Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2016.1163509

57 (2016) 26257–26266
November

Taylor & Francis
Taylor & Francis Group

Bioremediation of shrimp (*Litopenaeus vannamei*) cultured effluent using copepod (*Oithona rigida*) and microalgae (*Picochlorum maculatam* & *Amphora* sp.) —An integrated approach

S. Dinesh Kumar[a,b], P. Santhanam[a,*], R. Nandakumar[a], S. Ananth[a], P. Nithya[c], B. Dhanalakshmi[c], Mi-Kyung Kim[b]

[a]*Marine Planktonology & Aquaculture Lab., Department of Marine Science, School of Marine Sciences, Bharathidasan University, Tiruchirappalli 620 024, Tamil Nadu, India, email: viv.dinesh@gmail.com (S. Dinesh Kumar), Tel. +91 98942 23482; Fax: +91 431 2407045; emails: sanplankton@yahoo.co.in, santhanamcopepod@gmail.com (P. Santhanam), damselms@gmail.com (R. Nandakumar), ananth.happyness@gmail.com (S. Ananth)*
[b]*MCK Biotech Co., Ltd, Daegu R&D Fusion Center, Daegu 704 948, South Korea, email: mkkim@ynu.ac.kr (K. Mi-Kyung)*
[c]*PG and Research Department of Zoology, Nirmala College for Women (Autonomous), Coimbatore 641018, Tamil Nadu, India, emails: neethujeni@gmail.com (P. Nithya), dr.dhanalakshmi02@gmail.com (B. Dhanalakshmi)*

Received 13 October 2014; Accepted 2 March 2016

ABSTRACT

Nitrogenous compounds are major contaminants in aquaculture effluent and thereby needs a potential candidate for removing these nutrients. The present study tested the efficacy of immobilized microalga, diatom *Amphora* sp. and copepod *Oithona rigida* to remove excessive nutrients from the shrimp (*Litopenaeus vannamei*) cultured effluents. Nutrient removal was evaluated with five different combinations: (1) copepod, *O. rigida*; (2) immobilized *Picochlorum maculatum* and copepod *O. rigida*; (3) immobilized *P. maculatum*; (4) immobilized *Amphora* sp. and copepod *O. rigida*; and only (5) immobilized diatom, *Amphora* sp. Preliminary studies showed maximum reduction of about nitrate 86% and nitrite 88% in treatment 2–4, respectively. The maximum phosphate (69%) and ammonia (91%) removal was recorded in the treatment 3. In disparity, the phosphate concentration recorded was significantly higher (6%) at the end of the experiment in the treatment 1 than other experiments. While in the treatments 1, 2, and 4 the copepod showed significant (ANOVA: $p < 0.001$) increases in phosphate from the previous concentration, it might be due to the rapid release of this nutrient from excreta of filter-feeding organism. The results showed that immobilized green alga and copepod performed well in absorbing phosphate, ammonia, and nitrogenous compounds, respectively.

Keywords: Adsorption; Absorption; Bioadsorption; *Picochlorum maculatum*; *Oithona rigida*; Copepod

*Corresponding author.

1. Introduction

In recent decades, eutrophication caused by intensive aquaculture practices has become a constant concern for policy-makers and environmentalists, owing to the large amount of residues produced in aquaculture practices and direct discharge of wastewaters into coastal systems. This wastewater promotes organic enrichment, sedimentation, and toxicity due to fecal material and unutilized food substances resulting in deterioration of coastal ecosystems and in turn leading biodiversity loss [1–3]. The above condition emphasis on development of sustainable approaches to coastal aquaculture by promoting ecological practices in improving the ecosystem health [4]. Removal of excessive nutrients using physical and chemical techniques is highly expensive and energy-consuming effort. Therefore, use of biological organisms as nutrient extractors could be a valid alternative. Currently in India, exotic species called Pacific white shrimp *Litopenaeus vannamei* culture has been practiced intensively which unfortunately leads to high nutrient loading in the culture system owing to its high stocking and feeding physiology [5]. In this circumstance, to remediate wastewater a known technique of using marine microalgae and copepods could serve as a possible alternative approach for economically viable wastewater treatment [6,7]. Now biologists are looking for integrative culture of biological organisms in the wastewater, which is economically and ecologically efficient.

Integrated culture mainly aims to maximize food production by utilizing waste from aquaculture component to enhance production of extractive aquaculture, in effect increasing nutrient utilization, and consequently, reducing nutrient impacts. Some few researchers have used marine organisms such as clams, mussels, oysters, algae, and macrophytic plants which are relatively efficient in assimilating or extracting nitrogen and phosphorus from their environment [8–12]. In addition, several studies have shown the benefits of applying polyculture or composite culture to reduce the nutrient and suspended solid from aquaculture effluent [8,13].

In this background present attempt revealed integrative culture of microalgae and copepod in shrimp-cultured wastewater for excessive nutrients removal. Some studies were carried out by [14] on the culture of marine microalgae in aquaculture wastewater (either shrimp or fish) for nutrient removal purposes. However no reports exist on the culture of copepods in wastewater for removal of nutrients or other pollutants. It is well known that the copepods feed on microalgae if they are cultured in same tank. So, in the present experiment microalgae are changed into immobilized form by following immobilization technique [14–17]. When compared to the mobilized form of microalgal bioremediation, immobilized algae showed better nutrient removal efficiency and in limiting the problem like high expenses and time consumption for centrifugation or filtration. Cyclopoid, voracious, detritus and debris feeder and harpacticoid copepods, which have the potential to thrive in extreme conditions like salinity, temperature and nutrients can be utilized to consume fecal matter and food residues resulting from shrimp aquaculture. Further, cyclopoid and harpcaticoid copepods are potential live feeds for fish larvae. Hence, treating shrimp-cultured wastewater using different forms of marine microalgae and marine copepods is need of the hour.

To our knowledge, this study will be the first attempt on using copepods in shrimp culture wastewater for bioremediation. However, use of *Artemia* for bioremediation of aquaculture wastewater has been previously reported [18] with partial success (especially nitrogen). In the present study, the laboratory-scale bioremediation potential of the green alga *Picochlorum maculatum* (PSDK01) isolated from normal seawater from Muthukkuda coast, diatom *Amphora* sp. (PSDK15) isolated from shrimp aqua farm, Chennai, and the marine copepod *Oithona rigida* was examined with an attempt at finding mitigating solutions to the growing concern on eutrophication of coastal waters.

2. Materials and methods

2.1. Microalgae culture

Marine microalgae (PSDK01) (Accession number: KJ754560) and (PSDK15) were collected from Muthukuda coast (9° 51′ 48″ N; 79°7′ 15″ E) and Shrimp Aquaculture Farm (13° 19′ 43″ N; 80° 19′ 54″ E) Chennai, Tamil Nadu, India and isolated using agar plating technique [19]. Indoor algal stock culture was maintained using Walne medium [20] in special air conditioning room in 250 ml culture flasks. Seawater was filtered using filter bag (1 μm) sterilized using autoclave and after cooling, water was transferred to the culture flask plugged with cotton. Glasswares used for algal culture were sterilized properly and dried in an oven before use. About 10 ml of inoculum in the growing phase was transferred to the culture flasks and the culture is incubated in 12:12 h light and dark cycle provided with 200 μmol m^{-2} s^{-1} using fluorescent bulbs. After 5–8 d, the maximum exponential phase was obtained. Temperature and salinity were maintained in the range between 23–25°C and 28–30 psu, respectively, for the entire culture period.

2.2. Copepod culture

The copepod samples were collected from Muthupet estuary (11˚ 29′ 23″ N; 79˚ 46′ 35″ E) using plankton net (158 μm). From the collected copepod samples, the healthy adults of *O. rigida* were isolated using a fine capillary tube and kept overnight in 250 ml beakers containing filtered seawater (1 μm) of ambient salinity (34 psu) with vigorous aeration for starving prior to the experiment.

2.3. Aquaculture wastewater

The shrimp (*L. vannamei*) cultured wastewater was collected from Ennoor, Chennai, Tamil Nadu, India (13˚ 19′ 43″ N; 80˚ 19′ 54″ E), transported to laboratory, and kept undisturbed for settling suspended particles for 24 h.

2.4. Immobilization of microalgae

Microalga *P. maculatum* was harvested during the exponential growth phase using Milliphore filtering system (Model No. X10422050, 50 Hz) and they were immobilized according to Santos et al. [21] with minor necessary modification with respect to alginate and cation solution concentrations. To prepare 100 ml of alginate solution with the required alginate concentration, the alginate was first carefully dissolved by slow stirring in 70 ml of distilled water. In the remaining 30 ml of distilled water, 3.5 g sodium chloride was dissolved to obtain a 35 psu salinity final solution. When the alginate was completely dissolved, the two parts were mixed using a magnetic stirrer. Cation solutions were prepared in nanopure water and beads were formed by adding dropwise in the alginate solution, by means of a 20 ml syringe (0.8 × 40 mm needle; Braun, Melsungen, Germany), into the cation solution, from a height of approximately 15 cm and at a rate of approximately one drop per second. The beads were stirred in the cation solution for 45 min to allow complete hardening of the alginate, and washed several times with filtered (0.45 μm) natural seawater to eliminate the remaining cation. Diatom *Amphora* sp. culture was harvested and immobilized as similar to *P. maculatum*.

2.5. Experimental setup

In experimental system 15 borosil glass bowls of 3 L capacity was used. The experiment consisted of five treatments studied in triplicate. In the first treatment (1) the copepod *O. rigida* (100 adults) was used, in the second treatment, (2) immobilized *P. maculatum* beads (100 numbers) plus 100 adult individuals of *O. rigida*, in the third treatment, (3) only immobilized *P. maculatum* beads (100 numbers). The fourth treatment, (4) was immobilized *Amphora* sp. (100 numbers) plus 100 adult individuals of *O. rigida*, in the final treatment, and (5) only immobilized *Amphora* sp. (100 numbers) was used. The experiment lasted for 6 d, with monitoring of physicochemical parameters (turbidity, pH, salinity, temperature, total suspended solids (TSS), and nutrients) for every 48 h. To know the stability or resistance of immobilized microalgae beads, the beads surface was monitored every 48 h using Light microscope (Magnus MLX-B). The water samples were taken and algal cell counts were made for every two days once using haemocytometer under the light microscope to determine the algal cell leakages if any in wastewater [22]. The copepod mortality in wastewater was assessed by collecting a known volume of sample (5 ml) and number of dead copepods was counted under light microscope using Sedgewick counting chamber according to Santhanam et al. [23]. TSS in effluent were estimated by filtering 10 ml of effluent sample using Millipore filtering apparatus fitted with 0.45 μm GF/C filter paper [24]. TSS was calculated by subtracting the initial weight of the filter paper from final weight. The experiment was conducted outdoor at constant temperature (37˚C), salinity (35 psu), light exposure (250 m mol m^{-2} s^{-1}), and 12:12 h (light/dark) photoperiod. The turbidity of effluent water was measured according to Palin [25]. A known volume (3.5 ml) of effluent sample was filled in the (4.5 × 1.2 cm) cuvette and the absorbance was measured using UV–visible Spectrophotometer (Spectro 20D plus) at 425–580 nm. The inorganic nutrients (PO_4^{3+}, NO_3^-, NO_2^-, NH_4^+, TP, and TN) in the effluent water were analyzed according to Strickland and Parsons [26] and Jenkins and Medsken [27]. Phosphate was measured by the treatment of water sample with acidified molybdate reagent to give phospho-molybdate complex which was then reduced to a highly colored blue compound (phosphomolybdenum blue) using ascorbic acid as a reducing reagent. For the analyses of nitrate, the reaction between the alkaloid, brucine, and nitrate in acid medium produces a yellow color measured by standard spectrophotometric procedures [27]. The nitrite was determined after diazotising it with sulfanilamide and coupling with N(1-Naphthyl)-ethylene diamine-dihydrochloride. Ammonia—Nitrogen was determined by the indophenol blue method based on the principle that in a moderately alkaline medium, ammonia was allowed to react with hypochlorite in catalytic amounts of nitroprusside to form indophenol blue. The determination of total phosphorous and total nitrogen (TN) of effluent water sample was oxidized

with the help of strong oxidizing agent (alkaline perusulfate) by autoclaving in closed condition. The organic forms of phosphate and nitrate and also their inorganic forms in lower oxidation states are finally oxidized to inorganic phosphate and nitrate respectively.

2.6. Statistical analyses

All data are expressed as means ± SD. Differences between treatments (1, 2, 3, 4, and 5) and time (Initial, second day, fourth day, and sixth day) were analyzed using two-way analysis of variance (ANOVA). The Graph Pad Prism (Version 5.0) software was used for the statistical analyses. A simple linear correlation analyses were carried out to find out correlation between TSS, beads resistivity, mortality of copepod and nutrients removal.

3. Results

During the course of experiment, no significant variation was observed in the environmental variables such as water temperature (37˚C), pH (8.41), and salinity (37 psu). On the second and fourth day experimental period in Treatment (1) 75%, Treatment (2) 68%, and Treatment (4) 65% significant decrease in turbidity value was observed. After fourth day till the end of experiment the turbidity value remained relatively invariable ($p > 0.05$).

Fig. 1 shows the effect of immobilized microalgae (*P. maculatum* and *Amphora* sp.) and copepod (*O. rigida*) on nutrients removal in five treatments. The concentration of PO_4^{3-} varied significantly among treatments ($p < 0.001$). In treatments 1, 4, and 5 there were no differences among samplings. In treatment two, a significant reduction was observed on the second day (0.27 ± 0.002–0.09 ± 0.002 μmol L^{-1}) reaching an absorption efficiency of 69.32%. Though, after day two, nutrient concentration increased gradually (0.09 ± 0.02–0.19 ± 0.038 μmol L^{-1}) until end of the experiment, in treatment three, phosphate concentration showed decreasing values until the fourth day of the experiment (0.27 ± 0.002–0.08 ± 007 μmol L^{-1}). At the end of the experiment (sixth day), phosphate showed a slight increase from day four (0.08 ± 007–0.10 ± 0.04 μmol L^{-1}). The overall treatments show that the nutrient concentration decreased up to sixth day, after that it was found to be increased (Fig. 1).

Treatments on nitrate reduction did not show any overall significance ($p > 0.05$); however their reduction significantly varied among them ($p < 0.001$). The nitrate showed marked reduction in all the treatments throughout the experiment period. Among the treatments, treatment 1 and 2 showed considerable reduction by the end of sixth day and better nitrate reduction was noticed at the end of second day in all the treatments. The concentration of nitrate shows a sharp decrease after day 2 in both the treatments (1 and 2). A continuous reduction of nutrients (i.e.) nitrate absorption efficiency in Treatment 1 (83%),

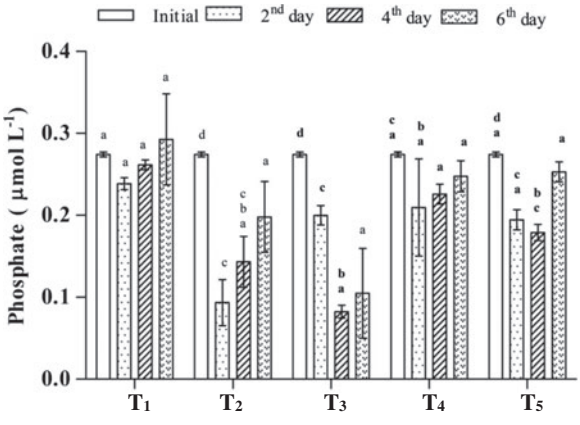

Fig. 1. Phosphate concentration (μmol L^{-1}) during the experimental period. Vertical bars represent ± SD for the means ($n = 3$).
Notes: T_1 *O. rigida*, T_2 *P. maculatum* + *O. rigida*, T_3 *P. maculatum*, T_4 *Amphora* sp. + *O. rigida*, T_5 *Amphora* sp.
[a,b,c,d,e]Values (Mean ± S.E.) within a treatment with different superscript letters are significantly different ($p < 0.05$).

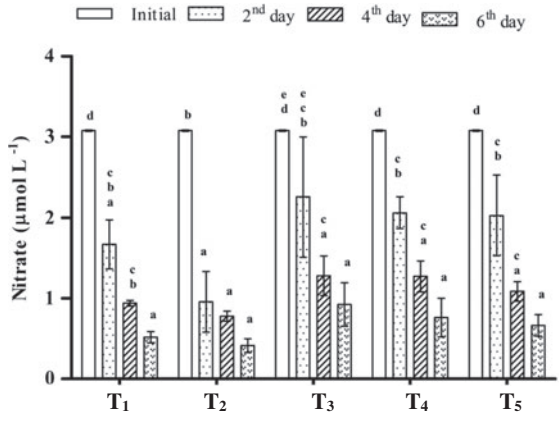

Fig. 2. Nitrate concentration (μmol L^{-1}) during the experimental period. Vertical bars represent ± SD for the means ($n = 3$).
Notes: T_1 *O. rigida*, T_2 *P. maculatum* + *O. rigida*, T_3 *P. maculatum*, T_4 *Amphora* sp. + *O. rigida*, T_5 *Amphora* sp.
[a,b,c,d,e]Values (Mean ± S.E.) within a treatment with different superscript letters are significantly different ($p < 0.05$).

Treatment 2 (87%) Treatment 3 (64%), Treatment 4 (75%), and Treatment 5 (78%) was observed in all treatments throughout the experimental period (Fig. 2).

In treatment 1 NO_3^- was decreased during the sampling periods ($p < 0.001$) and a maximum reduction (2.55 μmol L^{-1}) was noticed after sixth day; where the NO_3^- reduced from 3.07 ± 0.01 to 0.51 ± 0.1 μmol L^{-1}. During the experiment, significant reduction ($p < 0.001$) of nitrate was noted on the fourth day of treatment in treatment 2 (3.07 ± 0.01–0.41 ± 0.13 μmol L^{-1}), in treatment 3 (3.07 ± 0.01 to 0.92 ± 0.41 μmol L^{-1}), in 4 (3.07 ± 0.01–0.76 ± 0.37 μmol L^{-1}), and treatment 5 (3.07 ± 0.01–0.66 ± 0.020 μmol L^{-1}), respectively.

NO_2^- concentration was significantly reduced in all the treatments and among them ($p < 0.001$). In treatment 1, concentration of this nutrient showed a sharp decrease on day two (from 3.67 ± 0.02 to 1.24 ± 0.42 μmol L^{-1}) and in due course of experiment got increased. In treatment 2, nitrite concentration varied from 3.67 ± 0.02 to 0.51 ± 0.19 μmol L^{-1}. The maximum nitrite removal (86%) was observed on sixth day. The nitrite value significantly decreased in treatment 3 (3.67 ± 0.02–0.14 ± 0.45 μmol L^{-1}), treatment 4 (3.67 ± 0.02–0.42 ± 0.11 μmol L^{-1}), and treatment 5 (3.67 ± 0.02–0.51 ± 0.23 μmol L^{-1}). In all the experiments, reduction was observed till fourth day. After fourth day, nitrite was slightly increased in those (T_2, T_3, T_4, and T_5) treatments (Fig. 3). The nitrite removal percentage was found to be greater than 75% in four treatments (T_2, T_3, T_4, and T_5) than treatment 1 (66%).

With regard to NH_3, significant variations were found among the treatments ($p < 0.001$), as well as among the sampling days ($p < 0.001$). Among the five treatments studied, NH_3 showed a significant decrease in all treatments except one at the end of second day. After fourth day, ammonia concentration was slightly increased compared to previous concentrations in treatment 2, 3, 4, and 5. At treatment 1, NH_3 got decreased from the initial concentration 11.05 ± 0.04–9.64 ± 1.66 μmol L^{-1}. This treatment did not show any significant variation ($p > 0.05$) during the sampling periods. The ammonia was excreted by copepod nearly 1.579 μmol L^{-1} during sixth day. However, it should be noted that the reduction values in treatment 2 (11.05 ± 0.04–6.56 ± 1.46 μmol L^{-1}), treatment 3 (11.05 ± 0.04–0.95 ± 0.53 μmol L^{-1}), treatment 4 (11.05 ± 0.04–5.92 ± 0.67 μmol L^{-1}), and treatment 5 (11.05 ± 0.04–4.89 ± 0.40 μmol L^{-1}) were found to be excellent on using green algae, diatom, copepod as bioremediants. Here, the maximum absorption efficiency (91%) was found in treatment 3 on fourth day (Fig. 4). The other treatments such as 1, 2, 4, and 5 resulted 13, 41, 46, and 56% biosorption efficiency, respectively.

The total phosphorus (TP) test measures all the forms of phosphorus in the sample (orthophosphate, condensed phosphate, and organic phosphate). In this current observation, TP varied significantly in the treatments ($p < 0.001$) and sampling periods ($p < 0.001$). The maximum TP removal was recorded at treatment 2 (65%, $p < 0.001$) followed by treatment 3 (53%, $p < 0.001$) at second day and fourth day,

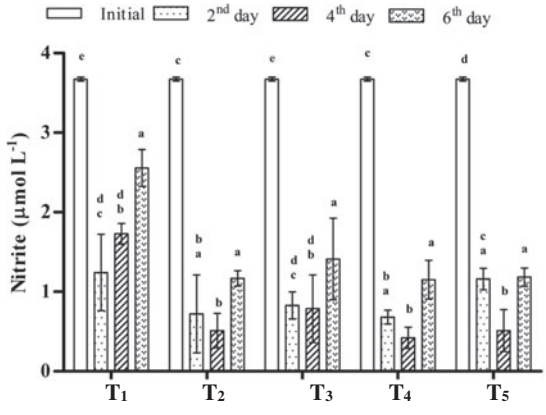

Fig. 3. Nitrite concentration (μmol L^{-1}) during the experimental period. Vertical bars represent ± SD for the means ($n = 3$).
Notes: T_1 *O. rigida*, T_2 *P. maculatum* + *O. rigida*, T_3 *P. maculatum*, T_4 *Amphora* sp. + *O. rigida*, T_5 *Amphora* sp.
[a,b,c,d,e]Values (Mean ± S.E.) within a treatment with different superscript letters are significantly different ($p < 0.05$).

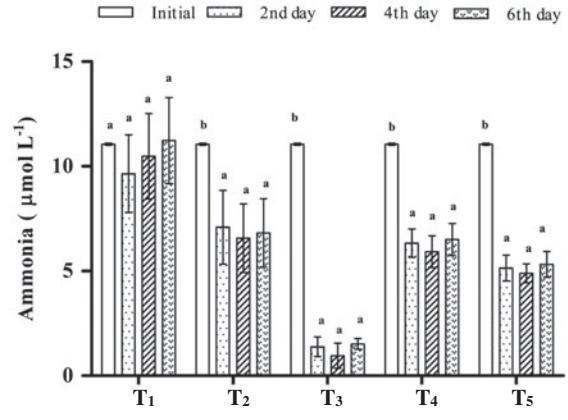

Fig. 4. Ammonia concentration (μmol L^{-1}) during the experimental period. Vertical bars represent ± SD for the means ($n = 3$).
Notes: T_1 *O. rigida*, T_2 *P. maculatum* + *O. rigida*, T_3 *P. maculatum*, T_4 *Amphora* sp. + *O. rigida*, T_5 *Amphora* sp.
[a,b,c,d,e]Values (Mean ± S.E.) within a treatment with different superscript letters are significantly different ($p < 0.05$).

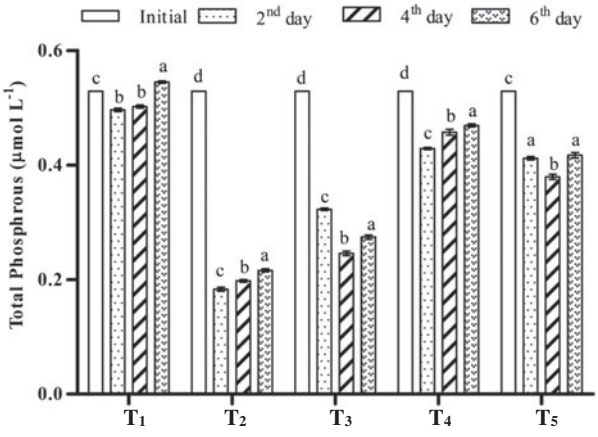

Fig. 5. TP concentration (µmol L^{-1}) during the experimental period. Vertical bars represent ± SD for the means ($n = 3$).
Notes: T_1 *O. rigida*, T_2 *P. maculatum* + *O. rigida*, T_3 *P. maculatum*, T_4 *Amphora* sp. + *O. rigida*, T_5 *Amphora* sp.
[a,b,c,d,e]Values (Mean ± S.E.) within a treatment with different superscript letters are significantly different ($p < 0.05$).

respectively (Fig. 5). Analyses of TN, which represents the sum of NH_3^+, NO_3^-, NO_2^-, showed significant differences among the five treatments ($p < 0.001$). In treatment 3, there was a considerable decrease in TN after 4 d, with a removal efficiency of around 83%. After this period, an increased TN concentration (~1.33 µmol L^{-1}) was observed similar to NH_3^+ concentration. Among the five treatments, the maximum TN removal (83%) was found in T_3 on fourth day

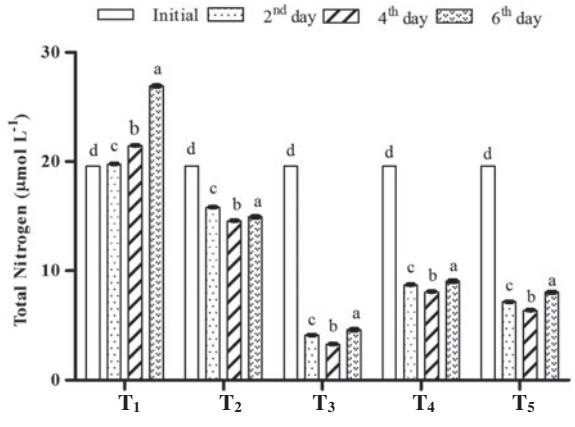

Fig. 6. TN concentration (µmol L^{-1}) during the experimental period. Vertical bars represent ± SD for the means ($n = 3$).
Notes: T_1 *O. rigida*, T_2 *P. maculatum* + *O. rigida*, T_3 *P. maculatum*, T_4 *Amphora* sp. + *O. rigida*, T_5 *Amphora* sp.
[a,b,c,d,e]Values (Mean ± S.E.) within a treatment with different superscript letters are significantly different ($p < 0.05$).

followed by T_5 (67%), T_4 (59%) and T_2 (26%) (Fig. 6). In contrast, in treatment 1, TN concentration was increased over time and reaching the values above 26 µmol L^{-1}.

The TSS was measured in T_1, T_2, and T_4. Among the three treatments, maximum (86%) TSS removal was noticed in T_2 on sixth day followed by T_1 (72%) and T_4 (47%). The TSS removal result negatively correlates with cell leakages. The cell leakages were found to be higher in T_2 compared to T4 which resulted in higher TSS removal. In the present study a positive finding was revealed while using copepod as bioremediant for the removal of nutrients. During the experiment, the TSS, beads resistivity, and mortality of copepod were measured. It was also noted that while decreasing TSS from the wastewater, nutrient concentration also decreased (Fig. 7), which proved that the correlation between suspended solids and nutrient removal was strongly negative (0.79) at the same time, the TSS, beads resistivity also decreased. The beads resistivity (0.90) and removal of TSS (0.99) were highly affected by mortality of copepod.

4. Discussions

The primary role of biofilters in aquaculture is to remediate the wastewater through uptake and conversion of toxic metabolites and pollutants [28]. Filter-feeding organism can consume particulate matter, thereby avoiding future release of nutrients from bacterial degradation of organic matter and also they feed on phytoplankton and bacteria, which use inorganic nutrients for their growth [18,28–30]. Martin [31] encouraged using filter feeder for the cleaning of wastewater at Delaware, New York, as they directly

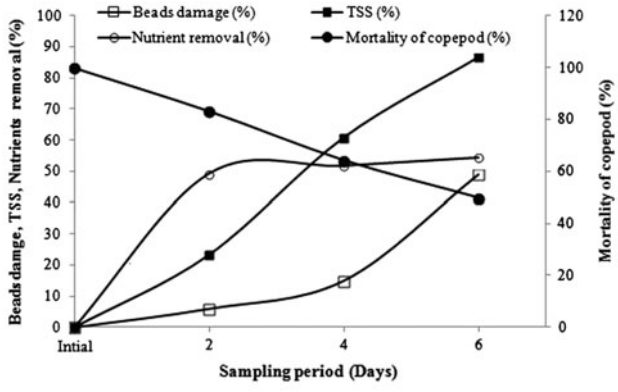

Fig. 7. Relationship between beads damage (%), TSS (%), Nutrient Removal (%) and Mortality of Copepods (%) during the experiment.

remove particulate matter from the water column and to improve the water transparency which leads light penetration for the growth and reproduction of organisms living in aquatic system. Finally he concluded that one cannot underestimate the role of filter feeders in nutrient equation. Immobilization of microalgae for wastewater treatment is based on the principle of keeping the living cells within a gel matrix metabolically active as long as possible, during which time they have very limited mobility. After absorption of the contaminants by the microalgae, the cleaner water diffuse out of the polymers and are collected and reused and the process is repeated for several cycles [32].

Van et al. [33] reported that the immobilized microalgal bioremediation carry the disadvantage of nutrient diffusion. But the present study did not initiate any phosphate diffusion and immobilized microalgae removed nearly 70% of the phosphate as agreed by previous workers [34,35]. The inorganic nutrients such as phosphate would be as liberally obtainable to immobilized microalgae as to their counterparts, it might be suitable to the nutrients must be spread through the alginate pores to the algal cells [35]. The increased phosphate concentration from second day onwards could be attributed to the increase in water temperature while conducting the experiment in outdoor conditions as agreed earlier by Motohashi and Matsudaira [36].

NO_3^- removal was found to be better in treatment 2 (immobilized *P. maculatum* + *O. rigida*) and treatment 1 (*O. rigida*) which suggest that nitrogen was removed by the filtration activity of copepod, whereas dissolved inorganic nitrogen was removed by immobilized microalgae. Moreover, the fact that the nitrate concentration in treatment 2, which was almost equal to treatment 1, explains the efficiency of *O. rigida*. Under stress conditions, significant amount of nitrogenous organic compounds is utilized as the respiratory substrates as agreed by Roman [37] and Marinho-Soriano et al. [18]. Nitrite removal was found to be highest (88%) in treatment 4 which contained immobilized *Amphora* sp. (PSDK015) and *O. rigida*. All treatments showed maximum removal of nitrite on fourth day except treatment 1. After fourth day, nitrite level showed a slight increase which might be due to the environmental conditions such as light intensity, temperature, and salinity which favors the nitrogen excretion by plankton growth [38,39].

In the present study, maximum ammonia removal was noticed in the treatment 3 (*P. maculatum*) and Treatment 5 (*Amphora* sp.). In all treatments ammonia showed slight increase ($p > 0.005$) by fourth day onwards except treatment 1, which showed increased NH_3^- on day two from the previous concentration (Fig. 4). Among the various nutrients studied, TN were considered as toxic excretory products of marine invertebrates [40]. Hence, maximum removal of ammonia in treatments 3 and 5 was noticed where the copepod was not involved (Stocked). Indeed, NH_3^- levels increased considerably in treatment 1, 2, and 4 at sixth day from previous concentrations. This excessive increase in NH_3^- during the experiment was attributed to the metabolic residues of copepod, considering that these filter-feeding organisms are ammoniotelic arthropods that excrete part of their final nitrogenated products in the form of NH_3^- and NH_4^+ [41].

Based on the previous report, cell leakages can limit the nutrient removal using algal immobilization techniques [34], one of the concern in using immobilized cells is the cell leakage from the matrix to the medium which obviously violates the prime aim of immobilization; and the same time relationship between the cell leakages and nutrients removal resulted strongly negative [22]. Cell leakages (monitored in the reactor containing copepods every sampling period under the microscope) and suspended solids played major role in present experiment. It was found that there was a positive correlation between nutrient removal and suspended solids while mortality of copepod and beads damage resulted in a negative correlation.

In the present study, we found maximum reduction of 69 and 65% of phosphate in treatment 3 and 2 respectively where the immobilized microalgae *P. maculatum* (PSDK01) and *O. rigida* served as bioremidiants. This result suggests that the removal of phosphate in the water column was contributed by microalgae. Marinho-Soriano et al. [18] stated that the reduction of phosphate is mainly ruled by algae only when compared to filter-feeding organisms (copepod). But while considering nitrite and nitrate removal, the maximum nitrogen removal (Nitrate-86%; Nitrite-88%) was obtained from the treatment 2 (Immobilized *P. maculatum* + Copepod) and treatment 4 (Immobilized *Amphora* sp. + Copepod), respectively, compared to algae alone in Treatment 3 and 5. Among the five treatments, treatment 2 (Immobilized *P. maculatum* + Copepod) showed maximum (81%) nitrate removal which was generally higher than the values previously reported [18,28]. In an integrated bioremediation system, the removal of nitrite is important because the accumulation of this nutrient in water may be toxic to aquatic organisms. It is inferred that the copepod significantly ($p > 0.001$) showed increased efficacy in the nitrite and nitrate removal from the effluent water compared to microalgae.

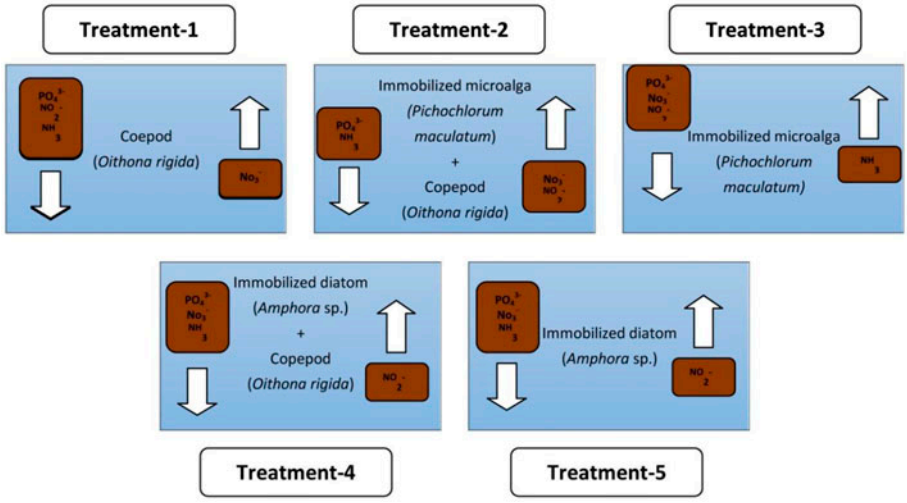

Fig. 8. Graphical conclusion of the present study (↓ Represents decreasing of nutrients. ↑ Represents increasing of nutrients).

5. Conclusion

The present study confirms that the immobilized green algae *P. maculatum* (PSDK01) and the copepod *O. rigida* are possible candidates for nitrogen removal (Fig. 8). This ability was confirmed by the significant decreases of nitrate in treatment 2 (86%) and nitrite in treatment 4 (88%), which encourage the employment of these organisms as biofilters. From this observation, for phosphate and ammonia removal we recommend treatment 3 (*P. maculatum* only) which recorded 69% and 91% of reduction. In conclusion, this study confirms the nutrient removal ability of *P. maculatum* and appoints copepod *O. rigida* as a new candidate to be integrated into bioremediation processes. The relations of these two organisms represent a capable and environmentally valid substitute for civilizing the environmental circumstances of coastal areas vulnerable to discharges of aquaculture wastewaters. With reference to above results and discussions it can be stated that both the microalgae and copepod could be considered as potential live feeds in aquaculture, these wastewater grown microalgae and copepod can be used as feeds for fish larviculture and bioremediation agents which could be environmentally benign and economically viable candidates for zero waste management.

Acknowledgement

The authors thank the Head, Department of Marine Science and authorities of Bharathidasan University, Tiruchirappalli-24, for the facilities provided and one of the authors (SD) greatly acknowledge the DBT, Govt. of India, for Junior Research Fellowship.

References

[1] M.C.M. Beveridge, Cage Aquaculture, second ed., Fishing News Books, Blackwell Science Ltd, Oxford, UK, 1996.
[2] J.K. Kim, D.J. Garbary, P. Corey, J. Duston, Marine finfish effluent bioremediation: Effects of stocking density and temperature on nitrogen removal capacity of *Chondrus crispus* and *Palmaria palmata* (Rhodophyta), Aquaculture 414–415 (2013) 210–216.
[3] T. Chopin, A.H. Buschmann, M. Troell, N. Kautsky, A. Neori, C. Yarish, C. Neefs, Integrating seaweeds into marine aquaculture systems: A key toward sustainabilitY, J. Phycol. 37 (2001) 975–986.
[4] B.A. Costa-Pierce, Ecological Aquaculture: The Evolution of the Blue Revolution, Blackwell Science, Oxford, UK, 2002.
[5] P. Ravichandran, Overview of *Litopenaeus vannamei* culture practice and importance of Govt. regulation measures, in: K.P. Jithendran (Ed.), Training Manual on Management of Emerging Diseases of Shrimp with Special Reference to Pacific White Shrimp, *Litopenaeus vannamei*, vol. 64, CIBA Special Publication, Chennai, 2012, pp. 64.
[6] K. Lorsdotter, Wastewater treatment with microalgae —A literature review, Vatten 62 (2006) 31–38.
[7] V. Aravinthan, N. Story, T. Yusaf, Nutrient removal of nursery and municipal wastewater using Chlorella vulgaris microalgae for lipid extraction, Desalin. Water Treat. 52 (2014) 727–736.
[8] B. Jones, N.P. Preston, W.C. Dennison, The efficiency and condition of oysters and macroalgae used as biological filters of shrimp pond effluent, Aquacult. Res. 33 (2002) 1–19.

[9] M.W. Lasalle, B.C. Posadas, Use of Constructed Wetalnds to Improve Water Quality in Finfish Pond Culture, Final Report, Coastal Research and Extension Center, Mississippi Agricultural Experiment Station, Mississippi, MS, 1997, pp. 32.
[10] M.F. Schwartz, C.E. Boyd, Constructed wetlands for treatment of channel catfish pond effluents, Prog. Fish. Cult. 57 (1995) 255–266.
[11] M.A. Abreu, R. Pereira, C. Yarish, A.H. Buschmann, I. Sousa-Pinto, IMTA with Gracilaria vermiculophylla: Productivity and nutrient removal performance of the seaweed in a land-based pilot scale system, Aquaculture 312 (2011) 77–87.
[12] M.H. Abreu, D.A. Varela, L. Henríquez, A. Villarroel, C. Yarish, I. Sousa-Pinto, A.H. Buschmann, Traditional vs. integrated multi-trophic aquaculture of Gracilaria chilensis C. J. Bird, J. McLachlan & E. C. Oliveira: Productivity and physiological performance, Aquaculture 293 (2009) 211–220.
[13] M.F. Schwartz, C.E. Boyd, Characterization and Management of Effluents from Aquaculture Ponds in the Southeastern United States, SRAC Final Project Report, vol. 600, Southern Regional Aquaculture Center, Mississippi, MS, 1998, pp. 42.
[14] S. Dinesh Kumar, P. Santhanam, T. Jayalakshmi, R. Nandakumar, S. Ananth, A. Shenbaga Devi, B. Balaji-Prasath, Optimization of pH and retention time on the removal of nutrients and heavy metal (zinc) using immobilized marine microalga *Chlorella marina*, J. Biol. Sci. 13 (2013) 400–406.
[15] L. Travieso, F. Benitez, P. Weiland, E. Sánchez, R. Dupeyrón, A.R. Dominguez, Experiments on immobilization of microalgae for nutrient removal in wastewater treatments, Bioresour. Technol. 55 (1996) 181–186.
[16] N.F.Y. Tam, Y.S. Wong, Effect of immobilized microalgal bead concentrations on wastewater nutrient removal, Environ. Pollut. 107 (2000) 145–151.
[17] R. Boonchai, J. Kaewsuk, G. Seo, Effect of nutrient starvation on nutrient uptake and extracellular polymeric substance for microalgae cultivation and separation, Desalin. Water Treat. 55 (2015) 360–367.
[18] E. Marinho-Soriano, C.A.A. Azevedo, T.G. Trigueiro, D.C. Pereira, M.A.A. Carneiro, M.R. Camara, Bioremediation of aquaculture wastewater using macroalgae and Artemia, Int. Biodeterior. Biodegrad. 65 (2011) 253–257.
[19] R.M. Knuckey, M.R. Brown, S.M. Barrett, G.M. Hallegraeff, Isolation of new nanoplanktonic diatom strains and their evaluation as diets for juvenile Pacific oysters (*Crassostrea gigas*), Aquaculture 211 (2002) 253–274.
[20] P.R. Walne, Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera Ostrea, Crassostrea, Mercenaria and Mytilis, Fish. Invest. 26 (1970) 1–62.
[21] M.M. Santos, I. Moreno-Garrido, F. Gonçalves, A.M.V.M. Soares, R. Ribeiro, An *in situ* bioassay for estuarine environments using the microalga *Phaeodactylum tricornutum*, Environ. Toxicol. Chem. 21 (2002) 567–574.
[22] M.S. Abdel Hameed, Effect of algal density in bead, bead size and bead concentrations on wastewater nutrient removal, Afr. J. Biotechnol. 6 (2007) 1185–1191.
[23] P. Santhanam, N. Jeyaraj, K. Jothiraj, Effect of temperature and algal food on egg production and hatching of copepod, *Paracalanus parvus* (Claus), J. Environ. Biol. 34 (2013) 243–246.
[24] APHA, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington, DC, 1998.
[25] A.T. Palin, Photometric determination of the colour and turbidity of water, Water Water Eng. 59 (1955) 341–345.
[26] S.C. Strickland, T.R. Parsons, A Practical Handbook of Seawater Analyses, Bulletin of Fisheries Research Board of Canada, Ottawa, 1972, pp. 185.
[27] D. Jenkins, L. Medsken, A brucine method for the determination of nitrate in ocean, estuarine, and fresh waters, Anal. Chem. 36 (1964) 610–612.
[28] A. Neori, T. Chopin, M. Troell, A.H. Buschmann, G.P. Kraemer, G. Halling, M. Shpigel, C. Yarish, Integrated aquaculture: Rationale, evolution and state of the art emphasizing seaweed biofiltration in modern aquaculture, Aquaculture 231 (2004) 362–391.
[29] P.Y. Qian, C.Y. Wu, M. Wu, Y.K. Xie, Integrated cultivation of the red alga *Kappaphycus alvarezii* and the pearl oyster *Pinctada martensi*, Aquaculture 147 (1996) 21–35.
[30] M. Troell, Integrated mariculture: Its role in future aquaculture development, in: A. Lovatelli, M.J. Phillips, J.R. Arthur, K. Yamamoto (Eds.), FAO/NACA Regional Workshop on the Future of Mariculture: A Regional Approach for Responsible Development in the Asia-Pacific Region, 7–11 March 2006, Guangzhou, China, vol. 11, FAO Fisheries Proceedings, Rome, FAO, 2008, pp. 323–325.
[31] J. Martin, Don't underestimate role of filter feeders in nutrient equation, Bay J., (2010). Available from: <http://www.bayjournal.com/article/dont_underestimate_role_of_filter_feeders_in_nutrient_equation_>.
[32] L.E. de-Bashan, Y. Bashan, Immobilized microalgae for removing pollutants: Review of practical aspects, Bioresour. Technol. 101 (2010) 1611–1627.
[33] D.E. Van, B.A. Faafeng, D.O. Hessen, T. Källqvist, Use of immobilized algae for estimating bioavailable phosphorus released by zooplankton, J. Plankton Res. 15 (1993) 761–769.
[34] P.S. Lau, N.F.Y. Tam, Y.S. Wong, Wastewater nutrients (N and P) removal by carrageenan and alginate immobilized *Chlorella vulgaris*, Bioresour. Technol. 18 (1997) 945–351.
[35] A. Ruiz-Marin, L.G. Mendoza-Espinosa, T. Stephenson, Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater, Bioresour. Technol. 101 (2010) 58–64.
[36] K. Motohashi, C. Matsudaira, Some problems relating to phosphate regeneration and oxygen consummation in seawater Phosphate regenerated by zooplankton during excessive grazing, Tohoku J. Agric. Res. 20 (1969) 81–88.
[37] M.R. Roman, Nitrogenous nutrition of marine invertebrate, in: E.J. Carpenter, D.G. Capone (Eds.), Nitrogen in the Marine Environment, Academic Press, New York, NY, 1983, pp. 347–383.

[38] S. Aslan, I.K. Kapdan, Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae, Ecol. Eng. 28 (2006) 64–70.
[39] P. Karthikeyan, K. Manimaran, P. Sampathkumar, L. Rameshkumar, Growth and nutrient removal properties of the diatoms, Chaetoceros curvisetus and C. simplex under different nitrogen sources. Appl. Water Sci. 3 (2013) 49–55.
[40] M.E.S. Bracken, J.J. Stachowicz, Top-down modification of bottom-up processes: Selective grazing reduces macroalgal nitrogen uptake, Mar. Ecol. Prog. Ser. 330 (2007) 75–82.
[41] C.A. Freire, H. Onken, J.C. McNamara, A structure function analysis of ion transport in crustacean gills and excretory organs, Comp. Biochem. Physiol. A Mol. Integr. Physiol. 151 (2008) 272–304.