Seaweed Res. Utiln., 27 (1 & 2) : 99 - 104, 2005

Influence of physicochemical parameters on the removal of nitrogenous compounds during treatment of fish effluent with seaweeds

C. SEEMA, REETA JAYASANKAR[1] AND GRACE MATHEW
[1]*Central Marine Fisheries Research Institute, Kochi - 682 018, Kerala, India*

ABSTRACT

Fish effluent was treated with *Gracilaria corticata* and *Ulva lactuca* in separate tanks. Effluent without seaweeds was kept as control. Physicochemical parameters such as pH, Biological Oxygen Demand, Dissolved Oxygen on nitrogenous compounds such as ammonia, nitrite and nitrate were monitored. The DO content was found to be 15 % more in the treatment tanks of *G.corticata* (5.44 ml/l) when compared to the control (4.05 ml/l) during same period, may account for the high photosynthetic activity and efficient utilization of dissolved carbon in the effluent. The BOD value was observed to be more in the treated tank of *G.corticata* compared to control on 20 days of treatment, could be due to the growth of associated bacteria in the system, which favoured the oxidation of ammonia to nitrite and then to nitrate. The pH was found to have significant positive correlation with nitrate content in all the treatments, whereas BOD exhibited a negative correlation with nitrate. The removal of nitrogenous compounds was found to be more in the effluent treated with *U. lactuca* followed by *G. corticata* than the control.

Introduction

The discharge of low-quality water from land-based mariculture facilities cause environmental and economic concerns, since fish excrete to the water 60-70% of their ingested protein nitrogen (Porter *et al.*, 1987). Effluents from intensive farming contain much organic matter, nitrogen compounds, phosphorus and other nutrients, lead to eutrophication. The water quality problem is associated with both physical and chemical factors such as high or low dissolved oxygen, high concentration of nitrogenous compounds (ammonia-N and nitrate-N) and high levels of hydrogen sulphide.

Biological treatment process appears to be economical, sound and scientifically acceptable, because of its simplicity, ease of operation and maintenance. The work on the use of biofilters in aquaculture system has been attempted by a few workers (Cohen and Neori, 1991; Rosenthal, 1991; Oswin and Rahman, 1997). The biological treatment of sewage by algae and bivalves was also studied by Ryther *et al.* (1972 and 1975). Duck weed culture in waste water has gathered much scientific concern, since the removal of Biological Oxygen Demand, nitrogen, phosphorus and metals by duckweed from waste water has been reported to be appreciably high. (Reed *et al*., 1998; Ayyappan, 2001). Large scale experiments on purification of nutrient rich effluents have been made with seaweeds, principally *Gracilaria* (Ryther *et al*., 1975 and Buschman *et al.*, 1994). In the present study, the influence of physicochemical parameters such as pH, dissolved oxygen, Biological Oxygen Demand and salinity on the removal of toxic nitrogenous compounds from fish effluents during biological method of treatment with seaweeds has been attempted.

Materials and Methods

Gracilaria corticata and *Ulva lactuca* were collected during low tide from Thonithurai ($9^0$17' N and $70^0$11'E), located on the south east coast of TamilNadu, near Mandapam. Samples were brushed off epiphytes, cleaned, well packed in perforated polythene bags and transported to CMFRI, Cochin. Fish effluents were collected from the Fisheries Harbour Laboratory of CMFRI, Thopumpaddy, Kochi where the grouper fishes were maintained in 5 ton capacity fibreglass tanks.

Experiments with two replications per treatment were set up in transparent rectangular perspex tanks of 100 l capacity inside the Marine Hatchery Complex under controlled environmental conditions with temperature ranging between 25–28^0 C and photoperiod of 16L: 8D. Illumination of 1500 lux was provided by fluorescent lights. Based on the preliminary experiments conducted, 200g of *Gracilaria corticata* and *Ulva lactuca* were kept on the nylon mesh just dipped in the effluent in two separate tanks respectively until the end of the experiment. Effluents without seaweed were treated as control. Continuous aeration was provided in all the tanks.

The experiments were carried out for 30 days. Regular sampling was done in control and treated tanks at 10 days intervals. Dissolved oxygen was estimated by Winkler's method of Strickland and Parson (1968). The pH was taken by a digital pH meter. Salinity was recorded by using a refractometer with reference to distilled water. Biological Oxygen Demand (BOD) was estimated by incubating the water sample in a BOD incubator for 5 days at 20^0 C under complete darkness. Ammonia estimation was carried out by the method of Solorzano (1969) and nitrate by the modified method of Morris and Riley (1963) with some modifications suggested by Grasshoff and Wood (1967). Nitrite was estimated by Shinn method (1941) modified by Bend schneider and Robinson (1952). Data were analyzed statistically by Analysis of Variance (ANOVA) and Pearson correlation using SYSTAT.

Results

The pH declined in *G.corticata* by 1% in 20 days and further increased by 4 % in 30 days. The analysis of variance did not show any significant difference in pH between the treatment and control tank throughout the experimental period (P> 0.01) (Fig.1). pH showed significant negative correlation with BOD (r = -0.71**) in *G.corticata*, (r = -0.95**) in *U.lactuca* and (r = -0.74**) in Control. While comparing with DO, significant positive correlation was observed *in U.lactuca* (r=0.93**). Concentration of ammonia was more in control than the treatment tanks till 30 days. The reduction was found to be 76.03% in *U.lactuca* and 66.9% in *G. corticata* on 20th day of treatment with respect to control. The decline of nitrate was found to be almost similar in both the treatments (55%) compared to control. The nitrite content was significantly declined in the treatment of *U.lactuca* by 98.6% and 74.9% in *G.corticata* (Fig.2). Correlation with nitrate was found to be negatively significant (r = -0.93** in GC, r = -0.91** in UL and r = -0.798** in Cont). It was also observed that ammonia and nitrite

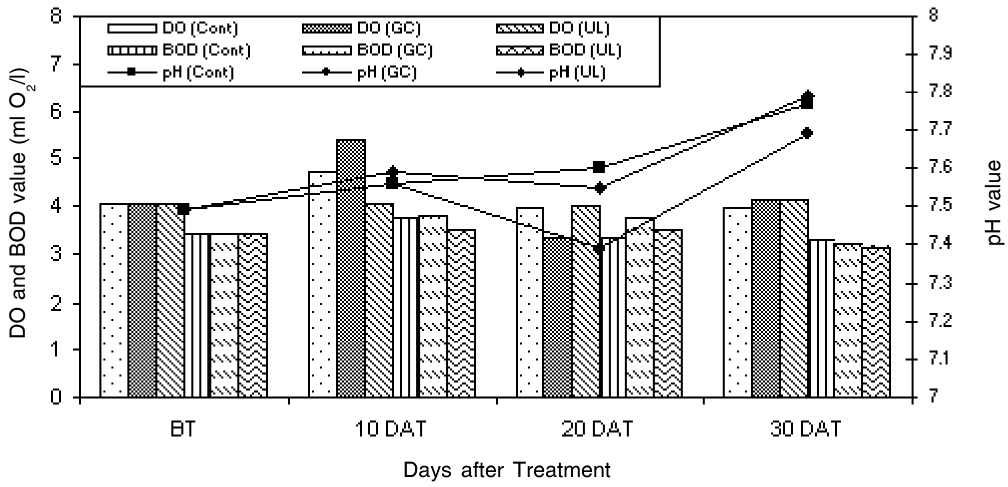

Fig. 1. *Physico chemical parameters of fish effluent treated with seaweeds*

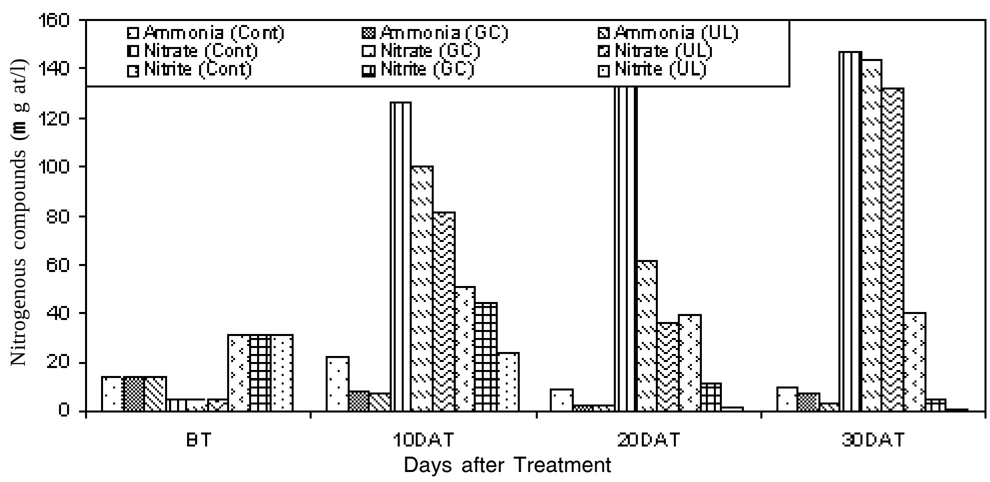

Fig. 2. *Nitrogenous compounds in fish effluents treated with seaweeds*

are negatively correlated with pH except in *G.corticata* , which showed a significant positive correlation with ammonia on 10 days (r = 1.00**). The correlation of ammonia and nitrite with pH was significant only in control tank (Table 1).

Dissolved oxygen (DO) content of the treated and control tank showed almost a similar trend. There was an initial increase of DO for 10 days, declined on 20 days and then increased on 30 days . The increase in DO was 33 % in treated tank of *G. corticata* but no change was observed in *U. lactuca* during 10 days of treatment. On 20 DAT the DO declined by 38 % in *G.corticata*, 16 % in control tank but only 2 % in *U. lactuca* with reference to 10 DAT. An increase in DO concentration was observed in all the tanks after 30 days. (Fig.1). Statistical interpretation did not show significant difference between the control and treated tanks ($P > 0.01$). Significant positive correlation was observed between DO and ammonia concentration in *G.corticata* (r=0.71**) and control (r=0.99**), whereas no significant correlation was noticed with *U.lactuca*. Similar pattern was observed between DO and nitrite (r= 0.84**) in *G.corticata* and (r=0.99**) in control. The nitrate concentration was found to have significant positive correlation with DO in *U.lactuca* (r=0.96**) whereas negative correlation was observed in control (r=-0.6*). DO concentration on 10 days of treatment was found to have significant positive correlation with BOD in *G.corticata* (r=1.00**) and control (r=0.99**). On the otherhand, significant negative correlation was observed in *U.lactuca* (r= -1.00**)

The Biological Oxygen Demand (BOD) also showed a similar trend like dissolved oxygen. There was an initial increase in BOD on 10 days of treatment which was more pronounced in *G.corticata* followed by a decline on 20 DAT both in control and treated tanks. The decline of the BOD was maximum in *G. corticata*. There was decline in BOD values in all the tanks during 30 days of treatment (Fig. 1). The analysis of variance table showed no significant difference in BOD values between treatment and control tanks throughout the experimental period ($P > 0.01$). BOD was found to have significant correlation with nitrate in *G.corticata* (r=0.82**) and *U.lactuca* (r=0.82**) treatments. Significant negative correlation was noticed in the control tank (r=-0.68*). With ammonia, BOD showed a significant positive correlation in control (r= 0.98**), whereas in *G.corticata* and *U.lactuca,* no significant correlation was observed. Nitrite with BOD exhibited significant positive correlation in control (r=0.97**) and *G.corticata* (r=0.68**), whereas no significance was observed in *U.lactuca*. The changes in different nitrogenous compounds in fish effluents during treatment with seaweeds are given in Fig. 2.

Table 1. *Correlation matrix of physicochemical parameters influencing removal of toxic nitrogenous waste from fish effluent*

Control	pH	DO	BOD	Ammonia	Nitrate	Nitrite
pH	1.00	-0.63*	-0.74**	-0.61*	-0.8**	-0.62*
DO	-0.63*	1.00	0.99**	0.99**	-0.61*	0.99**
BOD	-0.74**	0.99**	1.00	0.98**	-0.68*	0.97**
Ammonia	-0.61*	0.99**	0.98**	1.00	-0.63*	0.96**
Nitrate	-0.8**	-0.61*	-0.68*	-0.63*	1.00	-0.53
Nitrite	-0.62*	0.99**	0.97**	0.96**	-0.53	1.00
G.corticata						
pH	1.00	0.22	-0.71**	0.69*	0.93**	-0.09
DO	0.407	1.00	0.22	0.407	0.35	0.84
BOD	-0.71**	0.407	1.00	-0.26	-0.82**	0.68*
Ammonia	0.69*	0.71**	-0.26	1.00	0.59*	0.38
Nitrate	0.93**	0.35	-0.82**	0.59*	1.00	-0.53
Nitrite	-0.09	0.84	0.68*	0.38	-0.53	1.00
U.lactuca						
pH	1.00	0.93**	-0.95**	-0.15	0.91**	-0.39
DO	0.93**	1.00	0.8**	-0.06	0.96**	-0.15
BOD	-0.95**	0.8**	1.00	0.34	-0.82**	0.55
Ammonia	-0.15	-0.06	0.34	1.00	0.1	0.82**
Nitrate	0.91**	0.96**	-0.82**	0.1	1.00	-0.06
Nitrite	-0.39	-0.15	0.55	0.82**	-0.06	1.00

Discussion

The pH value in fish effluent did not show wide variation in the control and treated tank, but there was a marginal decline of pH in the treated tanks. *G.corticata* showed a decline of pH during 20 DAT, which may account for the efficient utilization of dissolved carbon in the effluent. Earlier report stated that *G.corticata* showed high photosynthetic activity in the initial period when kept under laboratory condition (Reeta and Kulandaivelu, 2000). The increase in dissolved oxygen in the treated tank of fish effluent by *G.corticata* (5.45 ml O_2/l) confirms the high photosynthetic activity of the plant. The higher carbon dioxide demand for *Gracilaria* was confirmed by Bidwell *et al.* (1985). Reeta and Kulandaivelu (2000) also explained the utilization of maximum carbon noticed in the ^{14}C uptake. On the otherhand, the effluent treated with *Ulva* did not show much changes in the DO content. The increase in DO content in the control tank of fish effluent may account for the phytoplankton bloom in the enriched effluent when they were supplied with optimal light and air.

The low BOD values observed in *U.lactuca* compared to *G.corticata* and control up to 20 days of treatment clearly indicate the antibacterial property of *Ulva spp*. that has been reported by several workers (Charles and Suzuki, 2000; Sergey and Pei-Yuan Qian, 2002). After 20 days, the plants were in a stressed condition and influenced the bacterial load. The efficient removal of toxic nitrogen even with the low BOD level confirms the ability of the *Ulva* spp. to utilize the nutrients within the system. On the otherhand, the high concentration of BOD in the treated tank of fish effluent by *G.corticata* may account for the encouraging bacterial growth. Being an agarophyte, *Gracilaria corticata* is considered as a very good substrate for bacterial communities (nitrifying bacteria) which helps in the oxidation of ammonia to nitrite and nitrate. Bacterial consumption of oxygen can rapidly outpace oxygen replenishment from the atmosphere and photosynthesis performed by algae and aquatic plants. The result is a net decline in oxygen concentrations in the water. Nitrates and phosphates in a body of water can contribute to high BOD levels. Nitrates and phosphates help in rapid growth of algae to grow quickly, so also enhanced aging contributing to the organic waste in the water, which is then decomposed by bacteria resulting in a high BOD level.

In the present experiment ammonia, nitrite and nitrate concentration was found to be at a lower level on 20 days of treatment in the treatment tanks of *U.lactuca* and *G.corticata* when compared to the control on the same day. Thereafter on 30 days, the difference between control and treatment were found to be very low. The effluents treated with *G.corticata* and *U.lactuca*, were kept in a confined environment,the non availability of dissolved carbon might have stressed the plants to efficiently photosynthesize and utilize the toxic nitrogen after 20 days of treatment. In the present experiment, accumulation of higher concentration of nitrate after 20 DAT might have also accounted for the toxicity in the system which exerts stress to the plant, thus affecting the physiological condition of the seaweed.

Acknowledgements

The authors are thankful to Prof. (Dr.) Mohan Joseph Modayil, Director, Central Marine Fisheries Research Institute, Cochin for providing necessary facilities to carry out the work. The first author is thankful to Central Institute of Fisheries Education, Mumbai for granting fellowship for Master in Fisheries Science Course.

Literature cited

Ayyappan, S. 2001. Duckweed and fish based aquaculture sewage treatment. In: *Summer School on Environmental Impact Assessment and Management of Coastal Zones : An integrated approach*. pp. 109-113.

Bendschneider, K. and R. J. Robinson 1952. A new spectrophotometric method for the determination of nitrite in seawater. *J. Mar. Res*., 11 : 87-96.

Bidwell, R.G.S., J. Mc Lachlan and N.D. Lloyd 1985. Tank cultivation of Irish moss *Chondrus crispus* Stackh. *Bot. Mar*., 28 : 87-97.

Buschmann, A. H., O. A Mora, P. Gomez, M. Bottger, S. Buitano, C. Retamales, P.A.Vergara and A. Gutierrez 1994. *Gracilaria chilensis* outdoor tank cultivation in Chile: Use of land-based salmon culture effluent. *Aqua. Eng*., 13 : 283-300.

Charles, S. V. and M. Suzuki 2000. Dynamics of total surface bacteria and bacterial species counts during desiccation in the Malaysian sea lettuce *Ulva reticulata* (Ulvales, Chlorophyta). *Phycol.Res.*, 48: 55.

Cohen, I. and A. Neori 1991. *Ulva lactuca* biofilters for marine fish pond effluent. Ammonia uptake kinetics and nitrogen content. *Bot. Mar*., 34 : 475-482.

Grasshoff, E.A.J. and E.D. Wood 1967. Determination of nitrate in seawater by Cadmium-copper reduction to nitrite. *J. Mar. Biol. Assoc. UK.,* 47 : 23-31.

Morris, A. W. and J. P. Riley 1963. The determination of nitrate in seawater. *Anal. Chem. Act.*, 29 : 272-279.

Oswin, S. D. and A. A. Rahman 1997. Impact of aquaculture on mangroves : In *Proceedings of the National Seminar on Water Quality Issues in Aquaculture Systems*. R. Santhanam, V. Ramadhas and Gopalkrishnan (eds.). pp. 15-22.

Porter, C., M.D.Krom, M. G.Robbins, L. Brickel and A. Davidson 1987. Ammonia excretion and total N budget for gilthead seabream *(Sparus aurat*a) and its effects of water quality conditions. *Aquacul.,* 66 **:** 287-297.

Reed, S. C., E. J. Middlebrooks and W. Crites 1988. *Natural Systems for Waste Management and Treatment.* Mc Graw-Hill, Inc., USA. pp.150-161.

Reeta, J. and G. Kulandaivelu 2000. Influence of different wavelengths of light on photosynthetic and pigment constituents and absorption spectra of *Gracilaria* spp. *J. Aqua. Trop*.,16(4) : 359-371.

Rosenthal, H. 1991. Water and waste water treatment. *Eur. Aqua. Soc. Spl. Publ.,* 14: 282.

Ryther, J. H., W. M. Danstan, K. R. Tenore and J. E. Huguenin 1972. Controlled eutrophication increasing food production from the sea by recycling human wastes. *Bioscience*., 22 : 144-152.

Ryther, J. H., J. C. Goldman, C. E. Gifford, J. E. Huguenin, A. S. Wing, J. P. Clarner, L. D. Williams and B.E. La Pointe 1975. Physical models of integrated waste recycling marine polyculture systems. *Aquacul.*, 5 : 163-177.

Sergey, V. D. and Pei-Yuan Qian 2002 Effect of bacteria associated with the green alga *Ulva reticulata* on marine micro and macrofouling. *Biofouling*, 18 (3) : 217-228.

Shinn, M. B. 1941. The reaction of nitrate with a primary aromatic amine. *Ind. Eng. Chem. Anal. Ed.,* 13 : 33-35.

Solorzano, L. 1969. Determination of ammonia in natural waters by the Phenol hypochlorite method. *Limnol. Oceanog.,* 14(5) : 799-801.

Strickland, J. D. H. and T. R. Parsons 1968. A practical handbook of seawater analysis. *Bull. Fish. Res.Bd. Canada*, 167 :1-310.