Biosciences, Biotechnology Research Asia Vol. **7**(2), 701-712 (2010)

Growth and nutrient removal rates of *Oscillatoria acuminata* and *Scenedesmus armatus* in aquaculture wastewater: A laboratory scale study

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(Received: August 10, 2009; Accepted: September 27, 2010)

ABSTRACT

One of the major concerns of aquaculture lies in the generation of polluted effluent. The accumulation of feed residues and fish excreta elevates the concentration of dissolved inorganic nutrients, organic suspended solids and particulate organic matters. These excess nutrients, particularly nitrogen and phosphorus and other contaminants, are the main cause of eutrophication of the receiving water bodies. Microalgae may perform tertiary treatment due to their ability to incorporate inorganic nitrogen and phosphorous for growth and also have the capacity to remove heavy metals as well as some toxic organic compounds. In the present study the aquaculture wastewater was treated with microalgae is an efficient of removal of most of the nutrients and organic contaminants.

Key words: Aquaculture, wastewater, micro algae, nutrient removal.

INTRODUCTION

Aquaculture is concerned with 'the propagation and rearing of aquatic organisms under complete human control involving manipulation of at least one stage of an aquatic organism's life before harvest, in order to increase its production. Fish catches from the marine environment have been steadily declining in many parts of the world due to over-exploitation and pollution. This development of aquaculture in our country has led to not only severe disease problems but also alteration of the quality of our natural habitats through increased effluent discharges from aquaculture systems, which contain high quantities of both organic and inorganic forms. Since, recent past it has been observed that the sustainable development of aquaculture sector can be achieved by adopting eco-friendly aquaculture practices by minimizing impact on the surrounding ecosystem. To maintain healthy tanks bioremediation is the best biotechnology process.

Aquaculture wastewater exerts adverse environmental impacts when the effluents from these systems are discharged to receiving water. The organic matter loading reduces dissolved oxygen levels and contributes to the build up of bottom sediments and high nutrient loading impairs water quality by stimulating excessive phytoplankton (Joyner, 1992). However, the discharge of nutrient-rich water, an environmental regulatory concern in many countries, may result in the deteriorated quality of receiving waters (Pillay, 1990). Means to minimize the environmental impacts of the pond effluents include minimizing the use of nutrients, managing drainage to retain most nutrients in the pond system and maximizing the use of surplus materials in sediments by fish during grow out. The type of wastes produced in aquaculture farms is basically similar. However, there are differences in quality and quantity of components depending on the species cultured and the culture practices adopted.

The waste in aquaculture farms can be categorized as:

- Residual food and faecal matter
- Metabolic by-products
- Residues of biocides and biostats
- Fertilizer derived wastes
- Wastes produced during moulting
- Collapsing algal blooms (Sharma and Scheena, 1999).

Algae are the basis of the entire aquatic food chain, production of renewable resources from fishing. Algal cultivation has been identified as a means of eliminating residual inorganic nutrients from secondary treated wastewater and represents a potential feed and food source. The value of phytoplankton and other algae as direct or indirect food for fish and their usefulness as indicator of water quality have long been well recognized. With the progress of inland fisheries in India, studies on the productivity and diversity of phytoplankton in inland water have gained considerable importance. High eutrophic condition results in algal bloom induction causing a great loss in fish production. The use of microalgae for wastewater treatment appears to be a feasible solution because of their ability to remove inorganic nutrients and other contaminants selectively and at a low cost (Pouliot and De la No¡e (1985); De la Noe, Laliberte and Proulx (1992). The main advantage of microalgal treatment is the fact that nutrients are recycled into high-quality biomass, which can be harvested and used for several purposes (De la Noe, Proulx, Guay, Pouliot and Turcotte, 1986).

Three main concerns are raised against the aquaculture industry: a) plant nutrients from fish farms have led to an increased occurrence of algal blooms; b) plant nutrients from fish farms have disturbed the natural ratios of nutrient elements when enters sea water so favouring the occurrence of toxic species over harmless algae and c) plant nutrients from fin fish farms have made potentially toxic algae more poisonous. Lack of long term observational data include direct comparison of nutrient and phytoplankton levels from fish farm development times and the present day. The present study aims to analyse physico-chemical properties of raw and treated aquaculture wastewater and to evolve effective and economic biological treatment method by using microalgae.

MATERIAL AND METHODS

Aquaculture wastewater was collected from Thiruvallur (a suburb of Chennai). In order to select organism for the treatment process, micro algal populations were collected at different places from where the wastewater was collected; isolated and identified by using the standard manual (Desikachary, 1959 and Anand, 1998) and were maintained in CFTRI medium (Singh, Dhar, Pabbi, Prasanna and Arora, 2002) and Bold Basal medium following Nicholas and Bold (1965).

The following taxa were collected and identified; *Chroococcus*, *Oscillatoria, Lyngbya, Spirulina*, *Scenedesmus* and *Chlorella* indicate the polluted status of the water body. Among the various microalgae *Oscillatoria acuminata* and *Scenedesmus armatus* alone acclimatized well with aquaculture wastewater. Based on the preliminary tests these algae were selected for large scale treatment process. To study the role of micro algae in aquaculture wastewater treatment, the following protocols were employed. i) Effluent without *Oscillatoria acuminata* and *Scenedesmus armatus* (control) and ii) Effluent treated with *Oscillatoria acuminata* and *Scenedesmus armatus*. Samples were periodically (every 6th day) analyzed for various physico-chemical parameters were analyzed by using standard methods (APHA, 2000). The experiment was carried out for 15 days. For dry weight, three 100 ml sample from each tank were filtered daily through whattman CF-C glass filter and dried at 60°C for 24 hrs. Biomass growth rate, *k* (day^{-1}) was calculated using the following equation:

$$k = \frac{\text{(maximal biomass- initial biomass)}}{\text{(Initial Biomass x d)}} \quad ...(1)$$

Where d- is the number of days between initial and maximal biomass measurements.

Growth of *Oscillatoria acuminata* and *Scenedesmus armatus* in aquaculture wastewater measured in terms of chlorophyll-a and protein as the biomass components (Arnon, 1949). Biochemical constituents of protein of the treated algae were estimated by Lowry *et al*., (1951).

RESULTS AND DISCUSSION

Based on the physico-chemical parameters of the aquaculture wastewater it is evident that the wastewater is very toxic and if discharged, can cause serious environmental disturbances and harmful effects on human beings, animals and aquatic organisms. However, bioremediation using micro algae can be a versatile, inexpensive and can potentially transform a toxic material into harmless end products.

The colour of the wastewater was yellow indicating that it may be due to presence of large variety of contaminants (Bindu and Anjaneyulu, 1997). These changes in colour and odour of the aquaculture wastewater may be due to the action of algae which decomposed the organic matter present in both untreated and treated wastewater was water clear and these findings are in accordance with the work of Verma and Madamwar (2002). In the present study when the wastewater was treated with *Oscillatoria acuminata* and *Scenedesmus armatus,* the colour of the wastewater changed from blackish to greenish yellow from 8th day onwards.

In the present study turbidity of the wastewater reduced to 96.58 percent by *Oscillatoria acuminata* and 60.11 percent by *Scenedesmus armatus* (Fig.1a). This may be due to settling, in addition to natural treatment by algae.

Total solids in the present study were reduced to 11.53 percent when the wastewater was treated with *Oscillatoria acuminata* and 11.24 percent with *Scenedesmus armatus* (Fig.1b). Dhamotharan *et al.,* (2008) reported 56.89 percent of reduction of total solids when the sewage water was treated with *Oscillatoria* sp and 58.03 percent by *Scytonema* sp.

Total dissolved solids in the effluent were found to be reduced to 90.35 percent when the effluent was treated with *Oscillatoria acuminata* and 0.30 percent by *Scenedesmus armatus* (Fig.1c). Similar kind of work was reported by (Kannan *et al*., 2004). The study confirmed that due to reduction of total dissolved solids the effluent is suitable for safe disposal on land through irrigation. Veeralakshmi *et al*., (2007) reported 19.16 percent reduction of TDS when the petroleum effluent was treated with *Oscillatoria sp.*

In the present study the total suspended solids in the wastewater were found to be reduced to 82.60 percent by *Oscillatoria acuminata* and 86.95 percent by *Scenedesmus armatus* (Fig.1d). In the present study large amounts of suspended particles were observed. One possibility was that as the algae decompose in the system the particles released become suspended in the water and move along with the flow of the system. Other possibilities were that there was an increase in bacteria in the system or that the molasses was not completely used up by the bacteria. All of these possibilities could have been responsible for the increase in suspended solid level. The high suspended solids caused steady temperature in the wastewater and could affect the productivity of aquatic biota, particularly fishes. Veeralakshmi *et al*., (2007) reported 32.75 percent reduction of total suspended solids when the petroleum effluent was treated with *Oscillatoria sp.*

Electrical conductivity of the treated wastewater was reduced to 9.06 percent by *Oscillatoria acuminata* and 10.85 percent by *Scenedesmus armatus* (Fig.1e). A high level of conductivity is due to increased concentration of salts.

In the present study, pH was found to increase in aquaculture wastewater when treated with algae, whereas there was no change in pH in control. Interestingly, the pH of the aquaculture effluent increased from 7.58 to 8.30 with *Oscillatoria acuminata* and 8.86 by *Scenedesmus armatus* (Fig.1f). Along with the limited nutrients from wastewater for their improvement over growth metabolism, they produce oxygen. The byproduct oxygen released during algal metabolism is utilized by the aerobic bacteria for biological oxidation of dissolved organics in effluent. Vijayakumar *et al.,* (2005) reported increase of pH in dye effluent, when treated with *Oscillatoria* sp.

In the present study, initially there was no carbonate, but fairly high levels of bicarbonates were present in the effluent. The removal of this carbon

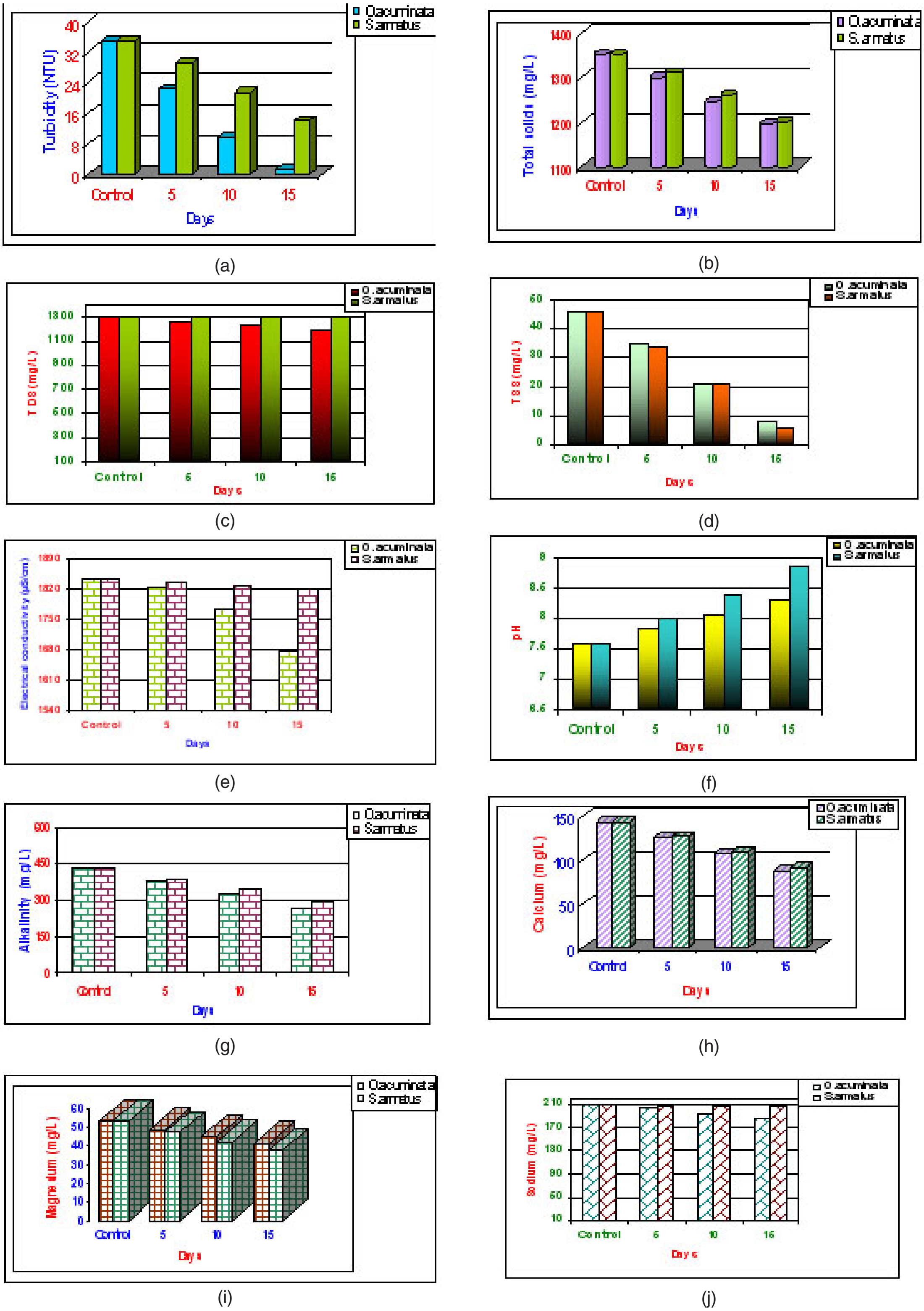

Fig. 1: Reduction of pollution load a) Turbidity b) Total solids c) TDS d) TSS e) Conductivity f) Increase of pH g) Alkalinity h) Calcium i) Magnesium j) Sodium

source effectively by *Oscillatoria acuminata* and *Scenedesmus armatus* was observed. In the present study it was found to be reduced to 38.53 percent when wastewater was treated with *Oscillatoria acuminata* and 32.11 percent by *Scenedesmus armatus* (Fig.1g). Alkalinity related techniques for bicarbonate estimation are well developed in applications where CO_2 is continuously generated by micro-organisms degrading organics. In the area of algal treatment, on the other hand, CO_2 is consumed by the algae, resulting in relatively low or even limiting concentrations of bicarbonate in the water samples. Inorganic carbon (IC) is of major importance, because it is the only carbon source used by algae. Vijayakumar *et al.,* (2005) reported reduction of carbonate in dye effluent, when treated with *Oscillatoria* sp.

Wastewater treatment processes generally have little effect on the hardness. Hardness in wastewater would make it unsuitable for industrial purpose as it may cause scaling of equipments (Goel, 2000). Total hardness was reduced up to 34.48 percent by *Oscillatoria acuminata* and 32.75 percent by *Scenedesmus armatus* proving efficient nutrient uptake of the algae. The reported values are similar to that of Ramasubramanian *et al.*, (2006). Calcium was reduced to 38.88 percent by *Oscillatoria acuminata* and 36.11 percent by *Scenedesmus armatus* (Fig.1h). Similarly magnesium was reduced to 22.64 percent by *Oscillatoria acuminata* and 28.30 percent by *Scenedesmus armatus* proving efficient uptake of both species (Fig.1i). The reduction was well noticed during 3-15 days of inoculation and after that it almost stabilized. Manoharan and Subramanian (1992b) on their study on sewage cyanobacteria interaction have found a 25 percent reduction of Ca^{++} by the blue green algae, *Oscillatoria pseudogerminata* var. *Unigramulata.* Although, calcium is undoubtedly required for cyanobacterial growth (Fogg *et al.*, 1973), substantial reduction in calcium level cannot be explained by uptake. Divalent cations such as calcium and magnesium are known to be essential for flocculation and would co-flocculate (Richmond and Becker, 1986).

In the present study, 11.42 percent of sodium was removed when the wastewater was treated with *Oscillatoria acuminata* and 2.38 percent by *Scenedesmus armatus* (Fig.1j) and 20 percent of potassium was removed by *Oscillatoria acuminata* and 36 percent by *Scenedesmus armatus* (Fig.2a). The treated wastewater contains decreased amount of sodium and potassium was due to the additions of nutrients in the treatment process (Somashekar *et al.*, 1984).

In the present study, 40.98 percent of iron was removed when the wastewater was treated with *Oscillatoria acuminata* and 60.65 percent by *Scenedesmus armatus* (Fig.2b). Similar observation was reported by Senthil Kumar (2001) and Kannan *et al.*,(2004). Murugesan and Dhamotharan (2009) reported 35.48 percent reduction of iron when the thermal wastewater was treated with *Pithophora* sp.

Fish excrete ammonia as waste from their gills, kidneys and normal respiration. Ammonia also develops from unconsumed feeds, shells moults of prawn and dead algae. The aquatic toxicity of ammonia increases dramatically with increased pH, promoting the formation of unionized ammonia, which is more toxic than the ionic form (CCREM, 1987). In the present study 88.88 percent free ammonia increased in aquaculture effluent treated with *Oscillatoria acuminata* and no change in *Scenedesmus armatus* (Fig.2c). The feed used as nutrients for the fish is proteinaceous. The decomposition of the residual unconsumed feed by nitrifying bacteria leads to the formation of ammonia, which is then converted into nitrate, thus increasing the ammonia and nitrate content of the effluent. Lee and Lee (2001) working with *Chlorella kestrel* reported the higher removal of ammonium from wastewaters. The toxicity of ammonia is dependent on pH, oxygen concentration and temperature (Cote, 1976). Martinez *et al.*, (2000), who described elimination of NH_4^+ (between 79% and 100%) after 188.25 hrs (about 8 days. There are obvious advantages of eliminating ammonium from wastewater using micro algae: (1) it does not generate secondary pollution of NH_3 and (2) the micro algal biomass can be harvested and used as a slow-release fertilizer or soil conditioner (de la noue *et al.*, (1992); Mallick (2002); Mulbry *et al.*, (2005).

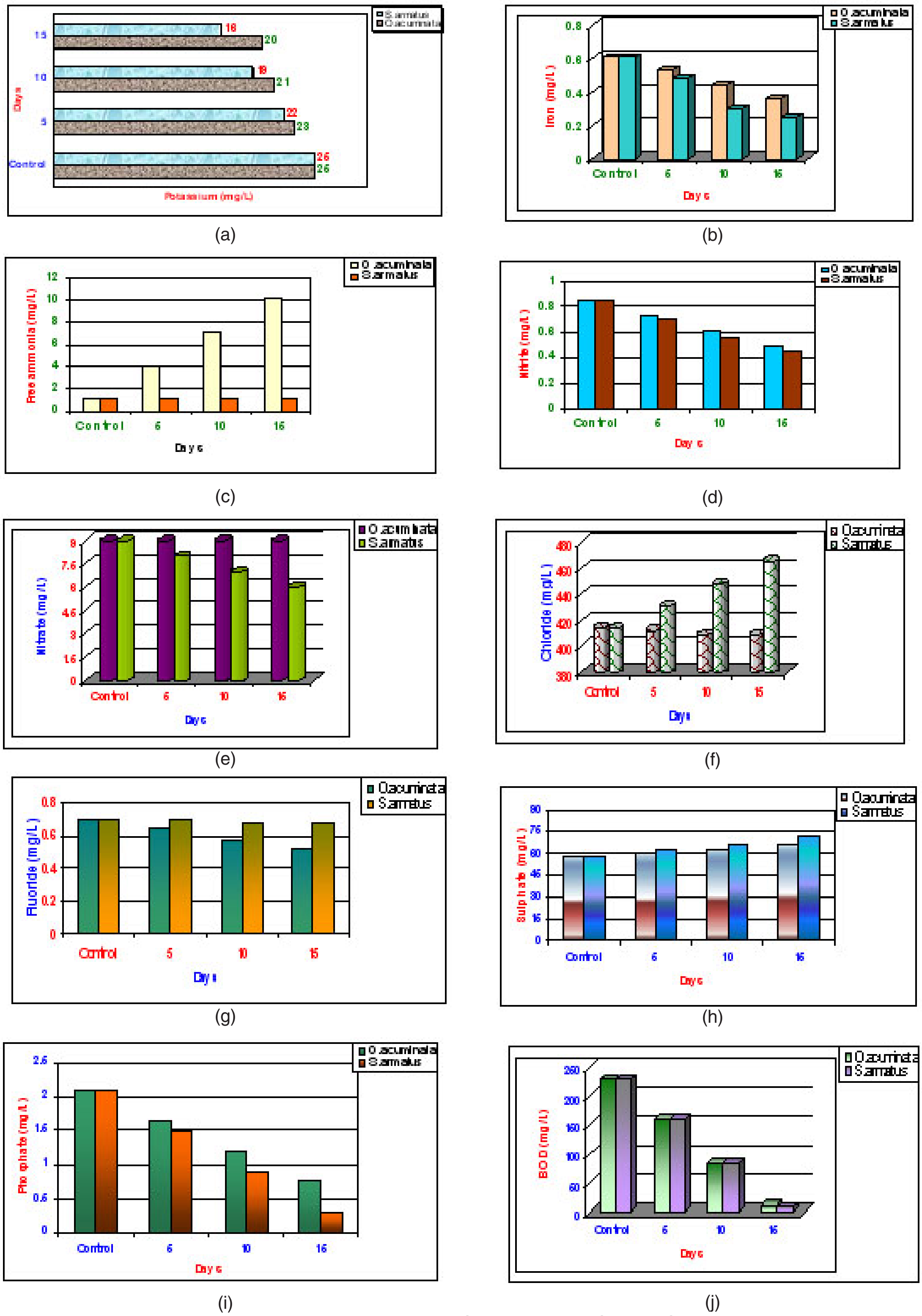

Fig. 2: Reduction of pollution load a) Potassium b) Iron c) Free ammonia d) Nitrite e) nitrate f) Chloride g) Fluoride h) Sulphate i) Phosphate j) BOD

Nitrite and nitrate may be produced during secondary wastewater treatment, when ammonia-N is biologically converted to the oxidized forms by nitrifying bacteria. Nitrogen uptake rates by microalgae depend on the concentration of nitrogen sources, and on environmental factors such as irradiance, temperature and water movements (Lobthobban and Harrison, 1994). The uptake rate of nutrients is influenced by several biological factors such as, various types of tissues, age of the plant, its nutritional history or nitrogen status of the thallus, and interplant variability. In the present study in the effluent treated with *Oscillatoria acuminata* 40.47 percent of nitrite was removed and 46.42 percent by *Scenedesmus armatus* (Fig.2d). Biological nitrogen removal of an effluent without substrate addition is possible if the effluent contains enough biodegradable organics to denitrify all its nitrifiable nitrogen content. Vilchez and Vega (1994) reported that alginate-entrapped *Chlamydomonas reinhardtii* cells provide a stable and functional system for removing nitrogenous contaminants from wastewater. As light intensity increased resulting in proportional increase in nitrate uptake was observed. On the other hand the effect of nitrate on ammonium uptake has received little attention, although a decrease in ammonium uptake in the presence of nitrate has been reported (Dortch and Conway, 1984). There is no change in the wastewater treated with *Oscillatoria acuminata*. Nitrate was reduced to 33.33 percent by *Scenedesmus armatus.* In the present study nitrate and low light favoured and increased the growth rate (Fig.2e). De La Noue *et al.*, (1980) showed that nitrogen uptake could be increased if the micro algae were preconditioned by starvation. Micro algae have the ability to take up various kinds of nitrogen (Kim *et al.*, 2000). The nitrate will be taken up by algae and either be converted to organic nitrogen in their cell tissues (algae assimilation) or will be reduced to elemental nitrogen (N_2) and lost as a gas by denitrification (Oswald, 1996).

Chloride is generally considered as one of the major pollutant in the wastewater which are difficult to be removed by conventional biological treatment methods. In the present study, 1.20 percent of chloride was removed when the effluent was treated with *Oscillatoria acuminata* and 11.15 percent of chloride was increased by *Scenedesmus armatus* (Fig.2f). In the present study amount of chloride, lowers the remediation activities of microorganisms. Vijayakumar *et al.,* (2005) reported 40 percent reduction of chloride in dye effluent, when treated with *Oscillatoria* sp.

Fluoride content in the present study was reduced to 24.63 percent when the wastewater was treated with *Oscillatoria acuminata* and 14.49 percent *by Scenedesmus armatus* (Fig.2g). In general cyanobacteria are highly sensitive to fluoride, whereas green algae appear almost resistant to fluoride concentrations up to 10mM (Bhatnagar, 1997). The threshold concentration at which toxicity is manifested varies between green algae and cyanobateria and is strongly pH dependent. The algal cell wall contains a high amount of polysaccharide and some of them are associated with proteins and other components (Ilhami *etal.*, 2005). These biomacromolecules on the algal cell surfaces have several functional groups (such as, amino, carboxyl, thiol, sulfydryl and phosphate groups) and biosorption phenomena depends on the protonation or unprotonation of these functional groups on the surface of the cell wall (Ilhami *et al.*, 2005). The presence of calcium and chloride reduce the toxicity of fluoride to fish (CCREM, 1987). Because of the limited effluent concentration data available for fluoride, it is recommended that it can be included in effluent surveys to expand the database, hence allowing for assessment of how significant a potential substance it may be. Kotteswari *et al.*, (2007) reported 45.28 percent reduction of fluoride when the dairy effluent treated with *Spirulina platensis.*

In the present study sulphate was increased to 12.12 percent when the wastewater was treated with *Oscillatoria acuminata* and 13.43 percent by *Scenedesmus armatus* (Fig.2h). The slowly degradable sulphate converted to sulphide which subsequently combine with the metal and form metal sulphide. This sulphide becomes toxic as well as corrosive in the degrading system. Moreover, the metal sulphide develops dark colour and foul smell. Kotteswari *et al.*, (2007) reported 74.82 percent reduction of sulphate in the dairy effluent treated with *Spirulina platensis.*

In the present study phosphate content in the wastewater was found to increase by 63.15 percent when the effluent was treated with *Oscillatoria acuminata* and 86.12 percent when the wastewater treated with *Scenedesmus armatus* (Fig.2i). The phosphorus assimilation rate was dependent on algal growth. Further, the cyanobacteria are known to absorb and store large amounts of phosphorus as polyphosphate granules (Fogg *et al*., 1973). Micro algae have the ability to absorb phosphorus (Adamsson *et al*., 1998). Kaya and Picard (1996) conducted an experiment with immobilized *Scenedesmus bicellularis* using high and low viscosity gels to enhance the stability of hardened gels during tertiary treatment of wastewaters containing high concentration of phosphate (1M). There are two major ways to remove phosphorus from wastewater: (i) direct cellular absorption under aerobic conditions, and (ii) sedimentation to anoxic conditions. Phosphorus removal in our, study under aerated conditions can be explained by its interaction with the nitrogen in the water. Because nitrogen is the limiting nutrient factor in the medium, the phosphorus concentration

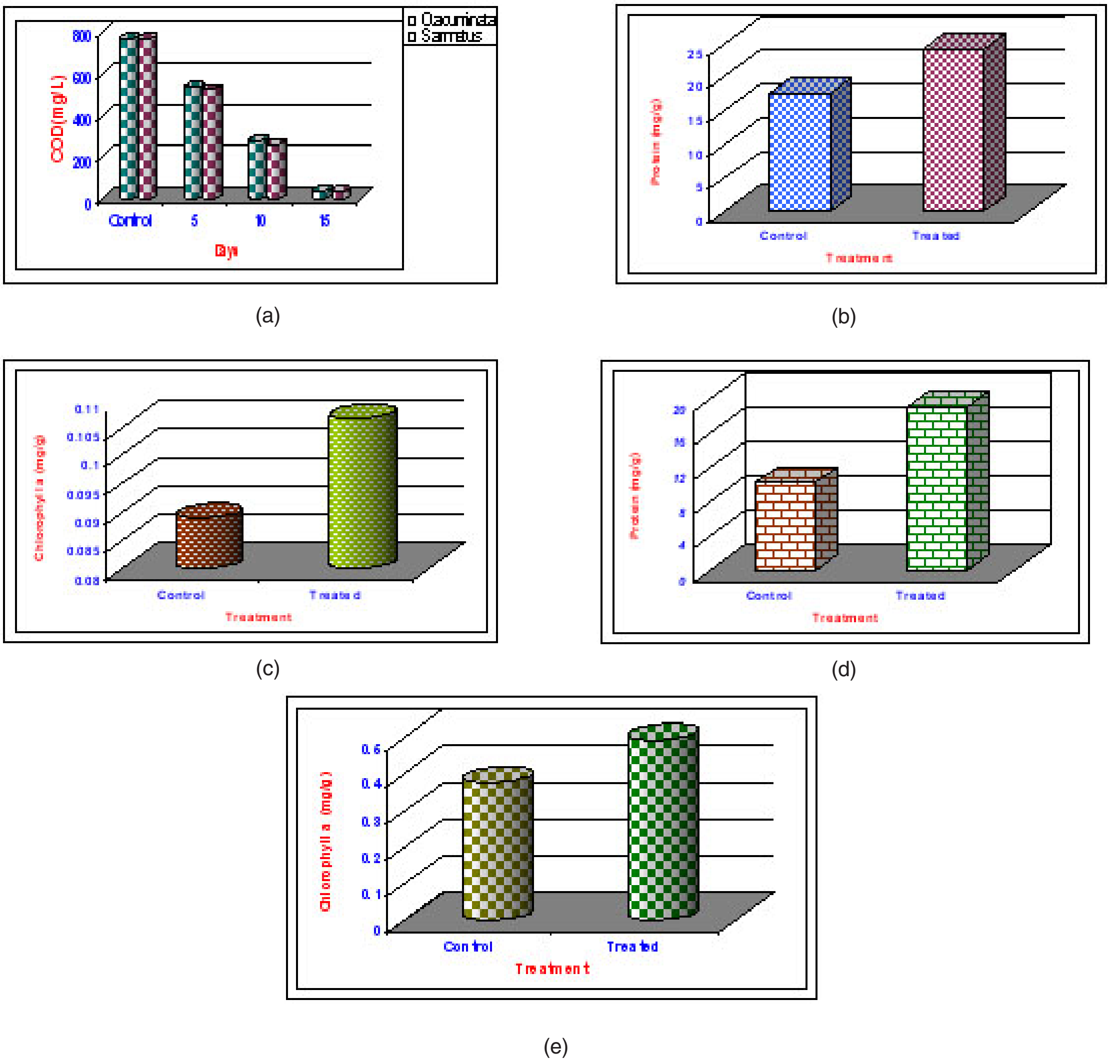

Fig. 3: a) Reduction of pollution load COD b) and c) Protein and chlorophyll-*a* content of aquaculture wastewater treated with *O.acuminata*. d) and e) Protein and Chlorophyll-*a* content of aquaculture wastewater treated with *S.armatus*

will still be high even after ammonia exhaustion. This leads to saturation in the phosphorus cellular absorption mechanisms (Chevalier and De la Noüe, 1985).

BOD indicates the quality of pollutant present in the waste that can be decomposed by bacteria under aerobic condition; hence BOD value increases with increase in organic content. The high content of organic matter results in high value of COD of wastewater because chemical oxygen demand measures the recalcitrant (non-biodegradable) organic matter in biologically treated industrial effluents (Malviya *et al.*, 2001). The BOD values in the wastewater were found to be reduced by 94.78 percent when treated with *Oscillatoria acuminata* and 96.08 percent by *Scenedesmus armatus* (Fig.2j). The COD values in aquaculture wastewater were found to be reduced by 95.72 percent when treated with *Oscillatoria acuminata* and 96.11 percent by *Scenedesmus armatus* (Fig.3a). Kotteswari *et al.*, (2007) reported 47.14 percent of BOD and 24.69 percent COD reduction when the dairy effluent was treated with *Spirulina platensis.* Similarly, Veeralakshmi *et al.*, (2007) reported 50.00 percent of BOD and 11.54 percent COD reduction when the petroleum effluent was treated with Oscillatoria sp. The presence of high suspended and dissolved organic matter increases the chemical oxygen demand (COD) and ammonia in the pond, thus rapidly deteriorating the water quality in intensive shrimp growing systems. These process accelerate the over blooming of phytoplankton in the presence of sunlight and oxygen. Algal over-blooming and consequent algal die-off not only cause the water quality to deteriorate but also lead to the proliferation of pathogenic bacteria in shrimp cultivation systems. Reduction of BOD and COD levels might occur due to the removal of dissolved organic compounds and derivatives to some extent from the effluent during the treatment process (Verma *et al.*, 1988).

The rates of increase in the biomass growth rates of *Oscillatoria acuminata* and *Scenedesmus armatus* cultivated in fish culture wastewater were k = 0.63 day^{-1} and k = 0.02 $^{\text{day-1}}$. Lower values were observed in the case of *Scenedesmus armatus* batch cultivation in nutrient medium (Lodi *et al.*, 2003). This was because the concentrations of inorganic nutrients in the medium used by Lodi *et al.*, (2003) were much less than that observed in the fish culture effluent. The results of increased chlorophyll- *a* (30.95%) and protein (85.03%) content of *Scenedesmus armatus* after 15 days of cultivation in fish culture wastewater (Fig.3d, e) are comparable with *Nostoc muscorum* grown in sewage and industrial wastewater (El-Sheekh *et al.*,2005). Aquaculture wastewater also increased the chlorophyll *a* and protein content of *Oscillatoria acuminata* 19.66 % and 37.75% respectively (Fig.3b, c). The above results suggest that the fish culture effluent supported the micro algal growth and induced the bioaccumulation and incorporation of nutritional elements present in effluent water into cellular macromolecules.

Sustainable growth of the aquaculture industry requires profitability, economic development, and waste management. Waste management decisions must be made on an individual basis due to site characteristics on the farm and within the watershed. Presently, no model considers the economic benefit of water treatment by biofilters, in as much as there is no cost associated with aquaculture discharge/effluent. This is a complex issue. The present study clearly indicates that unscientific fish farming has adverse effects on environment and that proper scientific method if properly employed; sustainable fish farming could be allowed to grow as per the Supreme Court directives on Dec 11th 1996.

There are several commercial products marketed for use in aquaculture to clean up the pond bottom, maintain good water quality and improve shrimp health, particularly for intensive aquaculture. The role of beneficial algae to control pathogens will become particularly important in aquaculture, especially in the light of the increasing number of antibiotic resistant strains of algae. Strict government regulation or environmental regulation of environmental treatments that is cost-effective. The present study has demonstrated the effectiveness of nutrient removal from aquaculture pond by algae the feasibility of rotation and co-culture of algae and fish. Both systems can recycle nutrients effectively within the environment friendly culture systems. Further research is needed to refine algae and fish co-culture system. Based on the results, it can be concluded that both *Oscillatoria acuminata* and *Scenedesmus armatus* have potential for use in aquaculture effluent treatment. The present study also showed that uptake of inorganic nutrients yielded a considerable amount of algal biomass, which can be a valuable byproduct of aquacultural activities. Further research is needed to evaluate the potential of microalgal biotreatment on the large scale basis with efficient harvesting methods.

REFERENCES

1. Adamsson, M., G. Dave., L.Forsberg and B. Guterstam., Toxicity identification evaluation of ammonia, nitrite and heavy metals at the stensund Wastewater. Aquaculture Plant. Sweden. *Wat. Sci. Technol*. 38: 151-157 (1988.
2. Anand, N., Indian Fresh Water Micro algae. Bishen Singh Mahendra Pal Singh, Dehra Dun. 1-94 (1998).
3. Arnon, D.T., Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol*. 24: 1-5 (1949).
4. Bhatnagar, M. 1997. Fluoride tolerance in micro algae and its ecological implications (Dissertation). Indian Agric. Res. Inst.New Delhi.
5. Bindu, H.V. and Anjaneyulu, Y., Removal of colour from industrial effluent by using waste biomass and reagent loaded polyurethane foam. International conference industrial pollution and control technologies (ICIPACT) ICI **065**: 457-463 (1997).
6. CCREM (Canadian Council of Resource and Environment Ministers). Canadian Water Quality Guidelines (1987).
7. Chevalier, P. & De la Node, J., Efficiency of immobilized hyper concentrated algae for ammonium and orthophosphate removal from wastewaters, *Biotech. Lett*., **7**: 395-400 (1985).
8. Cote, R.P., The effects of petroleum refinery liquid wastes on aquatic life, with special emphasis on the Canadian environment. National Research Council of Canada. NRC Associate Committee on Scientific Criteria for Environmental Quality, Ottawa, Ontario, Canada K1A 0R6, publication number 15021**,** 77 (1976).
8. De La No¡e J., Laliberte, G. and Proulx D., Algae and wastewater. Journal of *Applied Phycology.* **4**: 247-254 (1992).
9. De La No¡e J., Proulx D., Guay R., Pouliot Y and Turcotte, J, Algal biomass production from wastewaters and swine manure: nutritional and safety aspects. In: Microbial Mass Proteins (ed. by M. Moo-Young & K.F. Gregory), Elsevier Applied Science, London, UK, 141-165 (1986).
10. De La Noue, J. G.A. Picard, N.C. Peiette and C. Kirouae, C., Utilisation de l'algue *Oocystis* pout le traitement tertiarir des eaux usees. II. Effect du conditionnement prelable des cellules en cyclostat sur la vitesse de prise en chanrge de l' zote lors d' incubations de longue duree. *Water Res*. **14**: 1115-1130 (1980).
11. Desikachary T.V, Cyanophyta, Indian Council of Agricultural Research New Delhi. 686 (1959).
12. Dhamotharan, R. Manigandan, A and Murugesan, S., Biotreatment of sewage (cooum) wastewater by Cyanobacteria. *Biotechnology Research Asia*. **5**(1): 349- 354 (2008).
13. Dortch, Q and H.L. Conway., Interaction between nitrate and ammonium uptake: Variation with growth rate, nitrogen source and species. *Mar. Biol*.**79**: 151-164 (1984).
14. El-Sheekh, M.M. El-Shouny, W.A. Osman, M.E.H. and El-Gammal EWE., Growth and heavy metals removal efficiency of *Nostoc muscorum* and *Anabaena subcylindrica* in sewage and industrial wastewater effluents. *Environmental Toxicology and Pharmacology*. **19**: 357-365 (2005).
15. Fogg, G.E. W.D.P. Stewart, P. Fay and A.E. Walsby., The blue-green algae. Academic Press Inc. (London) Ltd., London (1973).

16. Goel, P.K., Water pollution, causes, effects and control New age international (P) Ltd., Publ. New Delhi 269 (2000).
17. Ilhami, T. B. Gulay, Y. Emine, B. Gokben, Equilibrium and kinetic studies on biosorption of Hg(II), Cd(II) and Pb(II) ions onto micro algae *Chlamydomonas reinhardtii*, *J. Environ. Manag*. **77**: 85-92 (2005).
18. Joyner, D., Aquaculture effluent regulation state regulatory perspective. Proceeding of National Livestock, Poultry and Aquaculture Waste Management Workshop. Vol.3. ASAE. Publication, Ste. Joseph. MI. pp. 27-31 (1992).
19. Kannan, N., Karthikeyan, Vallinayagam, G., and Tamilnselvan. N, A study on assessment of pollution load of sugar industry effluent. *ISEP* **24**(1): 256-262 (2004).
20. Kaya, V.M., Picard G. 1996. Stability of chitosan gels as entrapment matrix of viable *Scenedesmus bicellularis* cells immobilized on screens for tertiary treatment of wastewater. *Bioresource Technol* 56, 147–155.
21. Kim, M.H, W.T. Chung, M.K. Lee, J-Y- Lee, S-J. Ohh, J.H. Lee, D.-H. Park, D. J. Kim and H-Y. Lee., Kinetics of removing nitrogenous and phosphorus compounds from swine waste by growth of micro alga. *Spirulina platensis*. *J. Microbiol. Biotechnol*. **10**: 455-461.University Press, Cambridge, London. 163-209 (2000).
22. Kotteswari, M., Murugesan, S, Kamaleswari, J and Veeralakshmi, M, Biomanagement of Dairy effluent by using Cyanobacterium. *Indian Hydrobiology.* **10**(1): 109-116 (2007).
23. Lee. J.S. D.K. Kim, J.P. Lee, S. C. Park, J.H. Koh and S.J. Ohh., CO_2 fixation by *Chlorella* KR-1 using flue gas and its utilization as a feed stuff for chicks. *J. Microbiol. Biotechnol*. 11: 772-775 (2001.
24. Lobthobban,C.S, Harrison,P.J., Seaweed Ecology and physiology. Cambridge Lodi A., Binaghi L., Solisio C., Converti A. & Del Borghi M., Nitrate and phosphate removal by *Spirulina platensis*. *Journal of Industrial Microbiology and Biotechnology.* **30**: 656-660 (1994).
25. Lowry, O.H. N.J. Rosebrough, A.L. Faer and R.J. Randall 1951. Protein measurements with Folin-phenol reagent. *J. Biol. Chem.* **193**: 265-275 (2003).
26. Malaviya Piyush and V.S. Rathore., A correlation study on some physico chemical quality parameters of pulp and paper mill effluents. *Poll Res*. **20**(3): 465-470 (2001).
27. Mallick, N., Biotechnological potential of immobilized algae for wastewater N, P and metal removal from wastewater by carrageenan immobilized *Chlorella vulgaris*. *Water Sci Technol*. **38**: 185-192 (2002).
28. Manoharan C., and G. Subramanian., Sewage – cyanobacteria interaction. A case study. *Indian J. Environ pro*. **12**(4): 251-258 (1992b).
29. Martinez, ME, Sanchez S, Jimenex JM, El Yousfi F, Munoz L, Nitrogen and phosphorus removal from urban wastewater by the micro alga *Scenedesmus obliquus*. *Bioresour Tecnol*. **73**: 263-272 (2000).
21. Mulbry, W. Westhead EK, Pizaro. C, Sikora, L., Recycling of manure nutrients: use of algal biomass from dairy manure treatment as a slow release fertilizer. *Bioresour Technol*. **96**: 451-456 (2005).
22. Murugesan, S and Dhamotharan, R, Bioremediation of thermal wastewater by *Pithophora* sp. *Current World Environment*. **4**(1): 137-142 (2009).
23. Nicholas, H.W and Bold, H.C., *Trichosarcina polymorpha* gen.et .sp. F.*Phycol.* 1: 34-8 (1965).
24. Oswald W. J., Green F. B., Bernstone L. S., Lundquist I. J., and other. Advanced Integrated Wastewater Pond Systems for Nitrogen Removal. *Water Science and Technology*. **119**(12): 115-122 (1996).
25. Pillay, T.V.R., Aquaculture Principle and practices. Fishing Book News, London (1990).
26. Pouliot Y. & De La No¡e J., Utilisation des micro-algues pourle traitement tertiaire des eaux usee s. C.R. 7 Symp. Traitement Eaux Usee University Laval, Montreal 131-145 (1985).
27. Richmond, A. and E.W. Becker, Technological aspects of mass cultivation. A general outline, In CRC Handbook of micro algal mass culture. Ed A. Richmond, CRC Press, Inc. Boca Raton, Florida. pp. 245-263 (1986).

28. Senthilkumar, R.D., Narayanaswamy, R and Ramakrishnan, K., Pollution studies on sugar mill effluent – physiological characteristics and toxic metals. *Poll. Res*. **20**(1): 93-97 (2001).
29. Sharma, R and T.P. Scheena., Aquaculture wastes and its management. *Fisheries World*. 22-24 (1999).
30. Singh, P.K., Dhar DW, Pabbi, S. Prasanna. R & Arora, A., BGA. *Azolla* Bertilizers – A manual for the production. *Evaluation and Ut ilization*. Venus Printers and publishers, New Delhi (2002).
31. Somashekar., Gowda., S.L.N. Shettigar and Srinath, K.P., Effect of industrial effluents on crop plants. *Indian J. Environ. Hlth*. 26(2): 136-146 (1984).
32. Veeralakshmi, M. Kamaleswari.J, Murugesan, S and Kotteswari, M, Phycoremediation of Petrochemical effluent by Cyanobacterium. *Indian Hydrobiology.* **10**(1): 101-108 (2007).
33. Verma, P. and Madamwar, D., Comparative study on transformation of azo dyes by different white rot fungi. *Indian J. Biotechnol*. **1**(10): 393-396 (2002).
34. Verma, A.M. V.K. Dudani, B. Kumari and A.N. Kargupta., Algal population in paper mill wastewater. *Indian. J. Environ. Hlth*. **30**: 388-390 (1988).
35. Vijayakumar S., Thajuddin N., and C. Manoharan, Role of cyanobacteria in the treatment of dye industry effluent. *Poll. Res*. **24**(1): 69-74 (2005).
36. Vilchez. C., Vega, J.M., Nitrate uptake by *Chlamydomonas reinhardtii* cells immobilized in calcium alginate. *Appl Microbiol Biotechnol*. **41:** 137-141 (1994).