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Removal of nitrogen load in the experimental culture system of seaweed and shrimp

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Abstract

The green seaweed *Ulva reticulata* used as co-culture species for monitoring the changes in toxic nitrogenous wastes in the shrimp culture system was found to efficiently remove ammonical nitrogen from 249.5 to 17.39 µmol nitrogen/l (94%). The nitrate nitrogen reduced from 28.39 to 24.21 µmol nitrogen/l (5%) and nitrite nitrogen from 14.51 to 9.03 µmol nitrogen/l (22 %). The removal of total nitrogen from the aquaculture system was found to be 45 % when treated with seaweed. The concentration of toxic nitrogenous wastes was found to be always at a lower level in the integrated system when compared to the monoculture system. Seaweeds of economical importance can be used in aquaculture system to improve water quality and generate revenue for the industry.

Keywords : Seaweed cum shrimp culture, toxic nitrogen, bioremediation

Introduction

Aquaculture, the fastest growing food production sector in the world has come under increasing scrutiny and criticism because of coastal pollution. Loss of sensitive coastal habitats is threat to aquatic biodiversity. Only toward the end of the 20th century, when the assimilative capacity of natural ecosystems was being overloaded by monoculture systems of shrimp and fish (Primavera, 1993; Rajendran and Kathiresan, 1997), the interest in using algae as nutrient scrubbers in integrated aquaculture of finfish, shellfish and crustaceans was renewed (Gordin *et al.*, 1981; Chopin and Yarish, 1998). Algae, and in particular seaweeds, are most suitable for biofiltration and can be economically cultured (Gao and McKinley, 1994). An output from one subsystem in an integrated farming system, becomes an input to another subsystem resulting in a greater efficiency of desired products (Edwards *et al.*, 1988), create a favourable environment for animal growth (Shan and Wang, 1985) and high growth rate of seaweed (Harlin *et al.*, 1978). Integrated farming systems with seaweeds have been reviewed by Neori *et al.*, 2004.

The objective of the present work was to study the efficacy of the seaweed *Ulva reticulata* for removal of toxic wastes from the experimental culture system of shrimp and seaweeds.

Materials and methods

Samples of *Ulva reticulata* from Ashtamudi Lake (8°45'-9°28'N and 76°25'-77°17'E) near Dalavapuram in Quilon were collected during low tide in the morning, brushed off epiphytes and cleaned well in running water 3-4 times. Seaweeds were sorted out and packed in perforated polythene bags and transported to Cochin. They were maintained in the seaweed culture laboratory of CMFRI Marine Hatchery Complex for 1-2 days to overcome the transportation stress before starting the experiment.

Indian white shrimp, (*Fenneropenaeus indicus*) H.Milne Edwards (1837) were collected from Ajanta Shrimp Farm near Valappu of Vypeen Island, Kochi.

Experiments were set up in transparent rectangular Perspex tanks of 100 l capacity inside the Marine Hatchery Complex under controlled environmental conditions. The temperature was maintained between 25–28° C. Illumination of 1500 lux was provided from fluorescent tube. The photoperiod was maintained at 16: 8 light and dark cycle. Shrimp and seaweed in a ratio of 1:5 biomass (20:100g) were kept in tanks with 90 l water of 23 ppt salinity. The tank having shrimps without seaweeds was treated as control. The animals were fed daily with pelleted diet from Higashimaru @ 2% of their body weight. All the experiments were carried out in duplicate. Adequate aeration was provided from the compressor with control valves. All the experiments were conducted for 20 days. Regular sampling was done in control and treated tanks at initial period i.e., before treatment (BT), 10 days after treatment (10 DAT) and 20 days after treatment (20 DAT).

Water quality parameters were estimated by standard procedures. Ammonia estimation was carried out by the

Table 1 *Anova table for the nitrogenous compounds in the seaweed - shrimp polyculture system*

Source	Sum-of-Squares	df	Mean-Square
Ammonia			
Days	35239.78	2	17619.89 **
Treatment	50768.00	1	50768.00 **
Days*Treatment	28142.36	2	14071.18 **
Error	14.83	6	2.47
Nitrate			
Period	1282.64	2	641.32**
Treatment	297.08	1	297.09**
Period*Treatment	379.78	2	189.89**
Error	1.24	6	0.21
Nitrite			
Period	283.48	2	141.74**
Treatment	112.58	1	112.58 **
Period*Treatment	83.74	2	41.87 **
Error	0.12	6	0.02
Total Nitrogen			
Period	57586.63	2	28793.32 **
Treatment	64091.97	1	64091.97 **
Period*Treatment	33074.75	2	16537.37 **
Error	23.59	6	3.93

** Highly significant ($p < 0.01$)

method of Solorzano (1969). Nitrate was estimated 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). Results were interpreted by analyzing the data statistically by two-way Analysis of Variance (Table 1).

Results and Discussion

In the present system of shrimp and seaweed culture, the nitrate-nitrogen concentration registered an increase in the control tank ranging from 1.02 to 28.39 μmol nitrogen/l compared to 24.22 μmol nitrogen/l in the treatment tank. It was observed that the nitrate nitrogen increased by 33 % per day in the control tank within 0-10 DAT and marginal increase of 0.2 % per day from 10-20 DAT. On the other hand, in the treatment tank, the nitrate-nitrogen increased by 7 % per day from 0-10 days, but higher increase of 25 % per day was observed on 10-20 DAT (Fig.1). At the end of the experiment, the ratio of nitrate-nitrogen was 1:1.17 ($P < 0.05$) in the treated and control tanks. Significant difference in nitrate concentration was observed between treated and controls tanks

The nitrite load was found to be higher in control tank ranging from 0.119 to 14.51 μmol nitrogen/l, in contrast

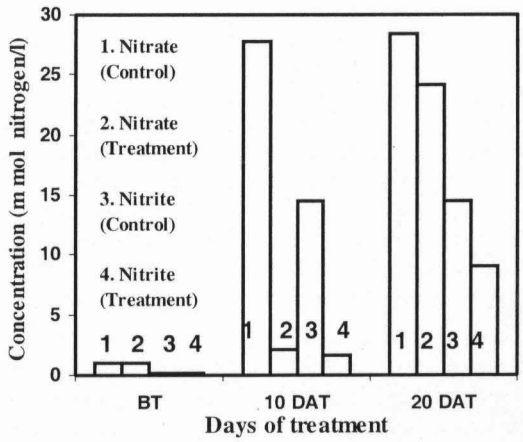

Fig.1 Nitrate and Nitrite concentration in the polyculture system

to the 9.03 μmol nitrogen/l in treatment tank after 20 days of experiment. The nitrite-nitrogen concentration increased by 48 % per day from 0-10 days and 0.005 % per day from 10-20 days respectively in the control tank. On the other hand, treatment tank registered an increase of 26 % per day from 0-10 days and 17 % per day from 10-20 DAT (Fig.1). The ratio of nitrite-nitrogen in control and

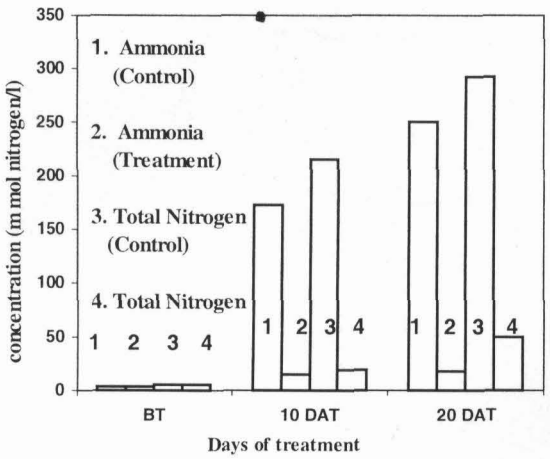

Fig. 2. Ammonia and total Nitrogen concentration in the polyculture system

treatment tank was found to be 1:1.61 ($P < 0.05$) at the end of experiment. The Analysis of variance showed significant difference in nitrite-nitrogen between treated and control tanks.

The ammonia concentration increased from 3.8 to 249.5 µmol nitrogen/l in the control tank and 17.2 µmol nitrogen/l in the treated tank after 20 days. The control tank recorded ammonia load @ 42 % per day till 20 days of culture period, compared to 15% in the treatment tank. Maximum load of ammonia nitrogen was observed within 10 days (38 %) and declined to 4 % from 10-20 days in the control tank. Similarly, the load was 14 and 1.5 % per day from 0-10 and 10-20 days respectively in the treatment tanks (Fig.2). At the end of the experiment, the ammonia nitrogen was found to be in the ratio of 1:14.5 ($P < 0.01$) in the treatment and control tanks. The Analysis of variance showed highly significant difference of ammonia-nitrogen in the control and treated tanks.

Ammonical-nitrogen contributed mainly to the total nitrogen content in the system. The total nitrogen content in the control tank was 38 % per day from 0-10 DAT and 3 % per day from 10-20 DAT. Whereas, in treatment tank, the increase was by 13 and 10 % per day from 0-10 DAT and 10-20 DAT respectively (Fig. 2). At the end of the experiment, the ratio of total nitrogen was found to be 1:5.79 ($P < 0.01$) in the treated and control tanks. The Analysis of variance showed highly significant variation between treated and control tanks.

In the closed polyculture system of shrimp and seaweed, the decline of ammonia concentration in the treatment tanks was found to be always more than nitrate and nitrite. This is due to the efficient utilization of ammonia by the seaweeds, as ammonium ion is frequently the preferred N source for growth of macroalgae. (D'Elia and DeBoer, 1978). According to Neori (1996) and Ahn *et al.* (1998), ammonia is assimilated as much as two to three times faster than the oxidized nitrate by many types of seaweeds. While comparing the utilization of nitrate and nitrite nitrogen in the treatment tank, the utilization was comparatively less from 10-20 days than 0-10 days of treatment. It may be mentioned here, that *Ulva reticulata* has high photosynthetic activity (Reeta, 2004), which can efficiently utilize the dissolved carbon in a closed system. During 10-20 DAT, plant may be stressed due to the non availability of dissolved carbon (Reeta and Sally, 2000) leading to bleaching by reduction in pigment concentration as observed earlier (Seema, 2002). *Ulva* species has antibacterial property (Selvin and Lipton, 2004) which might not have allowed the bacteria to grow in the system for 10 days when the plants were in healthy condition. Further, due to the unhealthy condition of seaweed, stress condition and increase in faecal matter of shrimps in the system enhanced bacterial load, oxidizing ammonia to nitrate and nitrite thereby increasing total nitrogen content in the system. Species of the genus *Ulva* have been described as opportunistic due to their thallus morphology, fast growth rates and rapid uptake of inorganic nutrients (Littler and Littler, 1980). Efficient nitrogen uptake even at low substrate concentrations has been reported for these species (Hein *et al.*, 1995; Pedersen and Borum, 1997).

The productivity of species in a mixed culture system is dependent on the performance of both the species in the system. When conditions are not optimal for both species, the co-culture system can produce negative results. Thus, environmental and physiological condition should be optimum. Maximum care should be taken in the stocking density of co-culture species.

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Isolation and screening of mucus-associated bacteria of the gastropod, *Drupa margariticola* for antagonistic activity

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Abstract

The mucus-associated bacteria of the gastropod, *Drupa margariticola* were screened for their ability to inhibit the human and fish pathogens. Out of the two hundred and eighty five bacterial strains isolated, 23% (65) were found to be pigmented, 71% (202) were identified as Gram-negative. A higher percentage of non-pigmented (77%) and Gram-negative (71%) strains were observed in the present investigation. 16% (46) of the isolates was found to have antagonistic activity against both human and fish pathogens tested and 63% of the Gram-negative strains (29) were found to be antibiotic producers. Antagonistic activity was found to be exhibited by pigmented strains too. A higher degree of inhibition was conferred by 3 of the isolates (D_{15}, D_{130} and D_{237}) against both human and fish pathogens. These strains exhibited full or complete degree of inhibition against *Escherichia coli*.

Key words: Gastropod, *Drupa margariticola* mucus-associated bacteria and antagonistic activity.

Introduction

Bacterial infections were considered won in the late 1960's but now antimicrobial resistance threatens to turn back. Resistance is spreading rapidly particularly where antibiotics are heavily used (Lech, 2004). The erroneous use of antibiotics both for therapeutic and aquaculture purposes has resulted in the advent of multiple drug resistance of the pathogenic bacterial strains. Hence the need of the hour is the search for novel antibiotics with lesser side effects. The ultimate source- the ocean, is a unique resource that provides a diverse of natural products primarily from bacteria and cyanobacteria and invertebrates such as corals, sponges, tunicates, bryozoans and molluscs. Many marine chemicals often possess quite novel structures which lead to pronounced biological activity and novel pharmacology (Lei and Zhou, (2001). A number of discovery efforts have yielded several bioactive metabolites which have been successfully developed by the pharmaceutical industry (Kong *et al*, 1994; Faulkner, 2001; Rosenfeld and Zobell, 1947; Fenical and Jensen; 1993 and Fenical, 1993). The study of marine bacteria has also led to the realization that microorganism from specific symbiotic relationship with marine organisms which may be responsible for the production of some bio-active compounds (Kobayashi and Ishibashi, (1993). The marine microorganisms have had a major impact on the development of medical science and these bacteria form highly specific symbiotic relationships with marine plants and animals, (Fenical, 1993). There are a number of works reporting the occurrence of bacteria associated with marine fauna, in particular, the gastropods (Kharlamenko *et al.* (2001), Distel, (1998), Belkin, *et al.* (1986), Stein *et al.* (1988) and Windoffer and Giere, (1997). Recent studies have shown that these antibacterial compounds are not only inhibiting the human pathogens but also fish pathogens (Strahl *et al.*, 2002).

Drupa margariticola is one of the commonest gastropods inhabiting the reefs and may be seen adhering the corals rocks in large numbers. Bacteria associated to the mucus of this organism are being less explored as potential sources of antagonistic compounds. This paper describes the isolation of the bacteria associated with the mucus of *D. margariticola* and testing the isolated strains for their ability to inhibit the growth of selected human and fish pathogens *in vitro*.

Materials and methods

Live *D. margariticola* (Stenoglossa: Muricidae) were collected from the intertidal region of the Tuticorin port area (Lat. 8°45'N and Long. 78°13'E). The animals were immediately transported to the laboratory and placed in the aquaria containing natural sediment and sea water. The animals were washed twice in sterile seawater and the