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Culture of Marine Microalgae in Shrimp Farm Discharge Water: A Sustainable Approach to Reduce the Cost Production and Recovery of Nutrients

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Abstract: Marine microalgal species such as *Skeletonima costatum* and *Chaetoceros coarctatus* were cultured in Discharge Water (DW) from shrimp culture pond to recover the organic and inorganic nutrients released as waste. Total nitrogen (N), nitrate-N and total phosphorus in the DW were observed significantly higher and their mean values were 95.261, 32.6 and 11.312 mg L⁻¹, respectively. Algal species were cultured in processed DW under 12 h light/12 h dark condition with light intensity of 6000-7000 Lux and compared with cultures made in standard Conway medium which served as a control. Cell density was obtained higher over the control by 30.1 and 20.0% in *S. costatum* and *C. coarctatus* respectively in DW. While the rate of nitrogen removal was between 42.3 and 47.2%, the phosphorus removal efficiency was 20.8 and 17.7% in *S. costatum* and *C. coarctatus* respectively. The present study inferred that, as it is a low cost technology for microalgal production as well as a mean for waste water treatment, marine microalgal culture in DW from shrimp farm will be a two in one approach towards sustainable utilization of aquatic resource.

Key words: Discharge water from shrimp culture, phytoplankton, cell density, nitrogen removal, phosphorus removal

Introduction

Microalgae possess a vast potential in pharmaceuticals, health foods, carotenoids, dyes, fine chemicals, biofuels, etc. (Faulkner, 1986; Radmer, 1996; Markov *et al.*, 1998; Borowitzka, 1999). Being a primary link in food chain, microalgae serve as an important live food for fin and shellfishes in hatcheries (Duerr *et al.*, 1998). Culture media play a major decisive role in microalgal culture as a cost factor. There are a number of conventional media, such as, F/2 media, Walne's or Conway, Scheiber's, Miquel's etc., being used for the culture and maintenance of microalgae in research laboratories as well as in fish hatcheries (Guillard, 1995; Fung Ip Po *et al.*, 2004). Normally the media contain inorganic recipes and procurement of the ingredient chemical is tedious and often expensive. Furthermore, microalgae can effectively fix atmospheric carbon by photosynthesis and remove excess nutrients in water (Hirata *et al.*, 1996; Hirano *et al.*, 1997; thereby they may be effectively utilized in waste water treatments (Takano and Matsunaga, 1995; Nakajima *et al.*, 1997). Microalgae utilize various organic compounds-especially eutrophic compounds containing nitrogen and phosphorus for their carbon sources. Therefore, culture of microalgae in waste water could serve as a possible sustainable approach towards waste water treatment (Craggs *et al.*, 1997; Lee and Gyun Lee, 2001). Discharge Water (DW) from shrimp ponds generally receives large amounts of high quality organic matter in the form of feed wastage, fertilizers and excretory products resulting in enrichment of receiving water body and ends

in eutrophication (Tacon and Forster, 2003; Alonso- Rodriguez and Paez-Osuna, 2003). Generally more intensive culture systems produce higher loads of nutrients such as N (nitrate) and P (Phosphate) in their discharge. Their concentrations depend mostly on the management aspects such as stocking density, water management, feeding rate and fertilizer application. The N/P ratio in shrimp culture discharge water ranges from 1.1 to 6.7 with values being more frequent between 1.1 and 6.8 mg L⁻¹ (Alonso- Rodriguez and Paez-Osuna, 2003). Hence shrimp farming become a curse in coastal areas due to their heavy discharge of used water with high organic and inorganic load and it has been regulated strictly under legislations (Tacon and Forster, 2003).

In the 1980s American scientists studied, at a laboratory scale, the possibility of recycling nutrients from secondarily treated domestic wastewater through artificial food chains (Adamsson, 1998). They found that species like *Scenedesmus* sp. and *Daphnia magna* were suitable organisms, both for their high growth potentials and also for their resistance to handlings in the low technology culture systems used. In a previous study, Lefebvre *et al.* (1996) demonstrated the feasibility of the batch production of pelagic diatoms (*Skeletonema costatum*, *Chaetoceros* sp.) from natural population assemblages using dissolved fish excretion as a source of nutrients once the Si/P ratio was adjusted >5.

In this backdrop, the present study presents the results on laboratory culture of two marine phytoplankton *Skeletonima costatum* and *Chaetoceros coarctatus* species, using DW obtained from commercial semi-intensive shrimp culture facility.

Materials and Methods

DW was collected from a 90-day-old semi intensive marine shrimp culture pond at the time of water exchange with stocking density of 17 ind m⁻². The farm is situated near our center in the bank of Vellar River (11° 29' N; 79° 47' E). Water quality parameters such as pH, salinity and dissolved oxygen were measured *in situ* using the Quanta Hydrolab (Austin, Texas), while chemical analyses of the water were accomplished in the laboratory. DW, with the exception of the samples for total nitrogen and total phosphorus analyses, were first filtered through a whatmann No.1 Glass Fiber filter (GF/C) and followed by 0.45 and 0.22 mm membrane filters to remove microorganisms and fine particulate matter. For processing the DW, initially water was stored in flat bottom container a dark room at -5°C for sediment settlement for minimum six days. After sedimentation the DW was exposed to ultraviolet radiation for 1.5 h. The samples were then tested for bacterial contamination by using the tryptone soy agar medium for total plate count (Seeley and VanDemark, 1972). Unprocessed and processed water samples were analyzed for Total Nitrogen (TN), ammonia-N, nitrate-N, nitrite-N and Total Phosphorus (TP) contents following Parsons *et al.* (1984). Silicate analysis was done using the Hach kit heteropoly blue method (Clesceri, 1989).

Two common species of marine diatoms, *Skeletonema costatum*, *Chaetoceros coarctatus* were used in the present study. Each species of algae was subsequently cultured in Conway medium at 24±2°C under 12 h light and 12 h dark cycle. The algal species were mass cultured in 250l transparent cylindrical fiber glass tank. Cultures were made in triplicates using DW and Conway medium in which the later one served as control. Each tank contained 200l of culture media and inoculated with an initial algal cell count of 10³. Continuous aeration was provided with help of diaphragm type aquarium air pump. The tanks were distributed randomly and day light fluorescent tubes (PHILIPS 40 W) were placed vertically around the sides of the tank at a photoperiod of 16 h light and 8 h darkness for seven days. The light intensity was between 4000 to 5000 Lux. The light intensity was measured by LX 101 Lux metre. The temperature of the room was controlled to 26±2°C. Algal concentration and pH were measured daily. One mL of sample from each tank was collected and preserved in Lugol's iodine solution and cell counts were made in using a haemocytometer. Chlorophyll content (mg m⁻³) and specific growth rates ($\mu = \ln (F_1/F_0)/(t_1-t_0)$, where F_1 = biomass at time of harvest t_1 and F_0 = biomass

at times zero, t_0) during the first 10 days of culture were calculated following the standard method (Clesceri, 1989). Data on cell growth was processed using one way ANOVA to test statistical differences between treatments at $p < 0.05$. The post culture water was analysed for TN and TP after remaining the algal cells by centrifugation at 3,000 rpm for 10 min. Finally, removal rates of TN and TP were calculated by comparing with post culture values with their initial values.

Results

The complete data on chemical characteristic of DW is presented in Table 1. The nutrients like TP, nitrate-N and TN values in processed and unprocessed DW samples were more or less similar. Result of microalgal growth was expressed concentration as cells/mL at interval of days up to 7 days in Fig. 1. On day five, *S. costatum* and *C. coarctatus* grown in DW showed a 30.1 and 20.0% higher cell concentration respectively than the cells grown in Conway medium as control. In supportive to the higher cell density, chlorophyll concentration (Fig. 2) and specific growth rate (Fig. 3) were also quite higher in cultures in DW and were significantly ($p < 0.05$) higher than the culture in Conway medium.

Table 1: Chemical characteristics of processed and unprocessed Discharge Water (DW) obtained from a 90-day old marine shrimp culture pond

Parameters	Processed DW	Unprocessed DW
Salinity (ppt)	31.2	30.0
pH	7.6	7.9
Dissolved oxygen (mg L^{-1})	NA	5.26
Ammonia nitrogen (mg L^{-1})	4.420	4.720
Nitrite nitrogen (mg L^{-1})	2.212	2.821
Nitrate nitrogen (mg L^{-1})	32.621	33.26
Total nitrogen (mg L^{-1})	95.261	95.812
Total phosphorus (mg L^{-1})	11.312	12.721
Reactive silicate (mg L^{-1})	11.262	11.386

NA- Not Available

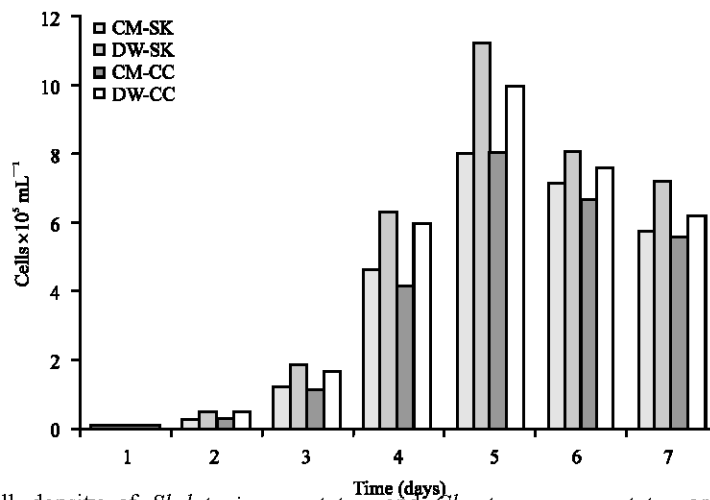


Fig. 1: Cell density of *Skeletonima costatum* and *Chaetoceros coarctatus* grown in shrimp culture discharge water (CM-SK = Conway medium *S. costatum*, CM-CC = Conway medium *C. coarctatus*, DW-SK = discharge water *S. costatum*, DW-CC = Discharge water *C. coarctatus*)

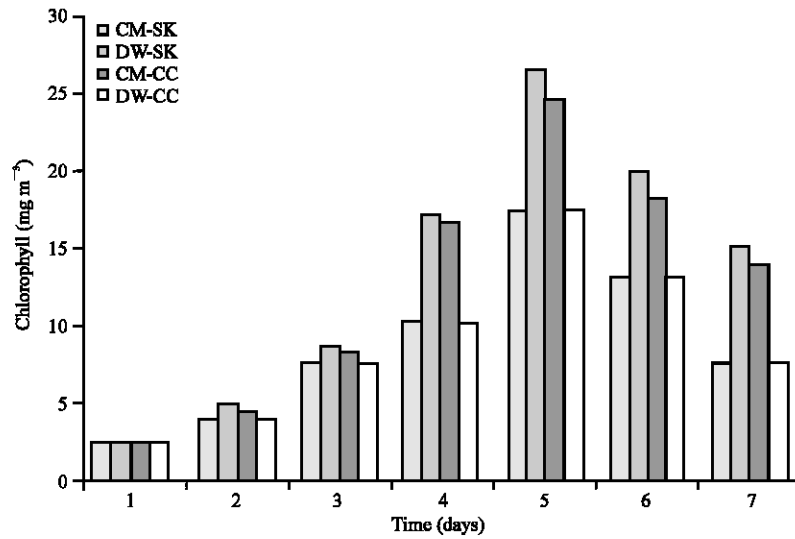


Fig. 2: Total chlorophyll content of *Skeletonima costatum* and *Chaetoceros coarctatus* grown in shrimp culture discharge water (CM-SK = Conway medium *S. costatum*, CM-CC = Conway medium *C. coarctatus*, DW-SK = Discharge water *S. costatum*, DW-CC = Discharge water *C. coarctatus*)

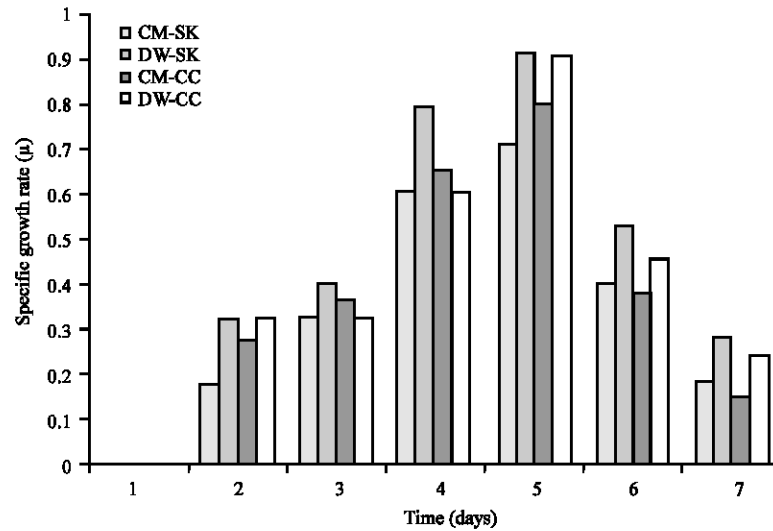


Fig. 3: Specific growth rate of *Skeletonima costatum* and *Chaetoceros coarctatus* grown in shrimp culture discharge water (CM-SK = Conway medium *S. costatum*, CM-CC = Conway medium *C. coarctatus*, DW-SK = Discharge water *S. costatum*, DW-CC = Discharge water *C. coarctatus*).

The rate of nitrogen removal from the discharge water by marine microalgae in the present study ranged between 42.3 and 47.2% (Fig. 4). *S. costatum* and *C. coarctatus* reduced the nitrate nitrogen from the initial level of 95.26 mg NO₃-N/l to 44.96 and 40.26 mg NO₃-N/l, respectively in 7 days.

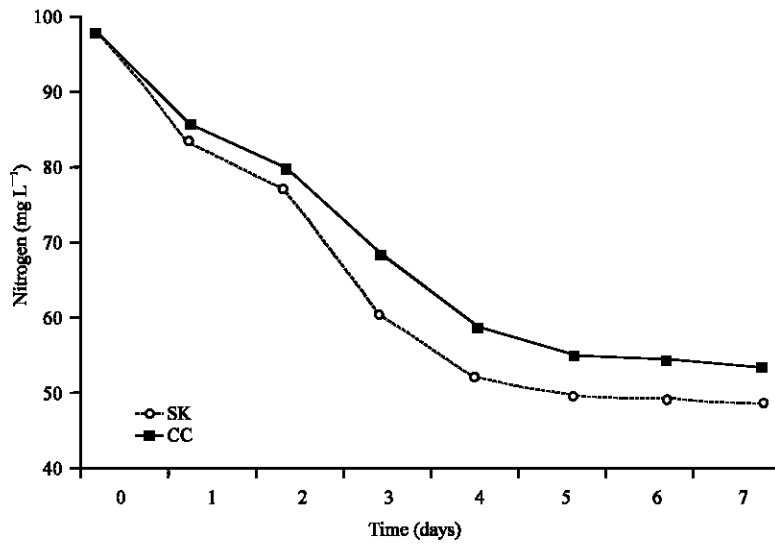


Fig. 4: Removal rate of nitrogen in discharge water by *Skeletonima costatum* and *Chaetoceros coarctatus* (SK = *S. costatum*, CC = *C. coarctatus*)

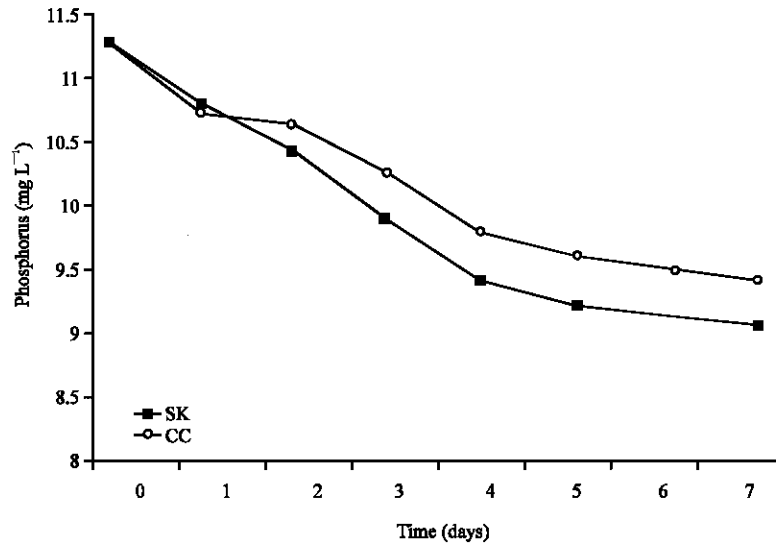


Fig. 5: Removal rate of phosphorus in discharge water by *Skeletonima costatum* and *Chaetoceros coarctatus* (SK = *S. costatum*, CC = *C. coarctatus*)

During the same period, the cell concentration was increased to about 1.2×10^6 cells mL⁻¹ and 10×10^5 cells mL⁻¹ in *S. costatum* and *C. coarctatus* respectively. Phosphorus concentration also was reduced significantly ($p < 0.05$) by microalgae (Fig. 5). Though the phosphorus removal ability of both phytoplankton was efficient, *S. costatum* (20.8%) showed a slightly better removal efficiency of phosphate-P than the *C. coarctatus* (17.7%).

Discussion

The values obtained in this study are comparatively higher than the values reported by Santhanam and Perumal (2003) in Vellar estuary, the main water source for the shrimp farm in these regions. Such higher nutrient content may be attributed to the high input of compounded feed and subsequent feed wastage, use of inorganic fertilizers and other management practices (Tacon and Forster, 2003). Naturally DW from shrimp farm harbours high level of nutrient and organic matter, which results in eutrophication of the receiving water body (Lopez Alvarado, 1997; McGoogan and Gatlin, 2000; Sugiura and Hardy, 2000; Cho and Bureau, 2001). Hence the higher cell density observed in the present study may be attributed to the higher inorganic nutrient content and organic carbon available with DW from the shrimp farm. In open waters Raman and Prakash (1989) have reported a direct relationship between cell densities of phytoplankton in Visakhapatnam near sewage disposal point. Adamsson *et al.* (1998) reported 2.5×10^6 cell mL^{-1} of *Scenedesmus acuminatus* as highest cell density in culture media containing human urine. Phytoplankton contains 5-10% nitrogen (Becker, 1994) and they can utilize nitrate as a nitrogen source for their growth. The reduction of nitrogen was due to possible removal of nitrogen compounds with help of light source by phytoplankton. The difference between the expected and the measured nitrogen amount under continuous illumination suggested that light can stimulate nitrogen consumption (Lee and Gyun Lee, 2001). Nitrogen containing compounds such as ATP and NADPH are produced actively when microalgae undergo photosynthesis, or when the cells are illuminated (Craggs, 1997). In a outdoor phytoplankton continuous culture in a marine fish-phytoplankton-bivalve integrated system, phosphate in the inflow water was removed at the rate of $0.3 \text{ g P m}^{-1} \text{ day}^{-1}$ (Fatimah, 2001). Such an assimilation rate was possible in the present study because of the high growth rate and high P requirements of the diatoms under culture (Finenko and Krupatkina-Akinina, 1974). The overall reduction of phosphate in the filtered water mixed with human urine on culture of *Scenedesmus acuminatus* was determined to be 36% for orthophosphates at the end of experiment (Adamsson *et al.*, 1998). The culture of phytoplankton provides a system in which the dissolved excretion from fishponds may be transformed from dissolved inorganic material into particulate phytoplankton organic material (Lefebvre, 2004). More studies are necessary to elucidate the mechanisms of phosphorus metabolism such as phosphate transport, phosphate hydrolysis and ATP formation from phosphate in algae. Many more parameters, such as light intensity, chemical constituents, temperature and pH, must be examined to elucidate the effect of phosphate metabolism in an algal wastewater treatment system.

Conclusion

In conclusion, the results of the present study proved that, two species of marine microalgae *S. Costatum* and *C.coarctatus* could be grown well in DW from marine shrimp culture pond. On day five, *S. Costatum* and *C.coarctatus* grown in DW showed a 30.1 and 20.0% higher cell concentration respectively compared to the cells grown in Conway medium which is most commonly used for large scale production in hatcheries. In supportive to the higher cell density, chlorophyll concentration and specific growth rate were also quite higher in cultures in DW and were significantly ($p < 0.05$) higher than the culture in Conway medium. By growing marine microalgae in such DW, large quantities of these commercially valuable algae could be produced in low cost. Also intensive shrimp farming a curse to the natural ecosystem could be made in to an eco-friendly system by adopting this kind of techniques in large scales.

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