:1, whereas reagent II was prepared by mixing Folin phenol with water in the ratio of 1:1 (Lowry *et al*., 1951).

Lipid analysis

Algal pellet was harvested by centrifuging 40 ml of microalgal sample for 10 min at 3000 rpm. To the pellets, 7.6 ml of chloroform:methanol:water was added in the ratio of 1:2:0.8 (v/v/v). This mixture was sonicated for 1 min at 20 kHz frequency;100 W using a sonicator (Labman Scientific Instruments) followed by vortexing for 30 s, separating three different layers, the upper methanol and bottom chloroform with lipid layer. The upper methanol layer was collected separately and extraction process was repeated. The chloroform layers were combined and dried in oven at 80°C for 24 h to evaporate the chloroform. The resultant lipid was weighed and expressed as lipid content per ml of microalgal culture (Rizwan *et al.,* 2017).

Statistical analysis

SPSS 22.0 (SPSS Inc., Illinois) was used to calculate the standard deviation and mean of the experimental data. One-way ANOVA was used in finding the difference between the treatments and control. Duncan's Multiple Range Test was used to find the mean difference at significance level of 0.05.

Results and discussion

Nutrient composition of EHSPs

The products derived from the enzymatic hydrolysis of seaweeds (EHSP-1 and EHSP-2) were found to contain high levels of macro, micro and trace nutrients to satisfy the nutritional requirement of marine microalgae. Nutrient level of the EHSP-1 (Table 1) was found to be high in all aspects than EHSP-2 (Table 2). From the results of both EHSPs, the levels of nutrients were found to be increasing till the 15th day of hydrolysis process and level got decreased on the 20th day. Hence the experiment was conducted for only 20 days and the product was filtered, centrifuged and the clear supernatant was used as nutrient media for microalgal culture. Among the two products, EHSP-1 was found to have significantly higher level of nutrients than EHSP-2. In both EHSPs, the dominant macro nutrients were sodium followed by potassium, calcium and nitrogen whereas the dominant trace nutrients were iron and zinc. In EHSP-1, the major nutrients like nitrogen, phosphorous and potassium were found to be at high levels *viz.,* 951, 128 and 6738 mg l^{-1} respectively, whereas in EHSP-2, the levels were significantly lower ($p<0.05$) *viz.,* 241, 158 and 6263 mg l^{-1} respectively. The levels of important trace elements, iron and zinc were found to be 146 and 133 mg l^{-1} in EHSP-1 and 74 and 114 mg l^{-1} and EHSP-2 respectively. Among the major nutrients, potassium level was very high in both EHSPs. In seaweed extracts, potash and other nutrients were found to be in enormous amount which are water soluble and hence easily available for absorption by plants thereby preventing the nutrition deficiency issue (Mohanty *et al*., 2013). Nasmia (2014) reported that seaweeds contain high levels of macro, micro and trace nutrients. Nutrients such as N, P, K, Ca and Mg play important roles in microalgal biomass and lipid productivity. From this we can conclude that EHSPs are suitable to be used as culture media for microalgae since these contain all essential nutrients that fulfil the requirement to achieve maximum biomass production of marine microalgae. EHSP-1 prepared from red seaweed (*G. corticata*) had significantly higher levels of essential macro and trace nutrients compared to EHSP-2, prepared from brown seaweed (*S. marginatum*). The present findings confirm with those of Raja *et al.* (2015) who observed higher amounts of nutrients in the seaweed extracts of *K. alvarezii* (red seaweed) when compared to that in *T. conoides* (brown seaweed).

Table 1. Nutrient composition of EHSP-1

Macro elements (mg l^{-1})	5th day	10th day	15th day	20th day
N	257	639	947	951
P	66	107	133	128
K	1087	5573	6711	6738
Ca	438	845	1394	1277
Mg	78	282	328	352
Na	4438	8148	9551	9566
Trace elements (mg l^{-1})				
Fe	53	116	142	146
Mn	NT	12	17	15
Cu	NT	1.2	3.8	3.2
Zn	46	129	127	133
Co	NT	NT	NT	NT
Mo	NT	NT	NT	NT

Table 2. Nutrient composition of EHSP-2

Macro elements (mg l^{-1})	5th day	10th day	15th day	20th day
N	110	217	223	241
P	68	146	169	158
K	1692	6007	6126	6263
Ca	1368	3173	3083	3179
Mg	18	344	342	358
Na	5394	7268	7395	7762
Trace elements (mg l^{-1})				
Fe	28	62	77	74
Mn	1.5	3	4.1	4.3
Cu	NT	2.7	2.6	2.72
Zn	86	103	112	114
Co	NT	NT	NT	NT
Mo	NT	NT	NT	NT

The production cost of one litre EHSP was 0.1 USD which is attributed to the cost of cellulase enzyme. Lam and Lee (2012) reported that organic fertiliser for microalgal cultivation costs around 1.20 USD per 400 g, whereas another documentation by Zheng *et al.* (2016) reveals that preparation of Kelp Waste Extract for microalgal cultivation costs around 0.28 USD. In comparison with the documented reports, the production cost of the EHSP in the present study was found to be cost effective.

Effect of EHSPs on growth rate of microalgae

For *N. salina* grown in EHSP-1, the maximum cell density of 26.74, 23.8 and 22.8×10^6 cells were observed at 8, 10 and 6% concentration respectively (Fig. 1) with a biomass production of 0.3794, 0.3377 and 0.3235 g l^{-1} respectively. The biomass production obtained at 8% concentration (0.3794 g l^{-1}) was significantly higher ($p<0.05$) than the control (F/2 media) *i.e.,* 0.3519 g l^{-1} (Table 3). The results suggest 8% concentration of EHSP-1 as the best media concentration to maximise the biomass production, even higher than the control. Zheng *et al.* (2016) reported similar results of enhancement in biomass production of four different microalgae *viz., Chlorella* sp., *Chlorella sorokiniana*, *Spirulina maxima* and *Phaeodactylum tricornutum* cultured using kelp waste extract (KWE). Another study by Lakshmi and Sheeja (2021) also stated that there was an increase in cell density of the microalga *Chlorella vulgaris* when grown in commercial media BBM supplemented with red seaweed extracts of *G. corticata* and *Grateloupia lithophila* in comparison with BBM exclusively grown microalgae. The present results are also in agreement with those reported by Cho *et al.* (1999) that the microalga *Isochrysis galbana* cultured in the extracts of seaweed *Monostroma nitidum* resulted in higher biomass productivity when compared with the control. Seaweed extracts not only contain enormous amount of essential nutrients but also growth promoting agents such as auxins, cytokinins and gibberlins which stimulate the growth rate of the microalgae (Crouch and Van Staden, 1993).

In EHSP-2 grown microalgae, the maximum cell density of 14.98, 13.8 and 12.44×10^6 cells were found at 4, 2 and 1% concentration respectively with a biomass production of 0.2125, 0.1958 and 0.1765 g l^{-1} respectively whereas in control, maximum cell density obtained was 25.92×10^6 cells (0.3614 g l^{-1}) (Fig. 2). The highest microalgal cell density obtained in EHSP-2 treatment was significantly lower than the control. The lower growth exhibited by the microalgae grown using EHSP-2 may be due to nutrient deficient condition, since the result of chemical composition clearly shows that levels of nitrogen and iron were significantly lower in comparison with EHSP-1. Another reason could be the dark colouration of EHSP-2, which would inhibit light penetration, thereby reducing the growth rate in the treatments. The results are comparable with the documentation of Malik *et al.* (2018) that the growth rate of the microalga *Chaetoceros gracilis* was decreased when grown under media with higher concentration of brown seaweed extract of *Sargassum* sp. Another study by Alvarado *et al.* (2008) also reported growth inhibition effect of higher dose of brown seaweed extract in *Chaetoceros muelleri*. Based on the results of the present study, EHSP-1 can be considered as an

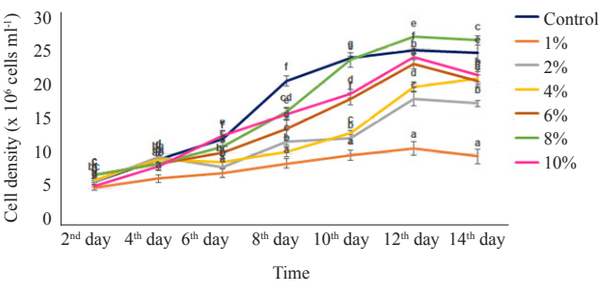

Fig. 1. Growth curve of *N. salina* at different concentrations of EHSP-1

Table 3. Growth parameters of *N. salina* cultured in EHSPs

EHSP-1	SGR (per day)	Doubling time (h)	Biomass concentration (g l^{-1})	Biomass productivity (g l^{-1} d^{-1})
Control	0.1698±0.0122	4.08	0.3519±0.0306	0.0251±0.0011
1%	0.0942±0.0042	7.35	0.1532±0.0528	0.0109±0.0038
2%	0.1399±0.0038	4.96	0.2531±0.0766	0.0181±0.0046
4%	0.1484±0.0056	4.67	0.2781±0.0441	0.0199±0.0082
6%	0.1622±0.0021	4.27	0.3235±0.0332	0.0231±0.0145
8%	0.1767±0.0109	3.92	0.3794±0.0176	0.0271±0.0059
10%	0.1661±0.0073	4.17	0.3377±0.0529	0.0241±0.0044
EHSP-2				
Control	0.1401±0.0254	4.95	0.3614±0.0515	0.0258±0.0083
1%	0.0930±0.0085	7.45	0.1958±0.0475	0.0140±0.0031
2%	0.0850±0.0185	8.15	0.1765±0.0168	0.0126±0.0075
4%	0.0993±0.0041	6.98	0.2125±0.0473	0.0152±0.0142
6%	0.0529±0.0026	13.09	0.1163±0.0116	0.0083±0.0016
8%	0.0518±0.0073	13.38	0.1146±0.0241	0.0082±0.0027
10%	0.0302±0.0053	22.96	0.0865±0.0175	0.0062±0.0076

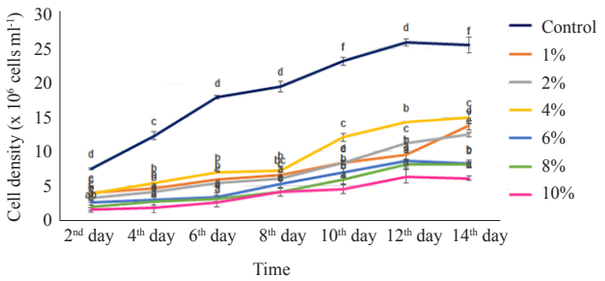

Fig. 2. Growth curve of *N. salina* in different concentrations of EHSP-2

effective culture medium with strong growth stimulation effect on *N. salina* in comparison with EHSP-2 as well as the commercial F/2 medium.

Effect of EHSPs on protein content

The media used for microalgae should enhance the biomass production without impacting the nutritional quality of the cultured microalgal species (Herrero *et al.,* 1991; Zhang, 1997). Among the two EHSPs tried in the present study, the microalgae cultured in EHSP-1 was observed to have significantly higher protein content than EHSP-2 and control. The maximum protein content of 0.84 mg ml^{-1} was found at 8% EHSP-1 followed by 0.74 and 0.71 mg ml^{-1} at 10 and 6% concentration of EHSP-1 respectively (Fig. 3). Maximum protein content was obtained from 8% EHSP-1 which was significantly higher ($p<0.05$) than the control (0.7439 mg ml^{-1}). The present study results clearly showed that the microalgal protein content increased when grown in EHSP-1. Similar results were reported by Rohani-Ghadikolaei *et al.* (2012) that the protein content of the microalga *I. galbana* was found to increase when the microalga was grown using commercial media F/2 supplemented with red and green seaweed extracts of *G. corticata, Ulva lactuca* and *Enteromorpha intestinalis.*

In EHSP-2 treatments, 4% concentration resulted in higher protein content *i.e.*, 0.51 mg ml^{-1} followed by 0.42 mg ml^{-1} in 2% concentration. The highest protein value obtained in EHSP-2 treatments was significantly lower than the control. The results of protein content positively correlated with the biomass concentration. Study by Alvarado *et al*. (2008) has shown similar results of decrease in protein content of microalga *C. muelleri* cultured using extracts of brown seaweed with amended silicate *i.e.,* the protein content was found to be 2-5% whereas in control (F/2 media) grown microalgae the protein level was 12%. Results of the present study clearly shows that red seaweed extract has better potential to increase the protein content of the cultured microalgae in comparison with commercial media and brown seaweed extracts.

Effect of EHSPs on pigment content

Analysis of pigment content of microalgae revealed that EHSP-1 had significantly stimulated the photosynthetic performance of microalgae. The maximum chlorophyll-*a* content of *N. salina* was 6.01 µg ml^{-1} observed in 8% followed by 5.5 and 5.21 µg ml^{-1} in 10 and 6% EHSP-1 (Fig. 4). The maximum value of pigment obtained was significantly higher ($p<0.05$) than the control (5.17 µg ml^{-1}). Similarly, the carotenoid value was higher in 8% treatment *i.e.,* 2.16 µg ml^{-1} followed by 10 and 6% *i.e.,* 2.08 and 1.84 µg ml^{-1}. The carotenoid value obtained from 8% EHSP-1 treatment was significantly higher ($p<0.05$) than the control (2.05 µg ml^{-1}) (Fig. 5). The present study results were in agreement with those reported by Raja *et al.* (2015) that the chlorophyll content of the microalga *C. muelleri* and *Dunaliella salina* increased when grown in the commercial media F/2 and Walne's media supplemented with extracts of *Kappaphycus alvarezii.* The stimulating effect of seaweed extracts on pigment synthesis could be attributed to the presence of the phytohormones, especially cytokinins which enhance the nutrient absorption/uptake capacity of microalgae thereby increasing the pigment synthesis (Zhang, 1997). In EHSP-2 treatments, the chlorophyll-*a* values recorded were 3.97, 3.59 and 3.1 µg ml^{-1} in 4, 2 and 1% EHSP-2 treatments respectively which were significantly lower than the values in control *i.e.,* 5.17 µg ml^{-1}. The maximum carotenoid values obtained for microalgae cultured in EHSP-2 were 1.39,13.6 and 0.78 µg ml^{-1} in 2, 4 and 1% respectively which were significantly lower than the carotenoid value of control (2.05 µg ml^{-1}). The reason behind the reduction in pigment synthesis of microalgae in EHSP-2 may be

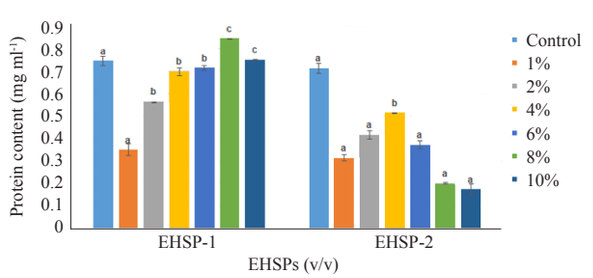

Fig. 3. Protein content of *N. salina* grown in EHSPs at various concentrations

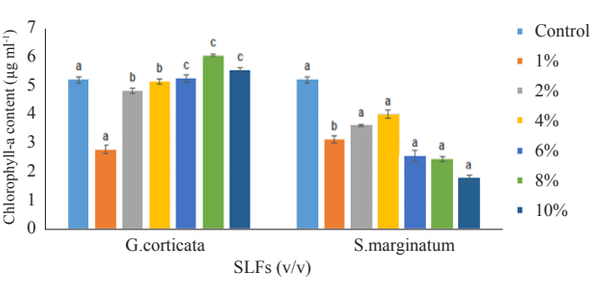

Fig. 4. Chlorophyll-*a* content of *N. salina* grown in EHSPs at various concentrations

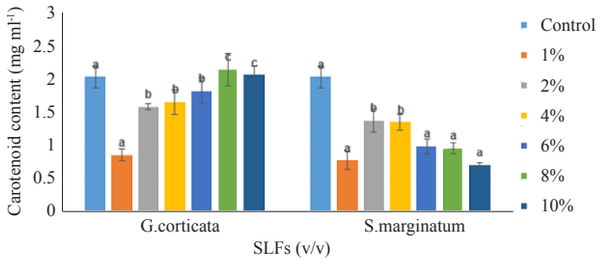

Fig. 5. Carotenoid content of *N. salina* grown in EHSPs at various concentrations

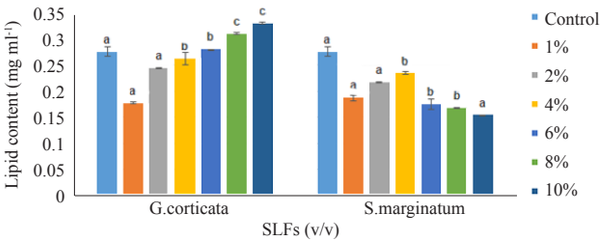

Fig. 6. Lipid content of *N. salina* grown in EHSPs at various concentrations

the low iron availability as iron serves as a main factor in increasing the photosynthetic performance of microalgae (Kong *et al*., 2014).

Effect of EHSPs on lipid content

EHSP-1 was found to stimulate lipid production in *N. salina* compared to F/2 medium. The maximum lipid yield was obtained at 10% followed by 8 and 6% which was 0.33, 0.31 and 2.28 mg ml^{-1} whereas in control the lipid yield obtained was 0.27 mg ml^{-1} (Fig. 6). The lipid yield of microalgae cultured using EHSP-1 was significantly higher than the control. The lipid yields of EHSP-2 cultured microalgae were 0.23, 0.21 and 0.18 mg ml^{-1} at 4, 2 and 1% respectively which were significantly lower than the control. Gireesh (2009) reported increase in lipid yield from *D. salina* cultured in seaweed liquid fertiliser compared to control (Conveyor Walne's media). Another study by Rohani-Ghadikolaei *et al.* (2012) also showed that microalga *I. galbana* cultivated in seaweed extract (SWE) had more lipid production than control (F/2 medium). The reason behind the potential of seaweed extracts in enhancing microalgal lipid yield was documented by Liang *et al.* (2009) that seaweed extracts contain enormous amount of carbon which has stimulation effect on lipid production and accumulation in microalgal cells.

Results of the present study clearly indicated that media prepared using enzymatic hydrolysis product of red seaweed (EHSP-1) was more efficient in enhancing the biomass and lipid yield of *N. salina* compared to brown seaweed (EHSP-2). An added advantage is EHSPs were prepared using indigenous seaweed resources following a simple enzymatic hydrolysis process. Enzymatic hydrolysis of biomass can derive an effective fermentable product with loaded nutrients (Karemore *et al*., 2013). EHSPs enhanced the biomass production of *N. salina* without compromising the nutritional quality of the microalgae, suggesting its potential application in aquaculture, biofuel and other related industrial sectors.

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References

Acien, F. G., Fernandez, J. M., Magan, J. J. and Molina E. 2012. Production cost of a real microalgae production plant and strategies to reduce it. *Biotechnol. Adv*., 30: 1344-1353. doi: 10.1016/j.biotechadv.2012.02.005.

Akila, N. and Jeyadoss, T. 2010. The potential of seaweed liquid fertilizer on the growth and antioxidant enhancement of *Helianthus annuus* L. *Oriental Journal of Chemistry*, 26: 1353. http;//www.orientjchem.org/p=23850.

Allen, R. J. L. 1940. The estimation of phosphorus. *Biochem. J*., 34: 858-865. doi: 10.1042/bj0340858.

Alvarado, D., Buitrago, E., Sole, M. and Frontado, K. 2008. Experimental evaluation of a composted seaweed extract as microalgal culture media. *Aquac Int.,* 16: 85-90.

Benavente-Valdes, J. R., Aguilar, C., Contreras-Esquivel, J. C., Mendez-Zavala, A. and Montanez, J. 2016. Strategies to enhance the production of photosynthetic pigments and lipids in chlorophycae species. *Biotechnol. Rep*., 10: 117-25. https://doi.org/10.1016/j.btre.2016.04.001.

Carioca, J. O. 2010. Biofuels: Problems, challenges and perspectives. *Biotechnol. J*., 5: 260- 273.

Chamovitz, D., Sandmann, G. and Hirschberg, J. 1993. Molecular and biochemical characterisation of herbicide-resistant mutants of cyanobacteria reveals that phytoene desaturation is a rate-limiting step in carotenoid biosynthesis. *J. Biol. Chem*., 268: 17348-17353. DOI:10.1016/S0021-9258(19)85341-3.

Chen, C. Y., Yeh, K. L., Aisyah, R., Lee, D. J. and Chang J. S. 2011. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. *Bioresour. Technol*., 102: 71-81. https://doi.org/10.1016/j.biortech.2010.06.159.

Cho, J. Y., Jin, H. J., Lim, H. J., Whyte, J. N. C. and Hong Y. K. 1999. Growth activation of the microalga *Isochrysis galbana* by the aqueous extract of the seaweed *Monostroma nitidum*. *J. Appl. Phycol*., 10: 561-567.

Crosby, N. T. 1968. Determination of ammonia by the Nessler method in waters containing hydrazine. *Analyst*, 93: 406-408.

Crouch, I. J. and Van Staden, J. 1993. Evidence for the presence of plant growth regulators in commercial seaweed products. *Plant Growth Regul*., 21-9.

Fogg, G. E. and Thake, B. 1987. *Algal cultures and phytoplankton ecology,* 3rd edn.The Universityof Winsconsins Press Ltd., London, UK.

Gireesh, R. 2009. Proximate composition, chlorophyll a, and carotenoid content in *Dunaliella salina* (Dunal) Teod (Chlorophycea:Dunaliellaceae) cultured with cost-effective seaweed liquid fertiliser medium. *Turk. J. Bot.,* 33: 21-26.

Griffiths, M. J. and Harrison, S. T. 2009. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *J. Appl. Phycol*., 21: 493-507. https://doi.org/10.1007/s10811-008-9392-7.

Hald, P. M. 1947. The flame photometer for the measurement of sodium and potassium in biological materials. *J. Biol. Chem*., 167: 499-510.

Herrero, C., Cid, A., Fabregas, J. and Abalde, J. 1991. Yields in biomass and chemical constituents of four commercially important marine microalgae with different culture media, *Aquac. Eng*., 10: 99-110.

Karemore, A., Pal, R. and Sen, R. 2013. Strategic enhancement of algal biomass and lipid in *Chlorococcum infusionum* as bioenergy feedstock. *Algal Res*., 2(2): 113-121. DOI: 10.1016/j.algal.2013.01.005

Kong, W., Liu, N., Zhang, J., Yang, Q., Hua, S., Song, H. and Xia, C. 2014. Optimisation of ultrasound-assisted extraction parameters of chlorophyll from *Chlorella vulgaris* residue after lipid separation using response surface methodology. *J. Food Sci. Technol*., 51(9): 2006-2013. doi: 10.1007/s13197-012-0706-z.

Lakshmi, D. and Sheeja 2021. Red seaweed extract as a biostimulant on growth and biochemical parameters of microalgae *Chlorella vulgaris*. *International Journal of Multidisciplinary Research In Arts, Science and Commerce*, 1(1): 26-37.

Lam, M. K. and Lee K. T. 2012. Microalgae biofuels: A critical review of issues, problems and the way forward. *Biotechnol. Adv*., 30: 673-690. doi: 10.1016/j.biotechadv.2011.11.008.

Levasseur, M., Thompson, P. A. and Harrison, P. J. 1993. Physiological acclimation of marine phytoplankton to different nitrogen sources. *J. Phycol*., 5: 587-595. https://doi.org/10.1111/j.0022-3646.1993.00587.x.

Liang, Y., Sarkany, N. and Cui, Y. 2009. Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions. *Biotechnol. Lett.,* 31: 1043-9. doi: 10.1007/s10529-009-9975-7.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Boil. Chem*., 193: 265-275. DOI:10.1016/S0021-9258(19)52451-6.

Malik, A. A., Khaeruddin, K. and Fitriani, F. 2018. The effect of *Sargassum* extract on culture medium to the growth of *Chaetoceros gracilis*. *Aquac. Indonesiana,* 19: 10-14. DOI: http://dx.doi.org/10.21534/ai.v19i1.115

Mohanty, D., Adhikary, S. P. and Chattopadhyay, G. N. 2013. Seaweed liquid fertiliser (SLF) and its role in agriculture productivity. *Ecoscan*, 3: 147-55.

Moran, R. 1982. Formulae for determination of chlorophyllous pigments extracted with N, N-dimethylformamide. *Plant Physiol*., 69: 1376-1381. https://doi.org/10.1104/pp.69.6.1376.

Nasmia 2014. *Prevention of ice-ice disease in Gracilaria verrucosa in an environmentally friendly manner using Caulerpa racemosa*. Dissertation, Hasanuddin University Agricultural Science Study Program. Makassar, Indonesia (In Indonesian).

Nayak, M., Rath, S. S., Thirunavoukkarasu, M., Panda, P. K., Mishra, B. K. and Mohanty, R. C. 2013. Maximizing biomass productivity and CO_2 biofixation of microalga *Scenedesmus* sp. by using sodium hydroxide. *J. Microbiol. Biotechnol*., 23: 1260-126. https://doi.org/10.4014/jmb.1302.02044.

Pacholczak, A., Nowakowska, K. and Pietkiewicz, S. 2016. The effects of synthetic auxin and a seaweed-based biostimulator on physiological aspects of rhizogenesis in ninebark stem cuttings. *Not. Bot. Horti. Agrobot. Cluj Napoca,* 44: 85-91. https://doi.org/10.15835/nbha44110061.

Raja, R., Hemaiswarya, S., Carvalho, I. S. and Arunkumar, K. 2015. Impact of industrial salt effluent and seaweed liquid fertilisers on three microalgae. *Rev. Bras. Bot*., 3: 547-553. https://doi.org/10.1007/s40415-015-0170-x.

Rizwan, M., Mujtaba, G. and Lee, K. 2017. Effects of iron sources on the growth and lipid/carbohydrate production of marine microalga *Dunaliella tertiolecta*, *Biotechnol. Bioprocess Eng*., 22: 68-75. DOI:10.1007/s12257-016-0628-0.

Rohani-Ghadikolaei, K., Ng, W. K., Abdulalian, E., Naser, A. and Yusuf, A. 2012. The effect of seaweed extracts, as a supplement or alternative culture medium, on the growth rate and biochemical composition of the microalga, *Isochrysis galbana* (P ark 1949). *Aquac. Res*., 43: 1487-98. https://doi.org/10.1111/j.1365-2109.2011.02951.x.

Spinelli, F., Fiori, G., Noferini, M., Sprocatti, M. and Costa, G. 2010. A novel type of seaweed extract as a natural alternative to the use of iron chelates in strawberry production, *Sci. Hortic*., 125: 263-269. https://doi.org/10.1016/j.scienta.2010.03.011.

Uchida, M. and Murata, M. 2002. Fermentative preparation of single cell detritus from seaweed *Undaria pinnatifida*, suitable as a replacement hatchery diet for unicellular algae. *Aquaculture*, 207: 345-57. https://doi.org/10.1016/S0044-8486(01)00792-X.

Zhang, X. 1997. *Influence of plant growth regulators on turfgrass growth, antioxidant status and drought tolerance.* Doctoral dissertation, Virginia Polytechnic Institute and State University, Virginia, USA.

Zheng, S., He, M., Jiang, J., Zou, S., Yang, W., Zhang, Y., Deng, J. and Wang, C. 2016. Effect of kelp waste extracts on the growth and lipid accumulation of microalgae. *Bioresour. Technol*., 201: 80-88. https://doi.org/10.1016/j.biortech.2015.11.038.

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