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An Experimental Study on Ammonia Assimilation through Cyanobacteria in Marine Ornamental Fish Culture

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Abstract

Blue - green algae (Cyanobacteria) are widespread in nature and they are the primary producers in marine environment. Carbon fixation by cyanobacteria is the most common process in natural waters and also contributing to ammonia assimilation to some extend. To find out the efficiency of cyanobacteria in ammonia assimilation in a marine ornamental fish culture tank was studied, the blue green algae such as *Oscillatoria nigra, Lyngbya majuscula, Spirulina plantensis, Chroococcus turgidus, Aphanocapsa delicatissama, Nitzschia palaceae* were identified, screened, developed as mass culture and used for the study. Two ornamental fish culture tanks were selected as control and experimental. The Physico-chemical parameters along with ammonia were monitored during the study period. In the control tank, little elevated level of ammonia was recorded (0.44 to 1.72mg/l) and in the experimental tank, the low value of ammonia was recorded (0.44 to 0.09mg/l). The present study revealed the clear uptake of ammonia by cyanobacteria and the growth of cyanobacteria was also monitored by analyzing the chlorophyll content in the water. The initial chlorophyll value in the control and experimental tanks both were recorded as 0.0µg/l. The higher level of chlorophyll (17.87µg/l) was recorded at the end of the study in the experimental tank than the control tank (1.2µg/l). The isolated and identified *micro algae* (cyanobacteria) were maintain the water quality parameters in fish culture tanks mainly assimilation of ammonia and reduce the toxic level and sustain the growth of fish.

Keywords: *Micro algae*, ammonia assimilation, toxic level, chlorophyll.

Introduction

Blue - green algae (Cyanobacteria) represent a wide range of organisms and have different morphological and biochemical characters in nature. In addition they have rich in protein, abundant in amino acids, vitamins, growth stimulating substances and other biologically active substances (Daniela Petrova unknown year). Marine cyanobacteria support fisheries and aquaculture production by providing food for aquatic animals, like fishes and mollusks (Baldia *et al.,* 2007) and also they are familiar for ammonia oxidation or assimilation in marine aquarium (Peter Hunnam, 1989). High ammonia level in captivity can also cause severe stress, whereas slightly elevated levels can contribute to chronic stress. In the natural environment the end product, nitrate will be incorporated by the plants and alga for their growth.

Algae can be utilized for the production of oxygen, essential to growth of bacteria and other organisms that break down the organic waste in captivity. Thus the availability of algae may become useful as only indicator but also purifiers (Tseng and Wang, 1981). In the culture system ammonia, is produced by fish through respiration, excretion and by the decomposition of waste products. It can be present as two forms; highly soluble toxic unionized ammonia (NH_3) or the less dangerous ammonium ion (NH_4+). Ammonia is a strong cell poison and can cause damage to the gills of the fishes. Clinical signs due to NH_3 toxic include increased mucus production, red or bleeding gills, darkening of body coloration, 'gasp' for air at the surface, increased respiration rate etc.

Plants and algae are assimilating ammonia directly for the biosynthesis and ammonia act as a protein sources (Ruckert and Giani, 2004). The simplest way of reducing ammonia level in culture system can be effected on exchange of water very often. Assimilation is the absorption of nitrate, ammonia, urea etc by some algal species. Since ammonia is thought to be an excellent source of nitrogen for algal growth (Morris, 1974), it was suggested that the introduction of cyanobacteria may be useful for the rapid reduction of ammonia in the fish culture tank. The *micro algae* and its application in marine aquarium is very much useful to prevent the high mortality rates by reducing ammonia like toxic substances.

The present investigation has been undertaken to study the efficiency of some selected cyanobacteria in assimilating ammonia in the ornamental fish culture tanks.

Materials and methods

Identification of *micro algae* through SEM

The out of the blue *micro algae* were found in the existing marine ornamental fish culture facility the samples were isolated from the same. The samples were taken for pure culture using BG-11 Medium under controlled illumination (4000 lux) with 16/8 h light/dark cycle at 28 ± 2°C (Narayan, 2006). The pure cultured samples were identified as follows. The micro algal samples were fixed in the primary fixative (3% glutaraldehyde) for Scanning Electron Microscopic studies. The fixed samples were given in three washes thoroughly using buffer. They were dehydrated through a graded series of acetone at 12-15 minute's interval at 4ºC up to 70%. Then 90 and 100% acetone were kept in room temperature for 2-3 hr interval. The dehydrated pieces were treated with critical control point drier were a stub and the specimen was gold coated. The samples were examined with Jeol Jsm- 56010LV with INSA-EDS and photomicrographs were taken selectively from the computer screen (Hayet and Falk 1980). *Micro algae* were identified by following the standard keys proposed by (Desikachary, 1959).

Setting up of ornamental fish culture tanks

The water used for both the experimental and control tanks were from a single source and confirmed its physico-chemical parameters as same at the time of introduction. Two FRP tanks were used, and the capacity is 1000 litres. One was made as control and another as experimental tanks. The cultured cyanobacteria (isolated from fish culture tanks) were introduced into experimental tank for assessing the rate of ammonia assimilation. 10 numbers of the clown fish, *Amphiprion sebae* with equal weight and length has been introduced in both tanks. The fishes were fed twice a day using clam meet with the same quantity in both tanks. The experimental period was one month to analyze the rate of increasing physico-chemical parameters by the growth of cyanobacteria.

Analysis of water quality

Physico-chemical parameters were measured before introducing the water into the tanks and subsequently it was measured at weekly intervals. The analysis of water samples was as follows. The water samples collected were filtered through Whatman filter paper (0.45 µm) GF/C filters, (under vacuum pressure lower than 75 mm Hg) and the same was allowed to frozen at -20ºC for later determination of nutrient contents (nitrite, nitrate, ammonia, phosphate, total phosphate and silicate). The water samples were also regularly analyzed for the general parameters such as pH, salinity and temperature with the standard equipments. Phytoplankton pigments (Chlorophyll-a) was analyzed using SHIMADZU UV-1201V spectrometer (Grassoff, 1999).

Determination of chlorophylls

Water samples were collected from the experimental and control tank was filtered through a glass fiber membrane (Whatman 4.5 cm dia, 0.45 µm GF/c filter paper) for chlorophyll extraction. Magnesium carbonate (2ml) was applied for making bed onto the glass fiber filter paper fitted in the filtering equipment. 1000 ml of sample was poured on to the funnel and applied low suction. After the filtration, the filter paper was ground with 5ml of 90% acetone by using tissue grinder. The content was poured in a black bag and kept in refrigerator for 20-24 hours and latter the same was centrifuged by 3000 RPM for 5 minutes. The clear supernatant extract was made up to 10ml with 90% acetone and measured the extinction of the extract at the wave length of 630 nm, 645 nm and 665 nm (Dharani, 1989 and Grassoff, 1999).

Results and Discussion

A total number of 6 species of *micro algae* belonging to the class of Cyanophyceae covering two families such as Oscillatoriaceae (3 species) and Chroococcaceae (3 species) were recorded (*Oscillatoria nigra, Lyngbya majuscula, Spirulina plantensis,* *Chroococcus turgidus, Aphanocapsa delicatissama, Nitzschia palaceae*). The micro photographs of the species were taken under Scanning Electron Microscope is shown in plate-1.

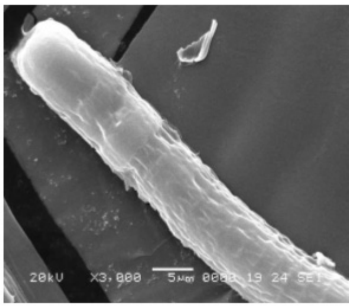


Fig. 1

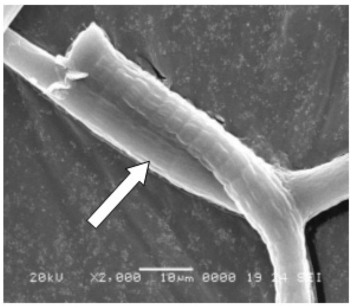
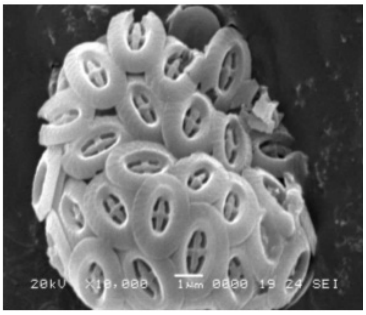


Fig. 2



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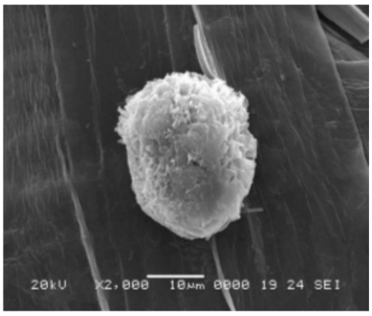

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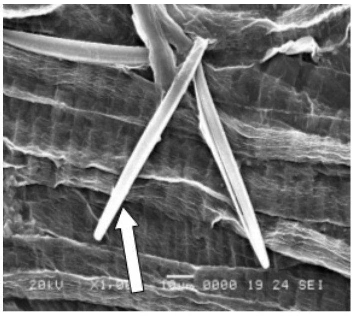

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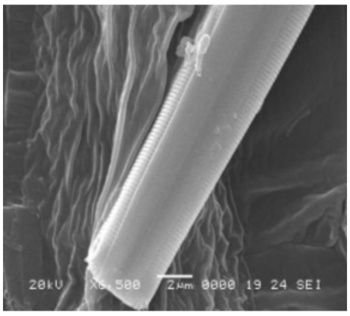


Fig. 6

Plate 1: Scanning electron micrograph photos of identified cyanobacteria. 1) Oscillatoria nigra, 2) Lyngbya majuscula (arrow showed), 3) Chroococcus turgidus, 4) Aphanocapsa delicatissama, 5) Spirulina plantensis(arrowshowed), 6) Nitzschia palaceae.

The species, *Oscillatoria nigra* had simple vegetative body and the trichomes are un- constricted, straight, rigid and thread-like without distinctly recognizable base or apex.

The species, *Lyngbya majuscula* had trichomes which grow singly or free, usually thick and firm sheath which may be homogenous or lamellated. The sheath often projects beyond the trichome and cylindrical with rounded apex.

The trichomes of the *Spirulina plantensis* are multicellular, cylindrical without sheath and loosely or tightly coiled into more or less regular coils. Cross walls are not distinct; the terminal cell is round and without calyptra.

Chroococcus turgidus are spherical or subspherical and occur in groups of 2-4, rarely 8-16 enclosed in a gelatinous or mucous matrix. Each cell may have an individual distinct firm and lamellated or homogenous sheath.

Aphanocapsa delicatissama are nearly spherical, loosely and irregularly arranged within a shapeless mass. Mucilage may be homogenous and colorless.

The percentage of *micro algae* in the experimental tank the dominant position is 60% oscillatoriaceae. The maximum production of *micro algae* found under the condition of the light intensity (4000 lux), temperature ranged "between" 28-29, salinity 28. Light and temperature is an important factor which regulates the biogeochemical activities of *micro algae* in the water. The level of nutrients such as ammonia, nitrate and nitrite were dramatically decreased when compared to initial level. The quantity of ammonia in the control tank, little elevated which was recorded (0.44 to 1.72mg/l) and in the experimental tank was found with lower value of NH_3 (0.44 to 0.09mg/l). The assimilation of ammonia has clearly utilized for its growth in the photo period (Table 1&2).

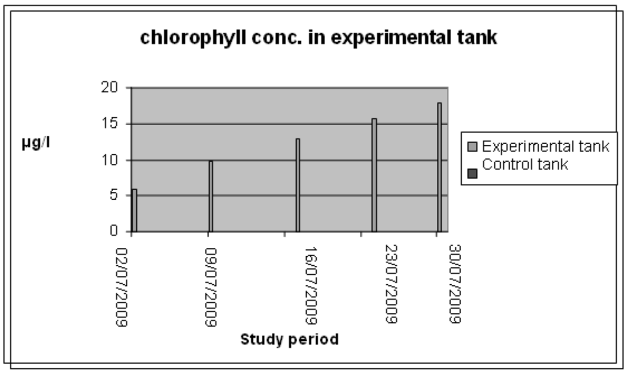
Table 1: Physico-chemical parameters of experimental tank.

Parameters/Date	2/7/2009	9/7/2009	17/7/2009	24/7/2009	30/7/2009
Temperature	28	29	29	28	28
Salinity (psu)	28.3	29	29	29.3	29.8
pH	6.9	7.5	7.9	8.2	8.4
DO (mg/l)	5.8	6.2	6.3	6.4	6.3
TSS(mg/l)	15.4	14.7	14.4	14.12	13.97
Nitrite(µg/1)	0.23	0.36	0.47	0.52	0.66
Nitrate(µg/1)	0.48	0.57	0.69	0.77	0.87
Phosphate(µg/1)	0.37	0.39	0.41	0.42	0.44
Silicate(µg/1)	0.21	0.22	0.25	0.27	0.28
Ammonia(mg/1)	0.44	0.39	0.27	0.16	0.09
Chlorophyll (µg/l)	5.97	9.76	12.9	15.6	17.87

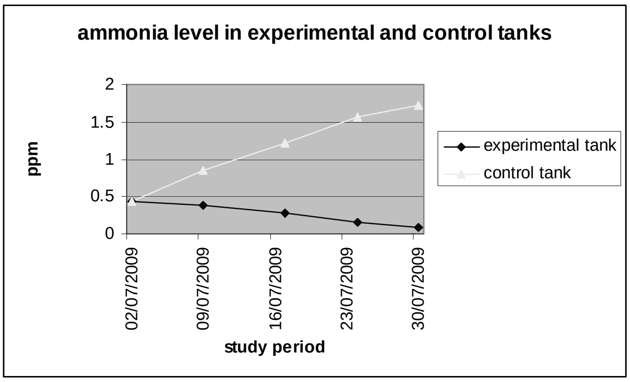
Table 2: Physico-chemical parameters of control tank.

Parameters/Date	2/7/2009	9/7/2009	17/7/2009	24/7/2009	30/7/2009
Temperature	28	29	29	28	28
Salinity (psu)	28.3	29	30	32	34
pH	6.9	7.0	7.2	7.4	7.8
DO (mg/l)	5.8	6.0	5.9	6.2	6.0
TSS(mg/l)	15.4	15.7	15.9	16.0	16.2
Nitrite(µg/1)	0.23	0.19	0.14	0.12	0.09
Nitrate(µg/1)	0.48	0.41	0.32	0.24	0.16
Phosphate(µg/1)	0.37	0.32	0.28	0.21	0.17
Silicate(µg/1)	0.21	0.19	0.16	0.14	0.12
Ammonia(mg/1)	0.44	0.85	1.22	1.56	1.72
Chlorophyll (µg/l)	0.00	0.00	0.00	0.00	0.00

In the control tank with out algal incorporation, the ammonia level increased the pH from 6.9 up to 7.8 in the control tank. The experimental tank was also shows high pH (6.9 to 8.4) because (essentially ammonia is a salt) when compared to the control tank which indirectly shows algal growth (Graph. 1). The important thing was observed when ammonia assimilation the nitrate and nitrite values were goes to high, because in the nitrogen cycle naturally the ammonia converts into nitrate and nitrite by cyanobacteria (Graph. 2).



Graph 1: Chlorophyll conc. of experimental tank was observed during study period.



Graph 2: The ammonia level in experimental tank and control period was observed during study period.

The cyanobacteria were primary producers of marine water which leads the stipulate of water quality parameters in the experimental tank. Generally, in marine aquarium the algal growth which has shown the degradation of organic substance and influences the nitrification and denitrification cycles. The analysis of Chlorophyll *a* concentration of experimental tank water shows, the maximum growth of algae, which shows the assimilation progression of ammonia that, condensed the toxicity level in fishes. The higher level of chlorophyll (17.87) was found at the end of the experiment than the initial period (5.97 µgl) condition.

The macro algae, *Kappaphycus alvarezii* is capable of assimilating nitrogenous waste and ammonium in particular and exhibits high growth rate in ammonium-rich waters (Rodrigueza, 2007). Nitrogen deficiency leads to the decrease in the efficiency of light reactions of photosynthesis, in the rate of photosynthetic fixation of carbon, and of population growth in algae. There is a vast information on the capacity of various algae to assimilate the organic substrata containing organic nitrogen. That previously reported by (Ilyash, 2007) on widely used approach for the determination of efficient photosynthetic light reactions and the estimation of fluorescent parameters of photoautotroph of marine *micro algae*.

Uptake and assimilation rate of ammonia is normally higher than for nitrate, because no enzymatic reduction is required for ammonia assimilation. It is also possible that cyanobacteria contribute to maintain undetectable nitrate levels in the upper layers of water column by the uptake of most nitrates that has been produced by the oxidation of ammonia present at very high concentration in the reservoir. Several studies indicated that nitrate uptake suffers inhibition by the presence of ammonium (Mccarthy, 1982).

The Marine planktonic algae *Pseudo-nitzschia delicatissima,Thalassisira weissflogii*, and *Tetraselmis viridis* are able to grow using urea and glycine as a sole source of nitrogen. These algae assimilate urea and glycine in the dark too (Ilyzsh, 2007).

Microcystis viridis was able to attain similar cell densities on both ammonia and nitrate. Nevertheless when it was growing on ammonia, high biomass values were reached more rapidly, presumably because of the higher growth rate thus driving to the conclusion that ammonia may lead or induce a population bloom (Ruckert and Giani, 2004). It is possible that the cyanobacteria take advantage over their ability to uptake ammonia for their maximal cell growth, however, can be achieved under high nitrate conditions.

P. amplissima assimilated both the forms of organic and inorganic N when presented simultaneously and removing NH+ six times faster than NO- even when NO- supply exceeds NH+ supply. There is general concurrence that growth on NH+ should in theory enable faster growth rates than NO- due to the energy savings associated with the direct assimilation of NH+ into amino acids (NO- must first be reduced to NH+) (George P. Kraemer 2005).

The growth rate increased with the nitrogen concentration up to 10 mg N.L-1, which indicated a direct relation ship of growth with nutrient concentration. There was no further increase in the growth yield of *M.aeruginosa* as phosphate concentration increased fro 0.22 to 10 mg P.L-1 (Baldia, 2007).

Conclusion

In this present study we conclude those micro algae (cyanobacteria) which we were isolated from fish culture tanks, beneficial to marine ornamental fish hatchery and not harmful. Cyanobacteria prevent severe stress, gills damage and mode of enter the microorganisms through reduce the un-ionized ammonia, which regulates the water excellence in fish tanks. Cyanobacteria also consume the misuse item for consumption and other organic substance in the tank. We would estimate the chlorophyll extent for their progress which indicates indirectly the decline phase of ammonia level. Cyanobacteria is perform excellent source of food for aquatic animals and support their growth.

Acknowledgements

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