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Investigation on nutrients and heavy metal removal efficacy of seaweeds, *Caulerpa taxifolia* and *Kappaphycus alvarezii* for wastewater remediation

R. Mithra[1], S. Sivaramakrishnan[1], P. Santhanam*[2], S. Dinesh Kumar[2] and R. Nandakumar[3]

[1]Department of Biotechnology, School of Life Sciences, Bharathidasan University, Tiruchirappalli-620 024, Tamil Nadu, India.
[2]Marine Planktonology & Aquaculture Division
Department of Marine Science, School of Marine Sciences, Bharathidasan University, Tiruchirappalli-620 024, Tamil Nadu, India.
* Corresponding author. Email: sanplankton@yahoo.co.in

Abstract

Aquaculture is source of significant amount of wastes, which generally leads to general deterioration of water quality. A simple alternative to this is use of biofilters to reduce the nutrient load. Hence, the marine macroalgae *Caulerpa taxifolia* (biosorpent) was screened for its nutrients and heavy metal absorption capacities at various initial concentrations and contact period in laboratory conditions. Likewise, the red alga *Kappaphycus alvarezii* was tested for nutrient utilization besides its growth in waste water. The experiment on nutrient absorption by seaweed *C. taxifolia* inferred that all time intervals (6, 12, 18 and 24 hrs) maintained at different pH (4-10) in the present study showed significant amount of nutrients absorption. Of these, 24 hours biosorption at pH 7 showed the utmost removal of all the tested nutrients. The maximum absorption of zinc was recorded in pH 7 and at 24 hours with 0.5 g concentration of the seaweed being optimum under laboratory conditions. The experimental study using *K.alvarezii* in shrimp waste water confirmed excellent potentials of the seaweed in absorbing nutrients and thereby aiding its growth.

Key words: biosorption, nutrients, heavy metal, waste water treatment, seaweed, bioremediation

Introduction

The rapid expansion of aquaculture has contributed to the excessive increase of nutrients, especially nitrogen and phosphorous, in aquatic ecosystems (Beveridge 1996). These nutrients generally originate from pond fertilization, feed and metabolic residues of the cultivated animals. In this sense, the major challenge has been to develop non-polluting strategies that minimize the negative effects of this activity. The most practical and economical approach to reduce the concentration of nutrients in aquaculture areas is to treat the effluents before it reach the sea. A potentially feasible alternative is the biological treatment of effluents, using macroalgae for nutrient removal (Chopin *et al*, 2001; Neori *et al*, 2004). Heavy metals like Fe, Zn, Ca and Mg have been reported to be of bioimportance to man and their daily medicinal and dietary allowances (Duruibe *et al.* 2007). Even for those having bioimportance, dietary intake have to be maintained at regulatory limits, as excesses will result in poisoning or toxicity, which is evident by certain reported medicinal symptoms that are clinically diagnosable (Fosmire 1990; Nolan 2003; Young 2005). Methods for removing metal ions from aqueous solution mainly consist of physical, chemical and biological technologies. The summarized the advantages and disadvantages of those conventional metal removal technologies. In recent years, applying biotechnology in controlling and removing metal pollution has been paid much attention, and gradually becomes hot topic in the field of metal pollution control because of its potential application. Alternative process is biosorption, which utilizes various certain natural materials of biological origin, including bacteria, fungi, yeast, algae, etc. These biosorbents possess metal-sequestering property and can be used to decrease the concentration of heavy metal ions in solution from ppm to ppb level. It can effectively sequester dissolved metal ions out of dilute complex solutions with high efficiency and quickly, therefore it is an ideal candidate for the treatment of high volume and low concentration complex wastewaters (Wang and Chen 2006).

Materials and Methods

Preparation of Biosorpent

Healthy and fresh sample of green alga *Caulerpa taxifolia* was collected from Palk Bay, Mandapam (9°16'47"N 79°7'12"E). The samples after washing were processed as powder according to Esteves *et al*. 2000.

Experiment setup on adsorption of nutrients and heavy metal (Zinc) by seaweeds

Nutrient stock solutions were prepared according to standards of Strickland and Parsons 1979. Analytical grade $ZnSO_4$. $7H_2O$ salt was dissolved in distilled water, as metal stock solution. Working solution was prepared by the dilution of zinc stock solution (100µg/l). All the nutrient and heavy metal stock solutions (100 ml) were taken in 250 ml conical flasks. The pH of the each solution was adjusted from 4-10 using NH4OH and HCL (pH meter, ELICO, INDIA). Algal powder of various quantities (0.1, 0.5 and 1g) was added to conical flasks. Then the flasks were maintained at 30°C in shaker for various time intervals (6, 12, 18 and 24 hrs) at 200 rpm. At the end of the experiment, the fluid was separated from the powder by filtration through whatman (47mm) filter paper. The amount of nutrient in the sample was measured spectrophotometrically (Shimadzu Model-2450) as per the standards (Strickland and Parsons 1979) and heavy metal was estimated by AAS.

Experiment on nutrients removal and growth characteristics of Kappaphycus alvarezii

The macroalgae *Kappaphycus alvarezii* was collected from Palk Bay region of Mandapam. It was then transported to the laboratory immediately whereupon epiphytes and encrusting organisms were cleaned of their surface. Later, they were placed in a container with sea water until the experiment began. The experiment was conducted over a period of five days. The wastewater used for this experiment was collected from shrimp culture pond, Parangipettai. The seaweed was stocked in cylindrical tanks filled with aquaculture wastewater. The tanks were kept indoor, illuminated with light (300 µ mol m^{-2} $s^{-1)}$, photoperiod (12:12 L: D cycle) and constant aeration. The physico-chemical parameters of wastewater were analyzed on all experiment days. The initial and final nutrient concentrations of wastewater were recorded. The nutrient removal efficiency of seaweed was estimated by subtracting the final nutrient concentration from initial concentrations. Similarly, the initial and final weight of seaweed was measured by using a weighing balance.

Results and Discussion

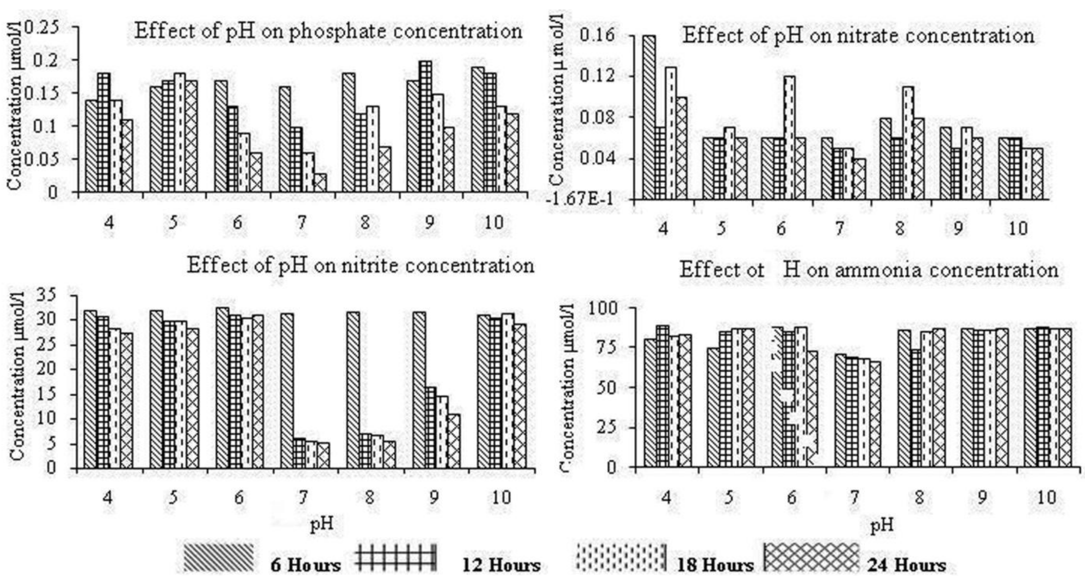
Effect of pH and time on nutrient absorption

Seaweeds are presented as very good sorbents, because the cell wall of green and brown algae contain alginate with its carboxyl and hydroxyl groups (Davis *et al*. 2003; Vieira and Volesky. 2000). Worse sorptive properties are suggested for red algae owing to its carrageen, exposing hydroxyl and sulfonate groups (Tsezos and Volesky. 1981). In this study the maximum phosphate removal was obtained at pH 7 where initial phosphate concentration of 0.4µmol/l was finally reduced to 0.03µmol/l at 24 hours duration with 0.06µmol/l final value at pH 6 being the second best (Figure 1), pH 5 had the lowest removal of phosphate in 24 hours with 0.17 µmol/l. The ammonia concentration decreased significantly with respect to pH ranges from 4-10. The lowest removal of ammonia (88.83µmol/l) was recorded at pH 4 at 12 hours; the highest removal was recorded (66.54µmol/l) at pH 7 and 24 hours duration. The concentration of nitrate decreased significantly at pH 4 and 10. The lowest removal of nitrate occurred at pH 5 with 0.06µmol/l and it was 0.05µmol/l at pH10. The highest nitrate removal was recorded at pH 7 where it reduced from 0.06µmol/l to 0.04µmol/l. The concentration of nitrite appreciably decreased at pH 4 and pH 10. There was a gradual decrease in the concentration of nitrite at all pH values. The maximum nitrite removal was reported as 4.98µmol/l at pH 7 whereas the lowest nitrite removal of 27.29µmol/l was observed at pH 4. From the present result, it is clearly understood that, the pH had a considerable effect on the reduction of nutrient concentration. It is clear that the optimum pH for the reduction of all nutrients (PO_4^{3-}, NH_3^{+}, NO_3^{-} and NO_2^{-}) was found to be pH 7 where the nutrients concentration decreased significantly, though a considerable amount of nutrient removal was also observed in acidic as well as basic pH concentration. The agitation of the nutrient solution also enhanced the adsorption of nutrients in seaweed. Similarly, the extension of experimental duration was also found to enhance the adsorption process. This is evident from the fact that maximum removal of nutrients was achieved at the 24 hours interval.

Table 1. Physico-chemical characteristics of waste water during the culture period

Parameters	Range	Mean ± SD
Temperature (°C)	28-29	28.2 ± 0.45
Salinity (psu)	22-30	25.6 ± 2.88
pH	7.66-8.08	7.94 ± 0.16
PO_4^{3-} (umol/l)	0.002-0.037	0.018 ± 0.016
NO_3- (umol/l)	12.281-27.017	21.35 ± 5.77
$NH3^-$ (umol/l)	14.865-26.012	21.18 ± 4.76
NO_2^- (umol/l)	0.215-3.102	0.99 ± 1.19

Figure 1. Effect of pH and time on nutrients removal

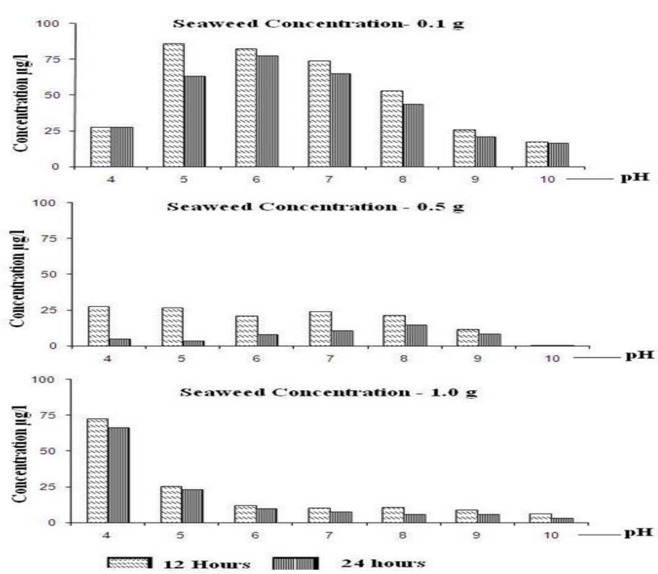


Effect pH, time and seaweed concentration on heavy metal adsorption

The effect of pH of the aqueous solution is a key controlling parameter in the biosorption of heavy metals. The pH ranging from 4 to 10 was tested for heavy metal absorption of seaweed. Results showed that heavy metal removal was directly proportional with pH (Figure 2). This experiment was conducted in 3 different concentrations with two different time intervals. The maximum removal (16.3µg/l) in zinc was observed in 0.1g seaweed concentration at pH 10 in 24 hours time period and minimum removal (86µg/l) at pH 5 in 12 hours. At pH 10, the maximum zinc removal was achieved with 0.5g seaweed concentration in 24 hours time period, the zinc concentration was observed as 0.3µg/l. 0.4µg/l the second best maximum removal of zinc in same seaweed concentration and observed at 12 hours.1g of seaweed concentration having significantly reduced the zinc. The minimum zinc removal was observed in 12 hours at pH 4

(72.65µg/l). The maximum removal of zinc was observed in pH 10 at 24 hours (3.3µg/l).The metal uptake increased with increasing pH. There was a gradual increase in the biosorption of heavy metals as the pH shifted from acidic to basic conditions. Though biosorption of heavy metals was recorded at both acidic and basic pH, heavy metal removal was not satisfactory in acidic conditions. Thus, from the present study, it is clear that pH above neutral conditions is capable of enhancing the heavy metal removal. This can be due to the presence of more number of free binding sites on biomass which took up the heavy metals from the solution at these pH concentrations. In the biosorption phenomenon, the pH value affects two aspects, metal ion solubility and biosorbent total charge, since protons can be adsorbed or released. This behaviour will depend on the functional groups present on the algae cell wall, which in turn determine the acidity constant. The increase in biosorption levels with an increase in pH can be explained by the influence of the number of negative charges, which depends on the dissociation of functional groups (Feng and Aldrich. 2004). At low pH values the concentration of hydrogen protons in the solution far exceeds that of zinc ions and hence, the protons competes with the metal ions in forming a bond with the active sites (functional groups) on the surface of the algae, leaving the metal ions free in solution. These bonded active sites thereafter become saturated and inaccessible to other cations. When the pH was increased, the competing effect of hydrogen protons decreased and the positively charged metal ions took up the free binding site. Hence, an increase in the sorption capacity or removal efficiency could be observed (Ajjabi and Chouba. 2009). The contact time is also evaluated as one of the most important factors affecting the biosorption efficiency. The biosorption efficiency increases with increases in contact time. From these experiments, the biosorption efficiency of biomass was found to maximum at 24 hours. The biomass concentration is another important variable during metal uptake. The effect of biomass concentration on the biosorption of zinc ions was studied using different dosage range (0.1, 0.5 and 1g) respectively. High biomass concentrations can exert a shell effect, protecting the active sites from being occupied by metal (Romera *et al*. 2007). But the metal uptake can also decrease with increasing the biosorbent dosage, which may be due to the complex interaction of several factors (Radojka N. Razmovski and Marina B. Šciban. 2007). This may be due to a partial cell aggregation that takes place at high biosorbent concentrations causing a decrease of the active sites. Esposito *et al*., 2001: Tangaromsuk *et al*. 2002 also observed this trend in their biosorption experiments. It has been suggested that electrostatic interaction between cells can be significant factor in the relationship between biomass concentration and metal sorption. In this connection, at a given metal concentration, the lower the biomass concentration in suspension, the higher will be the metal/biosorbent ratio and the metal retained by sorbent unit, unless the biomass reaches saturation.

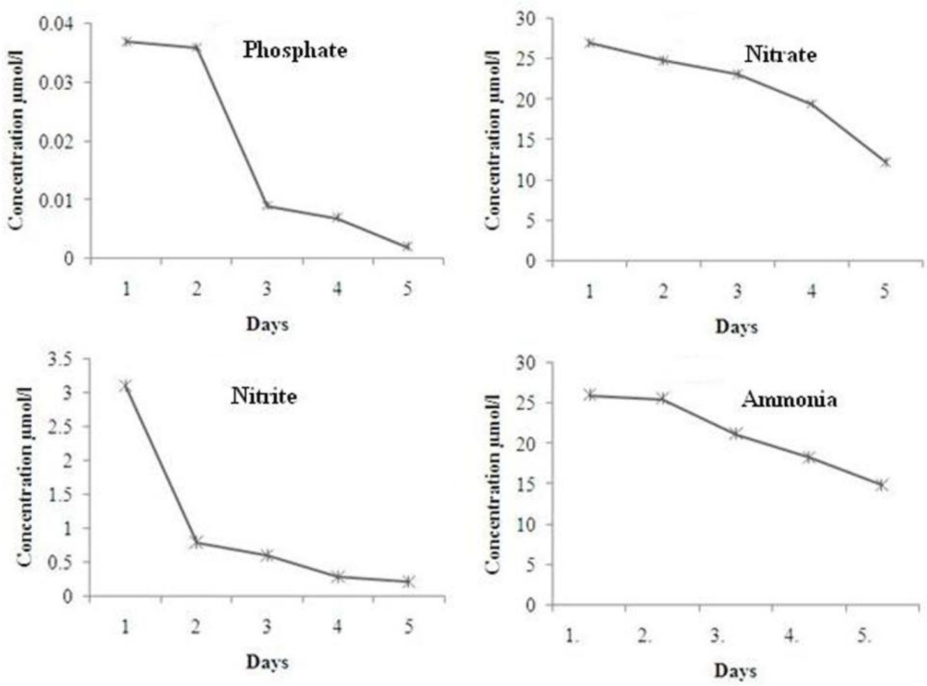
Figure 2. Effect of pH, time and seaweed biomass on heavy metal removal



Nutrient removal using live seaweed Kappaphycus alvarezii

The concentration of the four nutrients tested (NO_2-N, PO_4-P, NO_3-N and NH_3-N) decreased significantly during the experimental period (Table 1), indicating that macroalgae have large nutrient removal capacity (Figure 3). The concentration of $PO4^3$ decreased from 0.037µmol/l to 0.002µmol/l which corresponds to about 94.5% of nutrients removal during the five days of study. A considerable amount of reduction in NO_3 concentration was observed in the present study. The greatest reduction occurred from the fourth (19.474µmol/l) to the fifth day (12.281µmol/l), which corresponded to around 49% of nutrient removal. The nutrient removal ability for the whole period was approximately 54.54%. At the end of the experiment (fifth day) most of the NO_2 was absorbed, corresponding to 92.3% removal efficiency. There was also a considerable NH_3^+ removal (42%) during the experiment. There was a considerable decrease in NO_3 (12.281µmol/l) during the culturing period. This decrease may be related to the mineralization of the organic materials as reported earlier by Jones et al (2001). Previous studies have also shown that seaweed may be able to remove high concentrations of PO_4^{3-} (Neori et al. 1998; Troell et al. 1997; Zhou et al. 2006). However, only studies conducted by Jones *et al*. (2001) and Martinez-Aragon *et al*. (2002) have observed higher than our present study. The removal of NH_4^+ from the waste water by seaweed was gradual between the initial and end of the experiment (26.012µmol/l - 14.865µmol/l). The biofiltration capacity of NO_3^- obtained in this experiment was considerably higher. Since nitrate has a secondary source from the nitrification processes, with a high contribution from nitrite. Besides, all the other aspects must be considered for the variation in the removal efficiency of the seaweed.

Figure 3. Rate of nutrients removal in waste water seaweed culture system



Algal growth and discolouration of wastewater

The present study inferred that *K. alvarezii* grew well in nutrient-rich water. Presently, the initial weight of 250g increased to 295g at the final day of the experiment. The values of biomass and growth of *K. alvarezii* obtained in this study are an indicative of the possibility of culturing macroalgae using shrimp pond effluents. The biomass harvested can provide another value-added product (phycocolloids, human food and animal feed), thus offering to producers a new economic incentive. In addition, it can be an alternative way to reduce the nutrient load thereby the problems associated with nutrient overloaded aquaculture waste water The change in the colour of the waste water and increase in the weight of the seaweed have showed that *K.alvarezii* possess good nutrient removal potential meanwhile aiding its growth.

Conclusion

In conclusion the present observation inferred that the *C.taxifolia* and *K.alvarezii* showed significant removal in nutrient and heavy metal. Hence, both the species can be considered as potential candidates for bioremediation of domestic and industrial effluent which can cause environmental pollution. It is concluded that the green alga *C.taxifolia* and red alga *K.alvarezii* species can be cultivated in shallow coastal ponds and further used as bioremediant agent in the effluent treatment plants for conservation of nature and natural resources. Furthermore, the effluent cultivated seaweeds can be utilized as feedstock for production of biofuel, biofertilizers and additives for animal feed which can reduces the cost of wastewater treatment process.

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