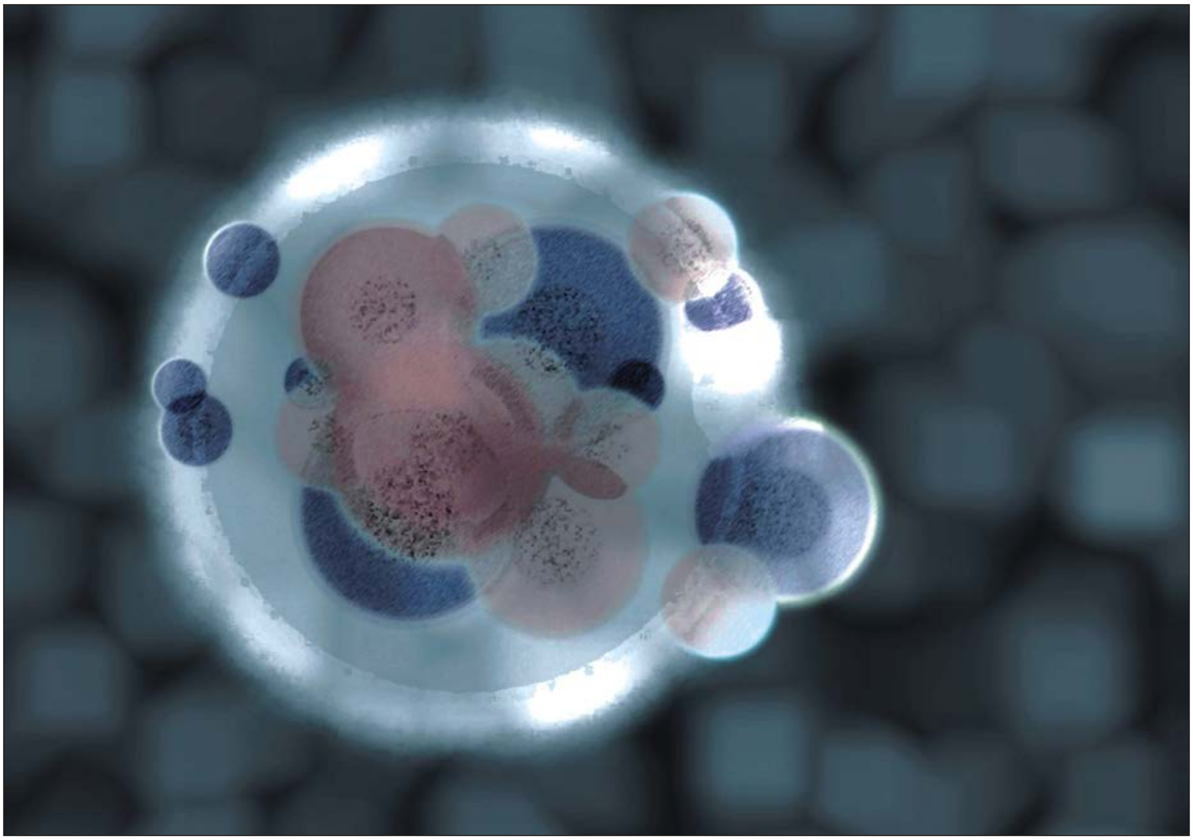

Journal of Biological Sciences

ISSN 1727-3048

Journal of Biological Sciences 13 (5): 400-405, 2013
ISSN 1727-3048 / DOI: 10.3923/jbs.2013.400.405

Optimization of pH and Retention Time on the Removal of Nutrients and Heavy Metal (Zinc) Using Immobilized Marine Microalga *Chlorella marina*

S. Dinesh Kumar, P. Santhanam, T. Jayalakshmi, R. Nandakumar, S. Ananth,
A. Shenbaga Devi and B. Balaji Prasath
Marine Planktonology and Aquaculture Lab., Department of Marine Science,
School of Marine Sciences, Bharathidasan University,
Tiruchirappalli-620 024, Tamil Nadu, India

Abstract: The interaction of phosphorus and nitrogen on their removal by microalgae can be of great concern during their role as bioremediant of waste water. Microalgae play an imperative role in nutrient and heavy metal removal in wastewater by their biosorption mechanisms. The study was attempted to optimize the pH and retention time for nutrient and heavy metal (zinc) removal from aqueous solutions using immobilized marine microalga *Chlorella marina* as beads and powder. The study inferred that pH 6 was found to optimum for removal of all nutrients except nitrite. However, for heavy metal removal, the pH 8 was found to be suitable for biosorbent studies. Further, the present experiment inferred that the 24 h incubation was enough for nutrients and zinc removal while using immobilized microalga *C. marina* beads.

Key words: Biosorption, nutrients, microalgae, heavy metal, immobilization

INTRODUCTION

Water pollution occurs when the water body gets affected adversely due to the addition of large amounts of materials. The greatest contributors to toxic pollution are herbicides, pesticides and industrial compounds. Organic pollution can occur when inorganic pollutants such as nitrogen and phosphorous accumulate in aquatic ecosystems. High levels of these nutrients can cause an overgrowth of algae. The process of rapid plant growth followed by increased activity by decomposers and a depletion of the oxygen level is called eutrophication. It is well known that the eutrophication causes severe biodiversity threats. In addition to nutrients, heavy metals also constitute a serious environmental problem as these substances are not biodegradable and are highly toxic to living organisms. Toxic metals have become an ecotoxicological hazard of prime interest and increasing significance owing to their tendency to get accumulating in the vital organs in humans and animals. Among these zinc is a key element for humans; free zinc ion in solution is highly toxic to plants, invertebrates and even vertebrate fish. Trace amounts of free zinc ions can cause heavy damage to the environment and even kill some organisms. Excessive intake of zinc can promote deficiency in other dietary minerals (Anirudhan and Radhakrishnan, 2011). Search of novel technologies involving removal of toxic compounds from wastewaters has significant benefit to the ecosystem function and management. There are many physical and chemical methods available for wastewater mitigation, but with economic loss and environmental destruction. Therefore, the need for cost effective and environmentally benign bioremediation techniques is of great interest. Bioremediation of waste water using natural biosorpents can be an efficient alternative (Matsuska *et al*., 2010), though it needs optimization before use (Das *et al*., 2008). Very stumpy number (Shimoda *et al*., 2011; Yen, 2008) of studies using marine microalgae for waste water bioremediation. *Chlorella marina* is a genus of single-celled green algae, belonging to the phylum called Chlorophyta. It is spherical in shape, about 2 to 10 μm in diameter and is devoid of flagella. *Chlorella marina* contains the green photosynthetic pigments called chlorophyll a and b in its chloroplast. Hence, the present attempt has been made using marine microalga *Chlorella marina* which is commonly occurring tropical species.

MATERIALS AND METHODS

The microalgal biosorbent powder was prepared according to Bishnoi *et al.* (2004). Algal biomass was

Corresponding Author: P. Santhanam, Marine Planktonology and Aquaculture Lab., Department of Marine Science, School of Marine Sciences, Bharathidasan University, Tiruchirappalli-620 024, Tamil Nadu, India Tel:+9198942-23482 Fax : +91431-2407045

harvested by filtering the culture through Millipore filtering equipment using filter paper (0.45 cm dia.). The obtained biomass was scrapped using sterile blades, washed twice with distilled water and dried. Algal biomass was also harvested by centrifugation at 3000 rpm for 10 min. The cell pellet was washed twice with distilled water. The collected cell pellets were dried in a hot air oven at 80°C for 40 min. The weight was determined gravimetrically (g L^{-1}) then stored in desiccators for biosorption experiments. The microalgal beads were prepared according to Santos *et al.* (2002) with modifications. The nutrient stock solutions were prepared according to method of Strickland and Parsons (1972). The metal solutions were prepared by using analytical grade $ZnSO_4$. $7H_2O$ salt. The salt was dissolved in distilled water to produce metal stock solution. The working solution was prepared by the dilution of zinc stock solution (1 ppm). About 4.397 g of $ZnSO_4$. $7H_2O$ salt was taken and dissolved in 1000 mL of distilled water to make stock solution. From the stock solution 0.1 mL was taken and dissolved in 100 mL of distilled water to make 1 ppm of zinc metal solution.

Experimental setup: The nutrient stock solutions were prepared with the following initial concentrations [nitrite (50 µmol L^{-1}), ammonia (10 µmol L^{-1}), phosphate (0.1 µmol L^{-1}) and nitrate (1 µmol L^{-1})] (Strickland and Parsons, 1972). The zinc stock solution was prepared with an initial concentration of 1 ppm. All the nutrients and heavy metal stock solutions (100 mL) were taken into 250 mL conical flasks. The pH of the each solution was adjusted between 4 and 10 using diluted NH_4OH and HCL and pH was estimated using pH meter (ELICO, INDIA). 0.1 g of microalga powder and 50 Nos. of algal beads was added in 250 mL of conical flask filled with 100 mL of aqueous solution. Then the flasks with aqueous solution and microalgal sorbents were kept in shaker at 200 rpm at 30°C temperature and microalgal biosorpent powder were incubated in different time intervals viz., 6, 12, 18 and 24 h for the estimation of nutrient and heavy metal removal rate. At the end of the experiment, the flasks were removed from the shaker and the nutrient and heavy metal solution was separated from the microalgal powder and beads by filtering the solution through Millipore filtering system fitted with Whatman 47 mm filter paper. Finally, the amounts of nutrients in the sample were measured using UV-spectrophotometer (Shimadzu Model-2450) as per the standard protocol (Strickland and Parsons, 1972). The initial and final concentration of zinc in aqueous solution and standard was estimated using Atomic Absorption Spectroscopy, 1983 (400/HGA 900/AS 800-Perkin Elmer).

RESULTS AND DISCUSSION

Nutrients removal using microalgae powder: A significant decrease in the concentration of PO_4^{3-} was observed at all the tested pH ranging from 4 to 10. The maximum phosphate removal was observed at 24th h at pH 6 where it reduced to 0.027 µmol L^{-1} from an initial concentration of 0.100 µmol L^{-1}. In case of nitrate the maximum (81%) removal was observed at pH 6 at 24 h incubation where its concentration reduced to 0.18 µmol L^{-1} from an initial concentration of 1.0 µmol L^{-1}. The maximum nitrite reduction (29.21 µmol L^{-1}) was observed at 18 h incubation at pH 7 while lowest removal (2.79 µmol L^{-1}) was found at pH 6 at 24 h incubation. No significant difference observed in the ammonium reduction. The least ammonia removal (0.99 µmol L^{-1}) was recorded at pH 7 until 6th h. After 6 h the ammonia concentration was found to increase irregularly. This could be attributed to ammonia demineralization from dead algal biomass. The nutrient adsorption efficacy of microalgae powder was shown in Fig. 1. Similarly, the time interval experiment on nutrient absorption of microalga *C. marina* inferred that all retention time (6, 12, 18 and 24 h) maintained presently showed considerable result on nutrients removal. Among these, 24 h biosorption at pH 6 resulted notable removal of nutrients. The nutrients removal at 24 h incubation in pH 6 was in the order: NO_3>PO_4^{3-} >NO_2>NH_3^+.

Heavy metal removal using microalgal powder: Biosorption process with immobilized biomass is analogous to an ion-exchange process and therefore, pH showed a significant effect on metal uptake. The pH influences both cell surface metal binding sites as well as metal chemistry in solution. At highly acidic pH values, metal cations and protons compete for binding sites on cell wall which results in lower uptake of metal (Doyle *et al.*, 1980; Garnham *et al.*, 1993). The highest removal of zinc was observed in pH 7 at 24 h, the resulting concentration being 0.030 ppm, while lower removal of zinc was recorded in pH 4 at 6 hours showing a final concentration of 0.456 ppm, with the initial concentration being 1 ppm for both. Overall result showed that incubation of 24 h at pH 7 (Fig. 2) could be a better approach for heavy metal removal using microalga *C. marina*. Our biosorption findings are in the agreement with earlier reports (Garnham *et al.*, 1993; Robinson, 1989). The results observed for the effect of pH on the sorption behavior of the metal ions studied are similar to those

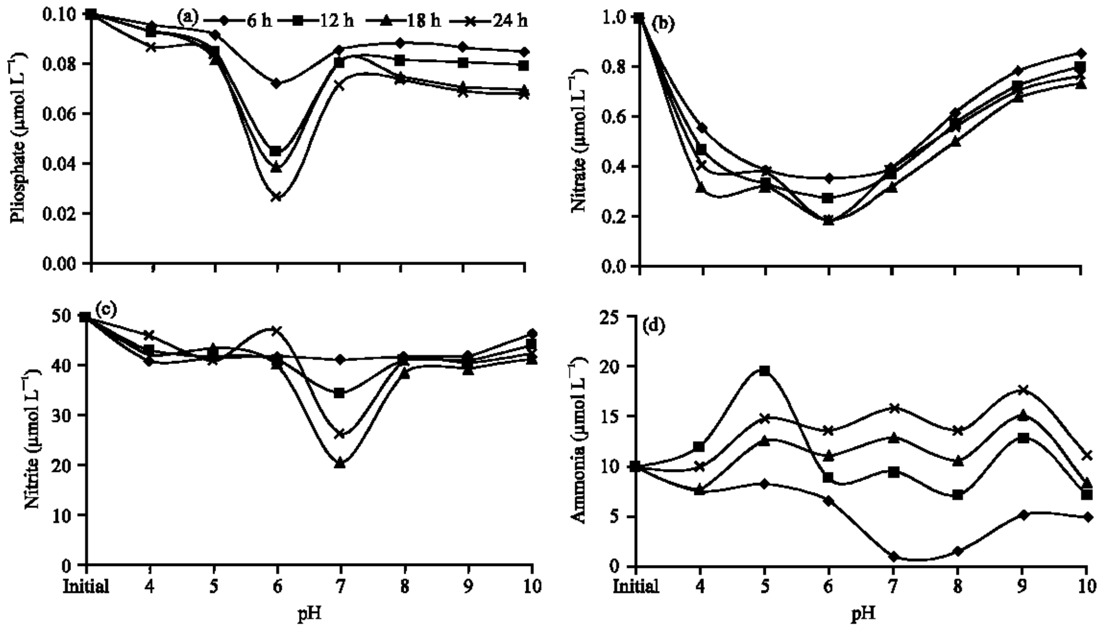

Fig. 1(a-d): Effect of pH (4 to 10) and retention time (6 to 24 h) on nutrients removal, (a) Phosphate, (b) Nitrate, (c) Nitrite and (d) Ammonia by marine microalga *Chlorella marina*, biosorbent powder. Experiment was conducted at 30°C in an orbital shaker at 200 rpm

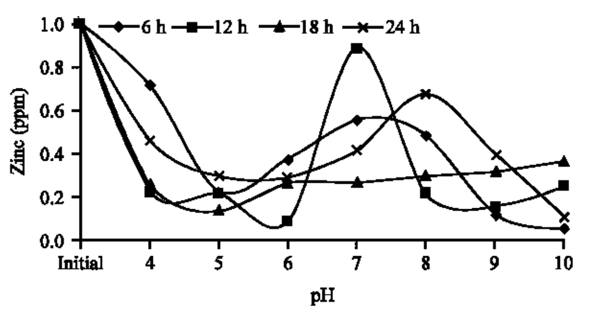

Fig. 2: Effect of pH (4 to 10) and retention time (6 to 24 h) on zinc biosorption by marine microalga *Chlorella marina* biosorbent powder, Experiment was conducted at 30°C in an orbital shaker at 200 rpm

reported by other workers (Harris and Ramelow, 1990; Zhou and Kiff, 1991). Though a temperature of around 80°C was used to dry the cells in the present study the heating process was not continuous. Intermittent heating was done to prevent over drying of the cells. However, there are reports supporting the use of high heating temperature (110°C) (Qiao *et al*., 2010).

Nutrients removal using immobilized microalgal beads: The reduction in the concentration of four nutrients (PO_4^{3-}, NH_3^+, NO_3^- and NO_2^-) were significantly better than control indicating that the microalga *C. marina* beads possess good absorbent capacity as shown in Fig. 3. The significant decrease in the concentration of PO_4^{3-} was observed at all the tested time duration ranging from 6 to 24 h. The maximum phosphate removal was obtained at 24 h where it was reduced to 0.04 μmol L^{-1}. This result was similar to early work (De-Bashan *et al*., 2002). The microalgal beads efficiently reduced the nitrate from 1.00 to 0.15 μmol L^{-1} at 24 h interval which was comparatively better than control where the removal recorded as 1.00 μmol L^{-1} to 0.88 μmol L^{-1}. The highest removal (9.064 μmol L^{-1}) of NH_3^+ was recorded at 24th hour, whereas the lowest removal was noticed during the 6th hour with 6.37 μmol L^{-1}. The lowest removal (17.12 μmol L^{-1}) of nitrite was obviously recorded during the 6th h. The maximum nitrite reduction was recorded at 24th hour where it was reduced from 50 to 27.81 μmol L^{-1}. Nitrogen is a crucial parameter and is becoming increasingly important in wastewater management because nitrogen can have many effects on the environment (Halling and Jorgensen, 1993). Nitrogen can exist in different forms because of various oxidation states and it can readily change from one form to another depending on the oxidation state present at the time. The

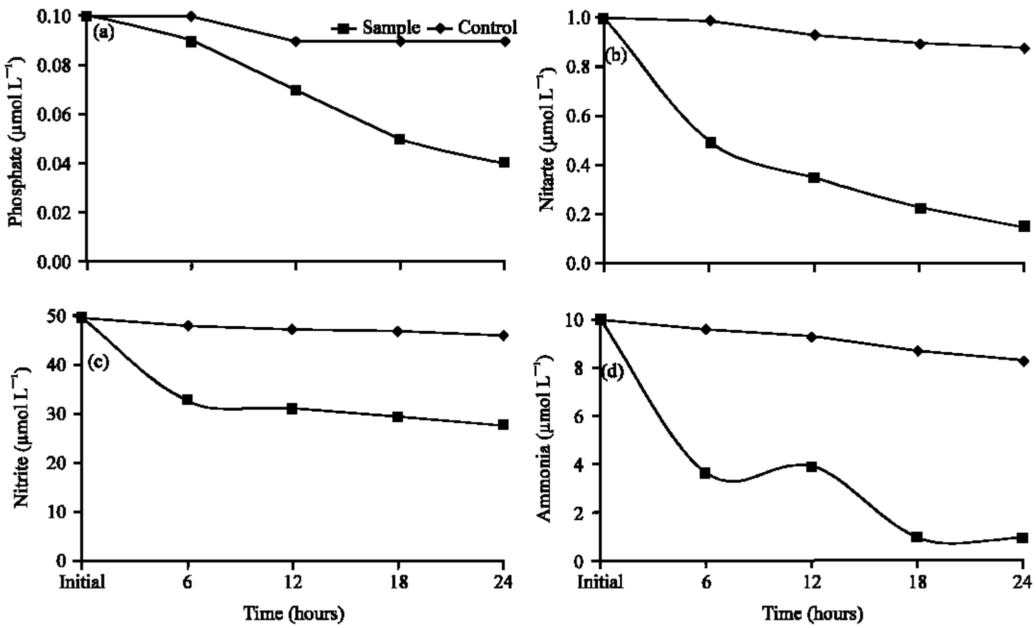

Fig. 3(a-d): Effect of retention time (6 to 12 hours) on nutrients removal, (a) Phosphate, (b) Nitrate, (c) Nitrite and (d) Ammonia by marine microalga *Chlorella marina* immobilized beads. Experiment was conducted at 30°C in a static condition

principal forms of nitrogen are organic nitrogen, ammonia (NH_4 + or NH_3), nitrite (NO_2-) and nitrate (NO3-). The presence of nitrogen in wastewater during discharge can be undesirable because it has ecological impacts and can affect public health. In our study, the maximum removal (74 %) of nitrogen was observed in immobilized algal form. Ammonia is extremely toxic and also an oxygen-consuming compound, which can deplete the dissolved oxygen in water. Although, nitrate itself is not toxic, its conversion to nitrite is a concern in the domain of public health. Nitrite is a potential public health hazard (Sedlak, 1991) and, in the body, can oxidise iron (II) and form methaemoglobin, which binds oxygen less effectively than normal haemoglobin. All forms of nitrogen are taken up as a nutrient by the microalga, although the most common nitrogen compounds assimilated by microalgae are ammonium (NH^{4+}) and nitrate (NH^{3-}) (Oliver and Ganf, 2000). In this study, *C. marina* was able to reduce all forms of nitrogen substantially and ammonia and nitrate levels, in particular, were drastically reduced. The reduction in ammonia values may also be result of it being stripped off into the air as a result of the increased pH values often found in algal cultures (Nunez *et al*., 2001). In this study, 56% of phosphate was successfully removed using immobilized algal forms. The phosphate removal can be a result of photosynthetic assimilation and calcium phosphate precipitation due to high pH levels caused by intense algal photosynthetic activity as reported earlier (Hammouda *et al*., 1995). Moreover, microalgae are able to assimilate phosphorus in excess, which is stored in the cells as polyphosphate granules and magnesium and potassium are co-transported along with phosphate (Bitton, 1999).

Heavy metal removal using immobilized microalgal beads: The pH is an important criterion during metal adsorption. The pH ranging from 4-10 and incubation duration 6 to 24 h (6, 12, 18 and 24) was studied for heavy metal absorption using immobilized microalgal beads (Fig. 4) Among the pH and time series tested presently, pH 6 for a time period of 24 h resulted the maximum (0.713 ppm) and pH 7 for a period of 6 hours resulted minimum (0.447 ppm) zinc adsorption. The biosorption capacity of the cell is sensitive to pH (Simine *et al*., 1998). The cell surface metal binding sites and availability of metal in solution are affected by pH. At low pH, the cell surface sites are closely linked to the H^+ ions, thereby making these unavailable for other cations. However, with an increase in pH, there is an increase in ligand with negative charges which results in increased binding of cations (Ahuja *et al*., 1999). The increase of pH can increase negative charge on the surface of the cell which can favor electrochemical attraction and adsorption of metal (Gourdon *et al*., 1990).

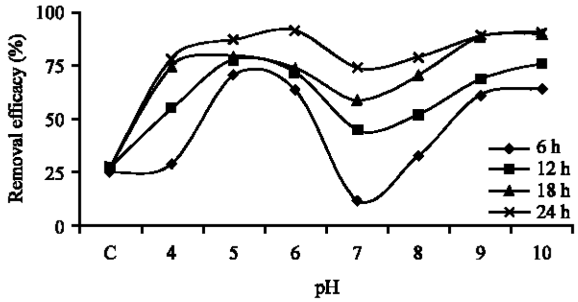

Fig. 4: Effect of pH (4 to 10) and retention time (6 to 24 h) on zinc biosorption by immobilized marine microalga *Chlorella marina* beads, Experiment was conducted at, 30°C in a static condition. C represents Control (Sodium alginate only)

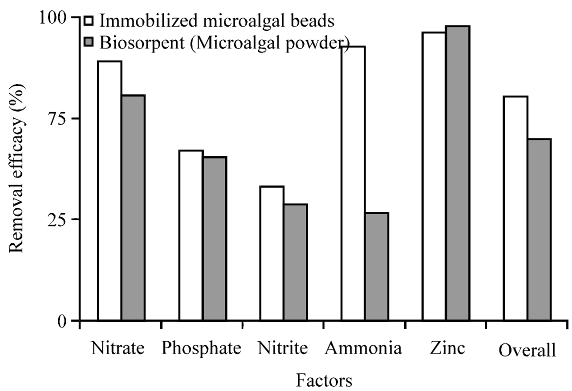

Fig. 5: Comparative nutrients and heavy metal removal rate of marine microalga *Chlorella marina* beads and powder

In comparison to live cells, the metal sorption capacity of dead cells may be greater, equivalent or less (Ozer *et al*., 2000). *Chlorella* sp. cells, killed by heat, accumulated greater amounts of uranium (VI) than living cells (Greene and Bedell, 1990). Earlier works found that the dead cells performed better than the live cells initially in copper removal and recovery (Tam *et al*., 1998). The zinc absorption of microalga *C. marina* increased with increasing pH and time interval. In the present study, the maximum absorption of zinc was recorded in pH 6 and at 24 h. The shorter contact period of microalgae biomass resulted in lesser metal absorption. The metal absorption of microalgae biomass can be attributed to the electrostatic interaction between cells and metal ions.

The results of the present study reveal that the adsorption capacity of the immobilized microalgae was greater for nutrients and heavy metal removal (Fig. 5). Dried biomass generally consists of small particles with low density, poor mechanical strength and little rigidity (Zaied *et al*., 2008). Hence the biomass is to be immobilized before being subjected to biosorption. The immobilized biomass offers many advantages including better reusability, high biomass loading and minimal clogging in continuous flow systems (Holan and Volesky, 1994).

CONCLUSION

The excessive nutrients and heavy metal loading from different wastewaters which finally find their way into sea is an ongoing threat to water quality, which leads to more stringent environmental regulations in different countries. In this aspect in mind, the broad application of the marine microalga *C. marina* was tested in different forms to evaluate its potentiality as a bioremediant. The present study demonstrated that pH has a strong impact on biosorption potential of *C.marina*. Among the pH studied, the pH 6 was found to report optimum for all the nutrients remediation except nitrite, where it was efficiently removed at a pH of 7. In case of heavy metal removal, the pH 8 was found to be suitable for biosorbent studies. Overall study concluded that the immobilized *C. marina* algal beads can efficiently removed the excessive nutrients in wastewater at 24 h incubation whereas removal of zinc was well aided by *C. marina* algal beads at pH 6 for 24 h.

ACKNOWLEDGMENTS

Authors are thankful to the Head, Department of Marine Science and authorities of Bharathidasan University for the facilities provided.

REFERENCES

Ahuja, P., R. Gupta and R.K. Saxena, 1999. News and Notes: Sorption and desorption of cobalt by *Oscillatoria anguistissima*. Curr. Microbiol., 39: 49-52.

Anirudhan, T.S. and P.G. Radhakrishnan, 2011. Adsorptive removal and recovery of U (VI), Cu (II), Zn (II) and Co (II) from water and industry effluents. Bioremed. J., 15: 39-56.

Bishnoi, N.R., A. Pant and Garima, 2004. Biosorption of copper from aqueous solution using algal biomass. J. Sci. Indus. Res., 63: 813-816.

Bitton, G., 1999. Wastewater Microbiology. John Willey, New York.

Das, N., R. Vimala and P. Karthika, 2008. Biosorption of heavy metals-An overview. Indian J. Biotechnol., 7: 159-169.

De-Bashan, L.E., M. Moreno, J.P. Hernandez and Y. Bashan, 2002. Removal of ammonium and phosphorus ions from synthetic wastewater by the microalgae *Chlorella vulgaris* co-immobilized in alginate beads with the microalgae growth-promoting bacterium *Azospirillum brasilense*. Water Res., 36: 2941-2948.

Doyle, R.J., T.H. Matthews and U.N. Streips, 1980. Chemical basis for selectivity of metal ions by the *Bacillus subtilis* cell wall. J. Bacteriol., 143: 471-480.

Garnham, G.W., G.A. Codd and G.M. Gadd, 1993. Accumulation of zirconium by microalgae and cyanobactefla. Applied Microbiol. Biotechnol., 39: 666-672.

Gourdon, R., S. Bhende, E. Rus and S.S. Sofer, 1990. Comparison of cadmium biosorption by gram-positive and gram-negative bacteria from activated sludge. Biotechnol. Lett., 12: 839-842.

Greene, B. and G.W. Bedell, 1990. Algal Gels or Immobilized Algae for Metal Recovery. In: Introduction to Applied Phycology, Akatsuka, I. (Ed.). SPB Academic Publishing, The Hague, The Netherlands, pp: 137-149.

Halling, S.B. and S.G. Jorgensen, 1993. The Removal of Nitrogen Compounds from Wastewater. Elsevier, Amsterdam, The Netherlands.

Hammouda, O., A. Gaber and N. Abdelraouf, 1995. Microalgae and wastewater treatment. Ecotoxicol. Environ. Safety, 31: 205-210.

Harris, P.O. and G.J. Ramelow, 1990. Binding of metal ions by particulate biomass derived from *Chlorella vulgaris* and *Scenedesmus quadricauda*. Environ. Sci. Technol., 24: 220-228.

Holan, Z.R. and B. Volesky, 1994. Biosorption of lead and nickel by biomass of marine-algae. Biotechnol. Bioeng., 43: 1001-1009.

Matsuska, O.V., R.P. Paranyak and Y.M. Gumnitskii, 2010. Adsorption of wastewater components by natural sorbents. J. Water Chem. Technol., 32: 218-222.

Nunez, V.J., D. Voltolina, M. Nieves, P. Pina, A. Medina and M. Guerrero, 2001. Nitrogen budget in *Scenedesmus obliquus* cultures with artificial wastewater. Bioresour. Technol., 78: 161-164.

Oliver, R.L. and G.G. Ganf, 2000. Freshwater Blooms. In: The Ecology of Cyanobacteria: Their Diversity in Time and Space, Whitton, B.A. and M. Potts (Eds.). Kluwer, Dordrecht, The Netherland, pp: 149-194.

Ozer, D., A. Ozer and G. Dursun, 2000. Investigation of zinc (II) adsorption on *Cladophora crispata* in a two-staged reactor. J. Chem. Technol. Biotechnol., 75: 410-416.

Qiao, J.W.L., X.H. Fu and G.H. Zheng, 2010. Comparative study on the NI^{2+} biosorption capacity and properties of living and dead pseudomonas putida cells. Iran. J. Chem. Chem. Eng., 29: 159-167.

Robinson, N.J., 1989. Alga metallothioneins: Secondary metabolites and proteins J. Applied Phycol., 1: 5-18.

Santos, M.M.D., I. Moreno-Garrido, F. Goncalves, A.M. Soares and R. Ribeiro, 2002. An in situ bioassay for estuarine environments using the microalga *Phaeodactylum tricornutum*. Environ. Toxicol. Chem., 21: 567-574.

Sedlak, R., 1991. Phosphorus and Nitrogen Removal from Municipal Wastewater: Principles and Practice. 2nd Edn. Lewis Publishers, New York, ISBN: 0-87371-683-3.

Shimoda, K., Y. Uchimura, H. Imai, M. Kitagawa, H. Hirano, H. Hamada and H. Hamada, 2011. Bioremediation of benzophenone by glycosylation with immobilized marine microalga *Chrysocampanulla spinifera* and *Amphidinium crassum*. Biochem. Insights, 2011: 35-40.

Simine, D.D., C. Finoli, A. Vecchio and V. Andreoni, 1998. Metal ion accumulation by immobilised cells of *Brevibacterium* sp. J. Ind. Microb. Biotechnol., 20: 116-120.

Strickland, J.D.H. and T.R. Parsons, 1972. A manual of sea water analysis. Canada Fisheries Research Board Bulletin no 167, pp: 310.

Tam, N.F.Y., Y.S. Wong and C.G. Simspon, 1998. Repeated removal of copper by alginate beads and the enhancement by microalgae. Biotechnol. Techniq., 12: 187-190.

Yen, A.M., 2008. Use of marine microalgae in bioremediation of balm oil mill effluent. M.Sc. Thesis, University of Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia.

Zaied, K.A., H.N.A. El-Mageed, E.A. Fayzalla, A.E. Sharief and A.A. Zehry, 2008. Enhancement of biosorption of heavy metals from factory effluents via recombinants induced in yeast and bacteria. Aust. J. Basic Applied Sci., 2: 701-717.

Zhou, J.L. and R.J. Kiff, 1991. The uptake of copper from aqueous solution by immobilized fungal biomass. J. Chem. Technol. Biotechnol., 52: 317-330.