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Culture of Nannochloropsis salina in Different Marine Grow Out Effluents

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ABSTRACT

Effluents from different indoor recirculatory marine grow out systems at the Fisheries Harbour Laboratory of Central Marine Fisheries Research Institute, Kochi, were tried as media for culture of Nannochloropsis salina. The experiment was laid out with seven treatments and three replications to arrive at the best medium for growth of *Nannochloropsis salina* indoors. The treatments tried were commercial grade farm chemicals (0.1g ammonium sulphate + 0.01g super phosphate + 0.01g urea in 1 litre), Walne's medium as well as culture effluents of rotifer (*Brachionus plicatilis*), clam (*Meretrix casta*), grouper (*Epinephelus tauvina*), crab (Scylla tranquebarica) and sea water (control). *Nannochloropsis salina* inoculum to medium ratio was 1:2. Comparison of the growth among different treatments showed that all media were definitely more productive than seawater. Grouper culture effluent produced highest concentration of *Nannochloropsis salina* (115 million ml^{-1}) followed by Walne's medium (95 million ml^{-1}). The third in performance was rotifer culture effluent which was on par with farm chemicals followed by crab culture effluent which in turn was followed by clam culture effluent. Stationary and declining phase continued till 50th day on which the experiment was terminated. Initial nutrient analyses of media and after 50 days of culture indicated whereever effluents possessed high initial content of NH_3-N, more than 95% of it was used up by *Nannochloropsis salina.* Grouper culture effluent had lower initial content of NH_3-N, out of which 52% was used up by *Nannochloropsis salina.* In the case of NO_3, more than 95% was used by *Nannochloropsis salina* in all the media tried. Except for Walne's medium, farm chemicals and seawater, more than 90% of NO_2 was used by *Nannochloropsis salina.* Irrespective of the medium used, phosphate utilization was more than 60% in all the cases.

Key words: Indoor, recirculatory, rotifer, grouper

INTRODUCTION

Untreated discharge of effluents from marine grows out systems cause environmental hazards as they contain high nutrient load. In closed or semi enclosed systems, effluent can be used for the production of microalgae which in turn can be fed directly to larvae of fin and shell fish and rotifers. This will save and economize the use of costly chemicals for culture of microalgae. *Nannochloropsis salina* is a widely used live feed for rotifer culture for larval rearing of marine finfish and shellfish culture. Among the microalgae used to feed rotifers *Nannochloropsis* sp. was found to support high rates of rotifer reproduction. It is well known that rotifer fed with *Nannochloropsis* sp. contain sufficient quantity of unsaturated fatty acids required for the larvae of finfish and crustaceans

(Hirayama *et al.*, 1979). Usually media with inorganic chemicals like Walne's medium (Walne, 1974) or commercial grade farm chemicals are used for culture of *Nannochloropsis salina*. If these chemical media can be replaced by the effluent water, the use of costly chemicals can be avoided and environmental contamination minimised. The growth pattern of *Nannochloropsis salina* in different media is studied to find out a suitable substitute for Walne's medium. Microscopic algae are efficient removers of nutrients from waste water effluents (Craggs *et al.*, 1997, Gonzalez *et al.*, 1997 and Lefebvre *et al.*, 1996). Ammonia and phosphorus removal efficiency of microscopic algae from effluents can be made use for standardizing a new medium consisting of waste water for the algal growth. The present investigation was initiated with these objectives. Use of rotifer culture effluent for culture of *Nannochoropsis* sp. has been tried elsewhere (Chebil and Yamasaki, 1998). Shirai *et al.*, (1998) examined the growth of different marine micro algae on soy sauce waste extract. The feasibility of using intensive fish farm effluents (sea bass, sea bream) was evaluated as a source of inorganic nutrients for continuous mass production of marine diatoms (Lefebvre and Hussenot, 1998).

MATERIAL AND METHODS

The experiment was set up in randomized block design with seven treatments and three replications. The treatments were T1 - Farm chemicals (0.1g ammonium sulphate + 0.01g super phosphate + 0.01g urea in 1 litre), T2 - Walne's medium (Walne, 1974), T3 – rotifer (*Brachionus plicatilis*) culture effluent, T4 – clam (*Meretrix casta*) culture effluent, T5 – grouper (*Epinephelus tauvina*) culture effluent , T6 – crab (*Scylla tranquebarica*) culture effluent and T7 – sea water (Control).

Grouper culture effluent was collected from a 5 ton fibre glass tank which had 7.5 kg of adult fish biomass. They were fed once in a day with small whole - fish at the rate of 3% of body weight. The uneaten, if any, were removed after half an hour of feeding. The faecal matter was removed once after feeding and once in the evening every day. The fish tank was provided with two immersed *in situ* biofilters equaling 1/14 of the tank area. Crab culture effluent was collected from 325 l culture water in a fibre glass tank in which two mud crabs having a total biomass of 1.11 Kg were reared. The crabs were fed daily once with fresh clam meat at the rate of 3% of body weight. The left over food and faecal matter were siphoned daily. Effluents from clam culture tank of 50 l culture water which had 362g (shell on) biomass was taken as treatment material. The clams were fed with *Nannochloropsis salina* twice a day. For the experiment rotifer culture effluent water was collected from a culture medium in which number of rotifer was maintained at a concentration of 65 ml^{-1} for a week and fed with *Nannochloropsis salina*.

Ten litre of effluent water from each of the said culture systems were simultaneously collected for the study and stored at -20°C in separate containers. Thereafter, only required quantity of effluent was taken out and used for experiment by bringing them to room temperature. Each experiment was conducted in 3 l round shaped transparent polythene containers. *Nannochloropsis salina* inoculum to medium was applied in the ratio of 1:2. The average cell size of the inoculum was 2–3 µ. The media were sterilized using 2 ppm available chlorine solution after adding the inoculum, to ward off ciliates. Temperature throughout was maintained in a range of 24 to 26°C. Continuous supplementary aeration was given uniformly to all treatments. Experimental containers were exposed to 12 hours of artificial light and 12 hours of darkness continuously in a day. The containers were kept 25 cm away from two fluorescent domestic tubes. The experiment was repeated three times.

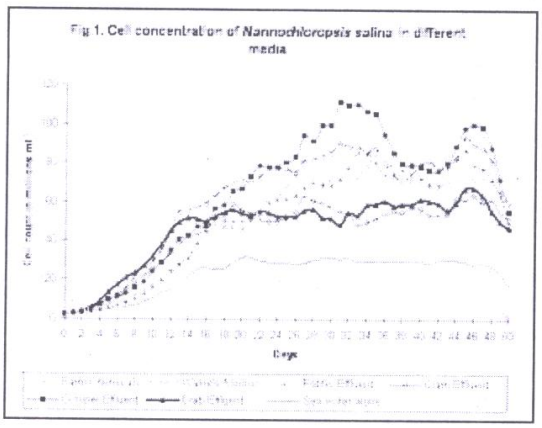
The number of microalgae was counted using a haemocytometer and daily counts (millions ml^{-1})

were taken similarly for the entire growth period in all treatments. Nutrient analysis was carried out at the beginning of experiment before adding inoculum and at the end of the experiment (50 days). To obtain accuracy for the nutrient analysis at the end of experiment, the microalgae in the culture were completely removed from the medium in order to get the true status of nutrients in the media alone. This was accomplished by filtering the culture through a water filter candle covered with polyester cloth in five drapes around. The drained water collected inside the filter candle (kept upside down) was siphoned out. This was tested for presence of microalgae if any, under a microscope and a nil count was obtained. This water was analysed for ammonia N, nitrite N, nitrate N and phosphate P content using standard methods (APHA, 1981).

Data on count of cells of *Nannochloropsis salina* in million ml^{-1} in different treatments were analysed statistically using SYSTAT 7.0. Suitable transformations of data were made to meet the normality assumption.

RESULTS AND DISCUSSION

The pattern of growth of *Nannochloropsis salina* in different media tried is depicted in Fig.1. Comparison of the growth among different treatments showed that all media were definitely more productive than seawater alone.



The statistical analysis of the data using ANOVA revealed that the treatments differ significantly ($p<0.01$). *Nannochloropsis salina* grown in grouper culture effluent produced highest concentration followed by that in Walne's medium. The efficiency of grouper culture effluent in culturing *Nannochloropsis salina* was on par with that of Walne's medium, whereas its growth performance in grouper culture effluent differed significantly from that of farm chemicals, rotifer culture effluent, crab culture effluent, clam culture effluent and control (Fig.1).

Walne's medium excelled all other treatments except grouper culture effluent, the difference in performance being statistically significant. The performance of farm chemicals was on par with rotifer culture effluent. The growth of *Nannochloropsis salina* in farm chemicals was significantly better than that in crab culture effluent, clam culture effluent and seawater alone. Rotifer culture effluent was found significantly better in efficiency in producing *Nannochloropsis salina* than crab culture effluent, clam culture effluent and seawater alone.

There was no significant difference in growth performance of *Nannochloropsis salina* in crab culture effluent and clam culture effluent, whereas control differed significantly from both of these (Fig.1). None of the media showed death phase during 50 days of experiment. Stationary and declining phase continued till 50th day when the experiment was terminated.

The chemical analyses of the media for nutrients were done before adding inoculum and at the end of the experiment. The amount of nutrients present initially in the media and the amount of nutrients utilized by *Nannochloropsis salina* as percentage over initial quantity is given in Table 1.

Table 1: Content of nutrients in different media and their usage by *Nannochloropsis salina*

Media	NH_3-N, µmol l^{-1}		NO_3-N, µmol l^{-1}		NO_2-N, µmol l^{-1}		PO_4 -P, µmol l^{-1}	
	Initial	% used	Initial	% used	Initial	% used	Initial	% used
Farm Chemicals	837.80	99.67	24.08	94.62	0.1338	86.79	25.80	79.15
Walne's medium	6.232	88.89	12.10	99.12	0.1325	71.43	32.37	99.29
Rotifer Effluent	1227.40	99.83	33.43	99.86	13.05	99.76	32.20	98.78
Clam Effluent	311.70	99.89	4.014	96.58	52.20	99.89	28.04	86.0
Grouper Effluent	10.72	51.68	11.67	98.90	0.63	93.01	29.27	89.77
Crab Effluent	24.75	98.60	35.57	99.65	9.66	99.54	25.18	77.23
Sea water	8.59	82.28	2.76	94.72	0.30	33.33	19.90	61.04

In the case of effluents possessing high initial content of ammonia N (in farm chemicals, rotifer culture effluent, clam culture effluent and crab culture effluent), more than 95% of it was used up by *Nannochloropsis salina.* In grouper culture effluent only 52% of ammonia N had been utilized by *Nannochloropsis salina.* In Walne's medium and control, more than 80% of ammonia N had been utilized by *Nannochloropsis salina.* In the case of nitrate, more than 95% was used by *Nannochloropsis salina* in all the media experimented. The phytoplankton utilized more than 90% of nitrite content in rotifer culture effluent, grouper culture effluent , crab culture effluent and clam culture effluent. Phosphate utilization by *Nannochloropsis salina* was highest in Walne's medium followed by rotifer culture effluent. Irrespective of the medium used, phosphate utilization was more than 60% in all the cases.

Effluent of the grouper culture tank was found to be the best for growth performance of Nannochloropsis *salina* and it was found to be on par with Walne's medium. The phosphate content of grouper culture effluent was less than that of Walne's medium. Even with this lesser nutrient content, grouper culture effluent could produce highest amount of *Nannochloropsis salina* possibly due to its organic nature. An organic medium influences the microbial activity and nutrient cycling. Though the percentage removal of ammonia N from grouper culture effluent was less, the production of *Nannochloropsis salina* was higher than that in Walne's medium. It shows the higher efficiency of this organic medium over Walne's medium (which is inorganic) for sustaining crop production.

When ammonia N content in media was higher, nearly 100% of its removal by *Nannochloropsis salina* had been shown. This is in tune with the observation of Craggs *et al.* (1997) in establishing the efficiency of marine microalgae for removal of ammonia N from waste water. Rotifer culture effluent was the richest in nutrient load, of which nearly 100% had been removed by *Nannochloropsis salina.* Though nutrient uptake from rotifer culture effluent was nearly 100%, its conversion efficiency into production of *Nannochloropsis salina* cells was less compared to grouper culture effluent and Walne's medium. The use of nitrate by phytoplankton was 95% or more in all the media tried. Though the phosphate utilization in grouper culture effluent was less than that of inorganic medium, the production was on par in both cases. This may be due to the bio-availability of the total phosphorus present in the grouper culture effluent by microbial action.

The uptake of ammonia-N, nitrite-N, nitrate-N and orthophosphate by marine micro algae for waste water treatment has already been established (Gonzalez *et al.*, 1997, Lefebvre *et al.*, 1996,

Craggs *et al.*, 1995). The production of algal biomass using waste water has been reported by de la None *et al.*, 1986. Extremely dense bloom of dinoflagellate *Alexandrium tamarense* was recorded in coastal lagoon ecosystems experiencing high level impact of waste discharge from intensive aquaculture (Sorokin *et al.*, 1999). Sarojini and Subbarangaiah (2001) reported higher density production of phytoplankton due to the effect of effluents from shrimp hatcheries.

The results of the present investigation indicate that grouper culture effluent may be used as an alternative for Walne's medium for the culture of *Nannochloropsis salina* after sterilizing this effluent with 2 ppm available chlorine against ciliates. It has been earlier proved by repeated trials that 2 ppm available chlorine does not kill *Nannochloropsis salina* but it kills the ciliates in algal culture medium (Prema and Thomas, unpublished). The results also give indication that effluents from the culture of marine organisms such as rotifer, clam and crab after sterilization can also be used for production of marine microalgae as live feed for hatcheries.

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REFERENCES

APHA, 1981. Standard methods for the examination of water and waste water. 15th edition, American Public Health Association, Washington D.C. pp. 1134.

Chebil, L. and Yamamasaki, S., 1998. Improvement of a rotifer ecosystem culture to promote recycling marine microalgae *Nannochloropsis* sp. *Aquacultural Engineering* 17 (1): 1 – 10.

Craggs, R. J., Smith, V. J. and McAnley, P. J., 1995. Waste water nutrient removal by marine microalgae cultured under ambient condition in mini ponds. *Water Science and Technology* 31 (12): 151 – 160.

Craggs, R. J., McAnley, P. J. and Smith, V. J. 1997. Waste water nutrient removal by marine microalgae grown on a corrugated raceway. *Water Research* 31 (7): 1701 – 1707.

De la None, J., Proulx, D., Guay, R., Pouliot, Y., Turcotte, J. 1986. Algal biomass production from waste waters and swine manure – Nutritional and safety aspects. *Microbial Biomass Protein.* 141 –167..

Gonzalez, L. E., Canizares, R. O. and Baena, S., 1997. Efficiency of ammonia and phosphate removal from Colombian agro-industrial waste water by the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus. Bioresource Technology* 60 (3): 259 – 262.

Hirayama, K., Takagi, K and Kimura, H., 1979. Nutritional effect of eight species of marine phytoplankton on population and growth of rotifer, *Brachionus plicatilis. Bull Jpn. Soc. Sci. Fish* 45: 11-16.

Lefebvre, S. and Hussenot, J., 1998. Biological treatment of intensive fish farm effluents for using mass phytoplankton production. Salt marshes and aquaculture : *Sustainable activity for conservation and exploitation of coastal wetlands and salt marshes*

(Symposium held in June 1996), IFREMER, Plouzane (France), 1998. P. 224-233.

Lefebvre, S., Hussenot, J. and Brossard, N., 1996. Water treatment of land based fish farm effluents by out door culture of marine diatoms. *Journal of Applied Mycology.* 8 (3): 193 – 200.

Sarojini, Y. and Subbarangaiah, G., 2001. Influence of effluents of shrimp hatcheries on the ecology of phytoplankton in coastal waters of Vishakhapatnam. *Seaweed Res. Utiln.* 23 (1&2): 143 – 148.

Shirai, F., Kunii, K., Sato, C., Terramato, Y., Mizuki, E., Murao, S and Makayama S., 1998. Cultivation of microalgae in the solution fro desalting process of soy sauce waste treatment and utilization of the algal biomass for ethanol fermentation. *World Journal of Microbiology and Biotechnology*, 14 (6), pp. 839 –842.

Sorokin, Y., Sorokin, P. and Ravagnan, G., 1999. Analysis of Lagoonal Ecosystems in the Po River Delta Associated with Intensive Aquaculture. *Estuarine, Coastal and Shelf Science* 48 (3) pp. 325 – 341.

Walne, P. R., 1974. *Culture of bivalve molluscs.* Fishing News (Books) Ltd. Surrey, p. 1-173.