Indian Journal of Geo-Marine Sciences
Vol. 44(1), January 2015, pp. 97-103

Excessive nutrients and heavy metals removal from diverse wastewaters using marine microalga *Chlorella marina* (Butcher)

Dinesh Kumar, S., P. Santhanam*., T. Jayalakshmi., R. Nandakumar., S. Ananth., A. Shenbaga Devi & B. Balaji Prasath
Department of Marine Science, Bharathidasan University, Tiruchirappalli-620 024, Tamil Nadu, India
*[E-mail: sanplankton@yahoo.co.in]

Received 8 July 2013; revised 5 August 2013

Present study consists the nutrients and heavy metals removal efficacy of mobilized marine microalga *Chlorella marina* cells using different wastewaters *viz.,* domestic sewage, industrial and aquaculture. Study indicated that as a result of seven days incubation in wastewater, *C. marina* cell concentration increased from 3×10^6 to 1.5×10^7 cells/ml and reduced 88% of nitrite, 75% of nitrate, 64% of ammonia and 51% of phosphorus in overall three different waste waters. Likewise, heavy metals such as chromium and lead were eliminated largely in the percentages of 89 and 87 respectively from aquaculture wastewater in particular.

[**Keywords:** *Chlorella marina*, Bioremediation, Wastewater, Nutrients, Heavy metals]

Introduction

Natural treatment of organic and inorganic nutrient rich wastewater is an accepted process for water purification and water reuse. Developing biological based treatment systems being considered as economically cheaper and more environment friendly[1]. A marine microalgae treatment is complementary and has not been implement in our country so far. Microalgae based treatment is considered to be one of the most efficient, eco-friendly, relatively low-cost and simple compared to physical and chemical treatments which are costlier and environmentally malignant. Microalgae can absorb molecules released during the early period development in wastewater treatment. Many species of marine microalgae are being practiced for tertiary wastewater treatment to eliminate different compounds of phosphorus, nitrogen, heavy metals, and toxic residues from wastewaters in temperate countries[1-4]. *Chlorella marina* is a unicellular alga commonly distributed in tropical estuaries and seas. *C. marina* cells lack an unbending cell wall, and the cell is enclosed solely by a thin elastic plasma membrane. As a consequence, the cell morphology is powerfully influenced by osmotic changes. Present study is to evaluate a chronological treatment rate of mobilized microalgae *C. marina* as a prospective absorbent for diverse wastewater effluents viz., domestic sewage, chemical industrial and aquaculture wastewaters.

Materials and Methods

For the present experiment, the wastewaters were collected from three different areas. Domestic wastewater was collected from Chennai Cooum (Lat.13° 4' N, Long. 80° 17' E), industrial wastewater from Cuddalore Sipcot (Lat. 11°43' N, Long. 79° 49' E) and aquaculture wastewater from shrimp farm located at Parangipettai (Lat. 11° 29' N Long. 79° 45' E), all the wastewaters are untreated. Stock culture of *C. marina* was maintained in special air-conditioning room followed by standard method[5]. Marine medium[6] was used for stock culture and 200 µmol m^{-2} s^{-1} light was provided. During the exponential phase, a known volume of *C. marina* was harvested and inoculated in to 1 litre plastic containers filled with three different (domestic, industrial and aquaculture) wastewaters and with one control (wastewater without microalgae). Initial algal density was 1.5×10^4 cells ml^{-1}. The fully transparent, round shape plastic containers were used under controlled conditions illuminated with light (300 µmol m^{-2} s^{-1}), photoperiod (12:12 L: D cycle) and constant aeration. Experiment lasted for 7 days. Water quality parameters such as temperature were measured using standard centigrade thermometer. Salinity was estimated with help of portable hand refractometer (ERMA, Hand Refractometer, Japan) and pH were measured using multiparameter probe (Deluxe pH meter, Elico-101, India), while the nutrients such as PO_4^{3-}, NH_4^+ and NO_2^- were analyzed according to Strickland and

Parsons[7]. Nitrate (NO_3^-) was estimated according to the method of Jenkins and Medsken[8] using UV- Vis Spectrophotometer (1800 Shimadzu UV). Chlorophyll 'a' was estimated by acetone extraction method[9]. 250 ml of water was filtered through a membrane filter (47 mm dia, 0.47µm pore size) using Millipore filtering system. Pigments were extracted from the concentrated algal sample in an aqueous solution of 90% acetone. Chlorophyll *a* concentration was determined spectrophotometrically by measuring the absorbance (optical density) of the extract at various wavelengths using UV- Vis Spectrophotometer (1800 Shimadzu UV).

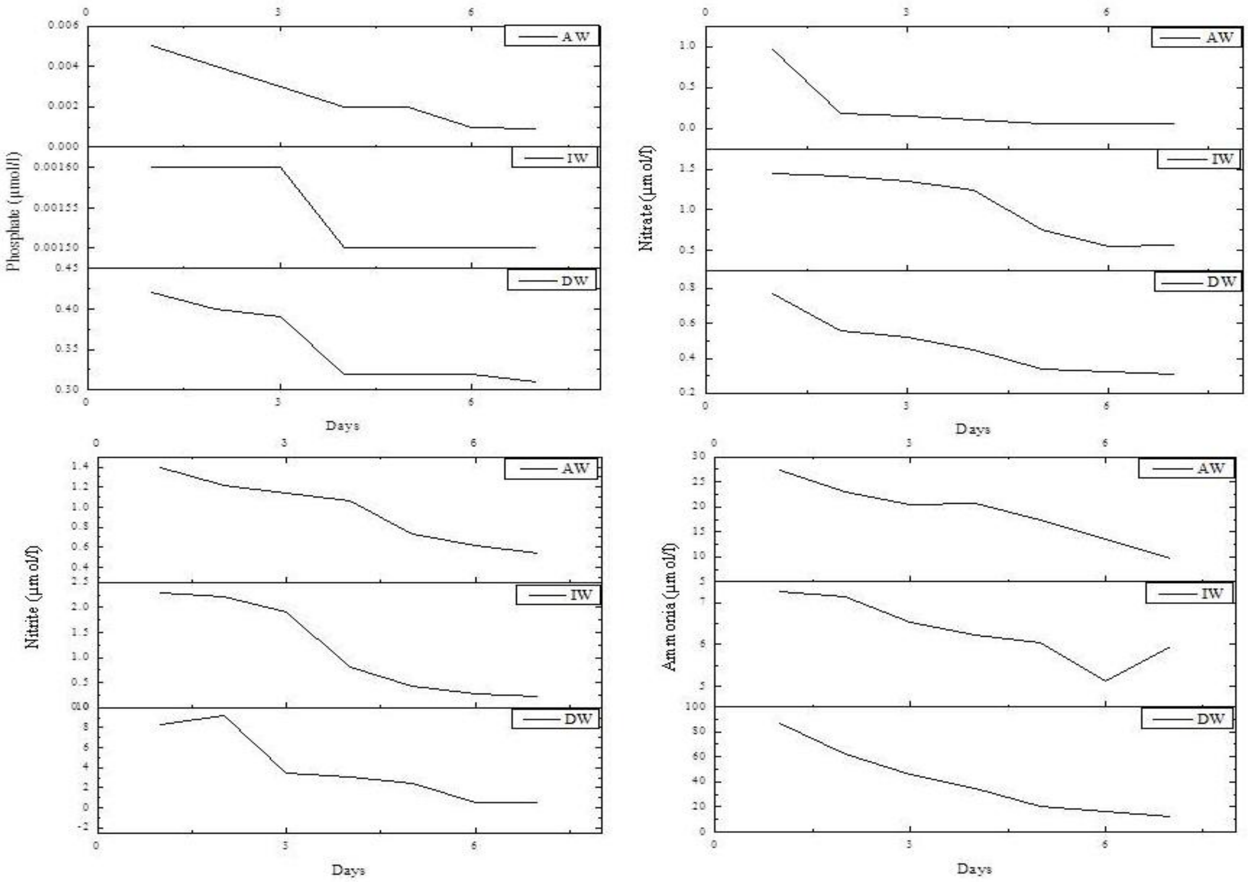
Physico-chemical parameters of wastewaters were analyzed every day. Initial and final heavy metals (Zn, Cr, Cu, Pb, and Cd) concentration in wastewaters was analyzed according to Brooks *et al*[10]. Filtered wastewaters (untreated and treated) (1 liter) was divided into two 500 ml aliquots and the pH was adjusted to pH 4±0.1 by careful drop-wise addition of 50% HNO_3. Heavy metals were pre-concentrated and separated from the bulk matrix by complexation with APDC and extraction into MIBK. Organic layer containing the metal chelates was collected and was back extracted with 50% HNO3, and diluted with metal free double distilled water to a minimum quantity (25 ml). Extracted sample containing metals were analyzed using Atomic Absorption Spectrophotometer (1983- 400 HGA 900/AS 800 Perkin Elmer) by using ICP multi- Element standard (MERCK-112837). Overall physico-chemical and biological characteristics of (untreated and treated) wastewaters values were given in a Table-1.

Results and Discussion

The recorded initial values of physico-chemical and biological parameters *viz.,*temperature, pH, salinity, phosphate, nitrate, nitrite, ammonia and chlorophyll 'a' in domestic wastewater were 28°C, 8.91, 12 psu, 0.55 µmol/l, 0.81 µmol/l, 19.0 µmol/l, 108.63 µmol/l and 4.39 µg/l respectively. At the end of the experiment the nutrients reduction was found significant with a considerable increase in chlorophyll 'a' content. Phosphate concentration was found to be dropped from an initial value of 0.55µmol/l to 0.31µmol/l. A considerable amount of reduction was also observed in nitrate concentration during the experiment with a drastic reduction from 0.81µmol/l to 0.31µmol/l occurring on second day. It is reported that the concentration of nitrate in wastewater decreased as the number of microalgae increased as agreed by Aziz and Ng[11]. Concentration of ammonia was decreased from 108.63µmol/l to 12.48µmol/l (Fig. 1). Similarly nitrite also decreased substantially from an initial concentration of 19.0µmol/l to a level of 0.47µmol/l. In summary, microalgae actively detached (consumed) the nitrogenous (nitrate, nitrite, and ammonium ions) and phosphate compounds from domestic wastewater as agreed by Oswald[12]. Algal cells were able to consume high concentration of nitrate ion and, therefore, can possibly contribute to purification of domestic wastewater[13].

Table 1 Physico-chemical and biological characteristics of untreated and treated wastewaters

Parameters	Domestic wastewater		Industrial wastewater		Aquaculture wastewater	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
Physico-chemical parameters						
pH	8.91	8.41	7.71	8.36	7.43	8.77
Salinity (psu)	12	21	35	40	23	24
Temperature (°C)	28	28	35	33	35	35
Macronutrients						
Phosphate (µmol/l)	0.55	0.31	0.0017	0.0015	0.010	0.000
Nitrate (µmol/l)	0.81	0.31	1.74	0.56	1.10	0.05
Nitrite (µmol/l)	19	0.47	2.39	0.22	2.30	0.54
Ammonia (µmol/l)	108.63	12.48	8.07	5.95	29.33	9.65
Biological Characteristics						
Chlorophyll 'a' (µg/l)	4.39	22.97	0.34	14.76	1.67	16.92
Heavy metals						
Zinc (ppm)	2.746	2.343	0.45	0.44	3.049	2.849
Chromium (ppm)	0.008	0.006	0.007	0.004	0.009	0.001
Copper (ppm)	4.625	4.194	3.897	1.007	2.262	0.654
Lead (ppm)	0.198	0.114	0.268	0.107	0.094	0.012
Cadmium (ppm)	0.092	0.041	0.023	0.013	0.035	0.011



Note: AW- Aquaculture wastewater; IW- Industrial wastewater; DW- Domestic wastewater

Fig. 1— Comparative nutrients removal rate of *C. marina* in different wastewaters.

Chlorophyll 'a' concentration was increased simultaneously from an initial value of 4.39µg/l to 22.97µg/l on the final day of experiment (7th day). Seven-day experiment showed that the concentration of metals such as cadmium, lead, chromium, zinc and copper were reduced by algae in the percentages of 55.43, 42.42, 25.0, 14.68 and 9.32 respectively. Microalgae were reported to be more efficient in sequestering metal species from solution than bacterial and fungal biomass[14]. Mechanism of the effectiveness in removing heavy metals from wastewater by microalgae is related to their large surface area and high binding affinity[15]. Different algal species have different sizes, shapes and cell wall compositions, which affect their metal binding efficiency[16], and the cell wall, in particular, is the main binding site for metals[17]. A mathematical model was previously done to describe the uptake of cadmium by algae, which includes two distinct steps in the process: an initial rapid uptake of metal ions due to attachment to the cell wall, followed by a relatively slow uptake due to membrane transport of the ion through the cell wall into the cell cytoplasm[14]. The present study shows that among the metals studied, zinc and copper were removed poorly as agreed by previous workers[18], who explained that microalgae cannot efficiently remove zinc from wastewater. However, in our study the maximum removal was procured with cadmium might be due to viability and metabolisms of *C. marina* cells[19] in binding cadmium.

Industrial wastewater treatment experiment showed an irregular variation in the values of pH where the maximum of 8.60 recorded on day 5 which increased from an initial value of 7.71 and on final day it was recorded as 8.36. Initial concentration of salinity was recorded 35psu and it increased up to a final value of 40psu. Increasing salinity in industrial wastewater treatment might be due to the presence of mycolic-acid containing actinomycetes which grow abundantly in industrial wastewater as opined by some workers[20, 21]. Recorded temperature was found ranged from 33°C to 35°C. The initial concentration of inorganic nutrients viz., phosphate, nitrate, nitrite and ammonia were decreased from 0.00169 to 0.00148, 1.74 to 0.56, 2.39 to 0.22 and 8.07 to 1.93µmol/l respectively (Fig. 1). In this experiment maximum ammonia was detached from the wastewater. Removal of ammonium ions from untreated wastewater is probably by an air-stripping mechanism[22]. In general, microalgal growth rate was reported lower in many industrial waste water due to low N and P concentration and high toxin concentrations as reported by earlier workers[23]. In this experiment, the removal of phosphate was very low compared to other nutrients as agreed by previous workers[24, 1]. However, the chlorophyll ‘a’ concentration was recorded to be increased from 0.34µg/l to 19.53µg/l with maximum concentration noticed on 4th day of experiment. A descending trend in chlorophyll ‘a’ value was noticed after 4th day can indicated the decline phase of algal growth. It is further confirmed that the ammonia level was found increased when the algae return to death phase as agreed by Thomas [25] who describes that the chlorophyll ‘a’ concentration been decreased when ammonia level increased in wastewater. Heavy metal removal rate of *C. marina* in chemical industrial wastewater was recorded high at copper where the alga removed 74.15 % followed by lead (60 %), cadmium (43.47%) chromium (42.85%) and zinc (2.06 %). It is clearly understood that the zinc was removed fewer compared to other metals and this could be attributed to the low viability of *C.marina* cells in adsorbing zinc[18].

In the present experiment, *C. marina* inoculated in aquaculture wastewater results the high phosphate removal where the concentration was reduced from 0.010µmol/l to 0.0003µmol/l followed by nitrate which reduces from 1.10µmol/l to 0.05µmol/l. The nitrite concentration was reduced from 2.30µmol/l to 0.54µmol/l whereas the ammonia reduced from 29.33µmol/l to 9.65µmol/l (Fig. 1). Daily chlorophyll ‘a’ concentration in wastewater culture shows the growing trend from an initial concentration of 1.67µg/l to 16.92µg/l. It is clear that the microalga can utilize the available nutrients in the wastewater for their cell growth, so that the nutrients in wastewaters can be reduced. However, the chlorophyll concentration in aquaculture wastewater was found to lesser than other wastewater studied presently. Generally aquaculture wastewater has an extremely soaring load of solid particles in suspension, which contributes to increased turbidity[26, 27]. This high turbidity can limits the light absorption by the microalgae, affects the photosynthetic capacity of these organisms and consequently their growth. Hence the low yield of chlorophyll ‘a’ was noticed in aquaculture wastewater (16.92µg/l) compared to chemical industrial (19.53µg/l) and domestic (22.97µg/l) wastewaters.

A seven days experiment on heavy metals removal of microalgal cell showed the efficient result with chromium which was removed 89% followed by lead (87%), copper (71%), cadmium (69%) and zinc (6.6%) (Fig. 2). Heavy metals in an aquaculture wastewater can arise from shrimp feed vitamin/ mineral supplements as well as from the corrosion of metal pipe fittings and other equipments[28]. It is understood that microalgae can take up metals from the environment and contribute to the accumulation of metals in the tropic chains[29-31]. Phytoremediation has recently become a subject of intense public and scientific interest and a topic of many recent researches[32-39]. Phytoremediation of heavy metals is a cost-effective green technology; there are more advantages, when it comes to the use of native and naturally growing plants.

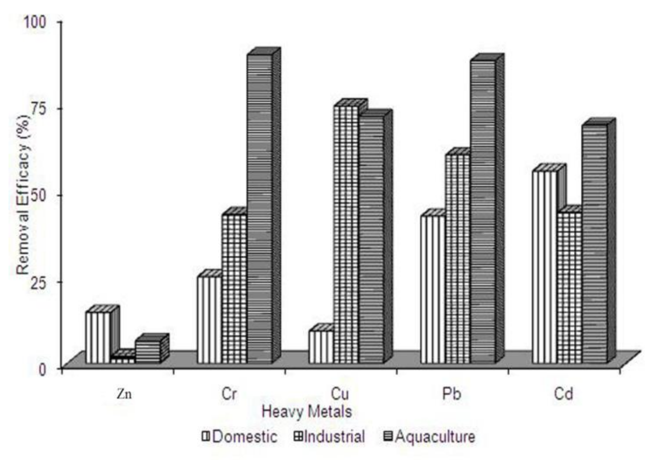


Fig. 2—Comparative heavy metal removal rate of *C. marina* in different wastewaters.

The Fig. 3 showed the recorded chlorophyll ‘a’ concentration in the different effluent water and these chlorophyll concentrations were finally compared with standard indoor algae culture. It is understood that the algal growth and thereby the chlorophyll concentration was mainly depend on available nutrients and suitable physical characteristics. Present study yielded an interesting result, with higher Chl ‘a’ concentration recorded in domestic wastewater (22.97μg/l) which was far better than Chl ‘a’ concentration at normal indoor culture (4.90μg/l). In fact all the wastewater effluents recorded higher concentrations of Chl ‘a’ than the standard indoor culture. Average chlorophyll concentration recorded in the various effluents were: 15.50μg/l (domestic wastewater), 10.82μg/l (industrial wastewater) and 8.81μg/l (aquaculture wastewater) whereas in control no chlorophyll ‘a’ was observed. Algal biomass productivity and photosynthetic efficiency could be increased further by mixing along with increased light/dark frequencies[40]. The nutrient removal and parallel microalgae growth was shown in Fig. 4.

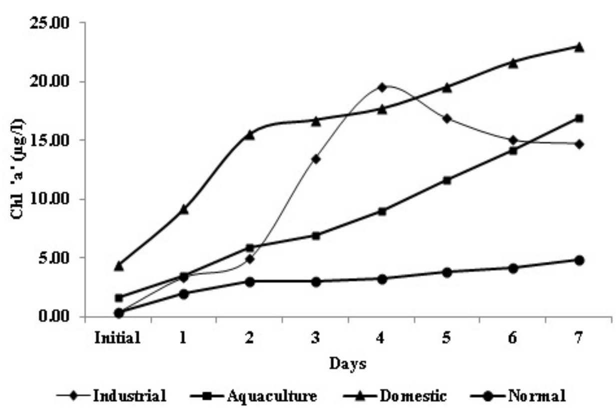


Fig 3. Comparative chlorophyll ‘a’ yield of *C. marina* in different wastewaters.

Microalgae *C. marina* could efficiently remove the nitrogen and phosphorus substances from wastewaters as the cell requires a high amount of nitrogen and phosphorous for protein, nucleic acid and phospholipid synthesis as reported by Martinez et al.[41,] and Kumar et al. [42] who stated that the multiple factors including light, pH, nitrogen and phosphorus ratio, temperature, carbon source and bacteria concentration were crucial to nitrogen and phosphorus removal efficiency by algae. Similar results are also obtained by previous workers[43] who stated that blue green algae are ideally suitable to play a dual role of treating wastewater in the process of effective utilization of different constituents essential for growth leading to enhanced biomass production.

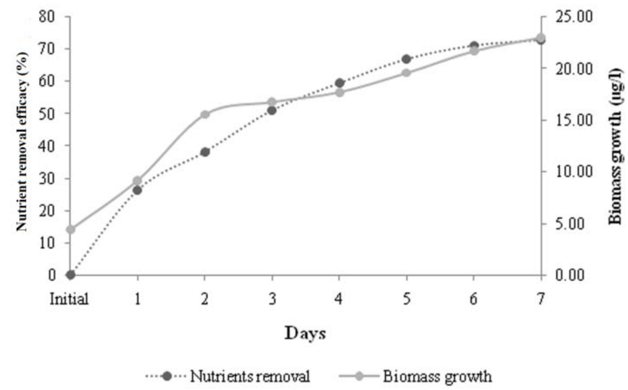


Fig 4. Relationship between nutrients reduction and *C. marina* biomass (Chl ‘a’) yield.

Conclusion

Result of this study suggests that *C. marina* has a remarkable ability on removal of excessive nutrients and heavy metals and perhaps considered as an excellent bioremediant for wastewater treatment. However, before commercialization and industrial scaling-up, further studies are needed on the effective utilization of wastewater growing algal biomass for zero waste management.

Acknowledgement

Authors are grateful to Head, Department of Marine Science and authorities of Bharathidasan University for the facilities provided. One of the authors (SD) thanks the DBT, Govt. of India, for Junior Research Fellowship.

References

1 Valderrama, L.T, Campo C.M.D., Rodriguez, C.M., De-Bashan, L.E. & Bashan, Y., Treatment of recalcitrant wastewater from ethanol and citric acid production using the microalgae *Chlorella vulgaris* and the macrophyte *Lemna minuscule, Water Res.*, 36 (2002) 4185-4192.

2 Gonzales, L.E., Canizales, R.O. & Baena, S., Efficiency of ammonia and phosphorus removal from a Colombian agro industrial wastewater by the microalgae *Chlorella vulgaris* and *Skenedesmus dimorphus*. Bioressource Technol., 60 (1997) 259-262.

3 Muhaemin, M., Toxicity and Bioaccumulation of Lead in *Chlorella* and *Dunaliella*, *J. Coast. Develop.,* 8(1) (2004) 27-33.

4 Muhaemin, M., The Initial Adsorption of $Pb2^+$ to *Dunaliella salina*. *J. Coast. Develop.,* 9(2) (2006) 97-105.

5 Gopinathan, C.P., Methods of culturing phytoplankton, *Manual of research methods for fish and shellfish nutrition*. CMFRI Spl. Publ., 8 (1982) 113-118.

6 Walne, P.R., 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.

7 Strickland, S.C. & Parsons, T.R., *A practical handbook of seawater analyses*. (Bulletin of Fisheries Research Board of Canada, Ottawa) 1972, pp. 185.

8 Jenkins, D. & Medsken, L., A Brucine Method for the Determination of Nitrate in Ocean, Estuarine, and Fresh Waters, *Anal Chem.,* 36 (1964) 61.

9 Mantoura, R.F.C. & Llewellyn, G.A., The rapid determination of algal chlorophyll and carotenoid and their breakdown products in natural waters by reverse-phase high-performance liquid chromatography, *Anal Chim Ada.*, 151 (1983) 297-314.

10 Brooks, R.R., Presley, B.J. & Kaplan, I.R., *APDC-MIBK extraction system for the determination of trace metals in saline waters by atomic adsorption spectroscopy*, Talanta.Bulletin of Fisheries Research Board of Canada., 14 (1967) 809-816.

11 Aziz, M.A. & Ng, W.J., Industrial wastewater treatment using an activated algal-reactor, *Water Sci. Technol.,* 28(7) (1993): 71-76.

12 Oswald, W.J., Ponds in the twenty-first century. *Water Sci. Technol.,* 31 (1995) 1-8.

13 Acien Fernandez, F.G., Garcıa Camacho, F., Sanchez Perez, J.A., Fernandez Sevilla, J.M. & Molina Grima, E., Modeling of biomass productivity in tubular photo bioreactors for micro algal cultures: Effects of dilution rate, tube diameter, and solar irradiance, *Biotechnol. Bioeng.,* 58(6) (1998) 605–616.

14 Khoshmanesh, A., Lawson, F. & Prince, I.G., (1996). Cadmium uptake by unicellular green microalgae. *Chem. Eng. J.,* 62(1) (1996) 81–88.

15 Roy, D., Greenlaw, P.N. & Shane, B.S., (1993). Adsorption of heavy metals by green algae and ground rice hulls. *J. Environ. Sci. Health Part A*, 28(1) (1993) 37–50.

16 Tam, N.F.Y., Wong, Y.S. & Simpson, C.G., Removal of copper by free and immobilized microalga, *Chlorella vulgaris*, In: *Wastewater treatment with algae*, edited by Y.S. Wong and N.F.Y. Tam (Berlin: Springer) 1997, pp. 17–36.

17 Wehrheim, B. & Wettern, M., Biosorption of cadmium, copper and lead by isolated mother cell walls and whole cells of *Chlorella fusca*, *Appl. Microbiol. Biotechnol.,* 8(4) (1994) 227–232.

18 Hammouda, O., Gaber, A. & Abdel-Raouf, N., Microalgae and waste water treatment, *Ecotoxicol. Environ. Safety,* 31 (1995) 205-210.

19 Torres, E., Cid, A., Fidalgo, P., Herrero, C. & Abalde. J., Long-chain class III metallothioneins as a mechanism of cadmium tolerance in the marine diatom Phaeodactylum tricornutum Bohlin, *J. Aquat Toxicol,* 39 (1997) 231-246.

20 De Azeredo, L.A. I., Da Cunha, C.D. & Rosado, A.S., New group-specific 16S rDNA primers for monitoring foaming mycolata during saline waste-water treatment, *Biotechnol. Lett.,* 28 (6) (2006) 447–453.

21 Alvarezm V.M., Marques, J.M., Korenblum, E. & Seldin, L., Comparative bioremediation of crude oil-amended tropical soil microcosms by natural attenuation, bioaugmentation, or bioenrichment, *Appl. Environ. Soil Sci.*, (2011)1-10. doi: 0.1155/2011/156320.

22 De la Noue, J. & Proulx, D., The potential of microalgae biotechnology: a review of production and uses of microalgae, *Biotechnol. Adv.,* 6 (1988) 725-770.

23 Pittman, J.K., Dean, A.P. & Osundeko, O., The potential of sustainable algal biofuel production using wastewater resources. *Bioresour. Technol.*, 102 (2011) 17–25.

24 Chevalier, P. & De la Noue, J., Wastewater nutrient removal with microalgae immobilized in K-carrageenan, *Enzyme Microbial. Technol.,* 7 (1985) 621-624.

25 Thomas, W.H., Effect of ammonium and nitrate concentration on chlorophyll increases in natural tropical pacific phytoplankton populations, *Limnol Oceanogr*, 15 (3) (1970) 386-394.

26 Zar, J.H., *Biostatistical analysis*. 4th edn. (Prentice Hall, New Jersey) 1999, pp. 718.

27 Jones, A.B., O'Donohue, M.J., Udy, J. & Dennison, W.C., Assessing ecological impacts of shrimp and sewage effluent: biological indicators with standard water quality analyses. *Estuar. Coast. Shelf Sci.,* 52 (1) (2001) 91–109.

28 Sharrer, M.J., Rishel, K. & Summerfelt, S.T., Evaluation of a membrane biological reactor for reclaiming water, alkalinity, salts, phosphorus, and protein contained in a high-strength aquacultural wastewater, *Bioresource Technol*, 101 (2010) 4322-4330.

29 Miretzky, P., Saralegui, A. & Cirelli, A.F., Aquatic macrophytes potential for the simultaneous removal of heavy metals (Buenos Aires, Argentina), *Chemosphere*, 57(8) (2004) 997-1005.

30 Rehman, A.& Shakoori, A.R., Heavy metal resistant *Chlorella* spp., isolated from tannery effluents, and their role in remediation of hexavalent chromium in industrial wastewater, *Bull. Environ. Contamin. Toxicol.,* 66 (2001) 542-547.

31 Munoz, R. & Guieysse, B., Algal-bacterial processes for the treatment of hazardous contaminants: A review, *Water Res*, 40 (2006) 2799-2815.

32 Raskin, P.B., Kumar, A.N. & Dushenkov, V., Bioconcentration of heavy metals by plants, *Curr Opin Biotec* 5 (1994) 285-290.

33 Cunningham, S.D., Berti, W.R. & Huang, J.W., Phytoremedia -tion of contaminated soils, *Trends Biotechnol*, 13 (1995) 393-397.

34 Salt, D.E., Blaylock, M., Kumar, N.P.B.A., Doshenkov, V., Ensley, B.D., Chet, C. & Raskin, I., Phytoremediation A novel strategy for the removal of toxic metals from the environment using plants, *J. Biotec.*, 13 (1995) 468-474.

35 Cunningham, S.D. & Ow, D.W., Promises and prospects of phytoremediation. *Plant Physiol.,* 110 (1996) 715-719.

36 Ike, R.S., Ono, H., Murooka, H. & Yamashita, M., Bioremediation of cadmium contaminated soil using symbiosis between leguminous plant and recombinant Rhizobia with the MTL4 and the PCS genes, *J. Chemo.,* 66 (2007) 1670-1676.

37 Kumar, M.S. & Jaiswal, S., Bioaccumulation and translocation of metals in the natural vegetation growing on fly ash lagoons: a field study from Santaldih thermal power plant, West Bengal, India, *Env. Mon. Ass.*, 116 (2007) 263-273.

38 Muneer, B., Shakoori, F., Rehman, A. & Shakoori, A.R., Chromium resistant yeast with multi-metal resistance isolated from industrial effluents and their possible use in microbial consortium for bioremediation of wastewater, *Pak. J. Zoo.,* 39 (2007) 289-297.

39 Sun, L.X., Zhao, H.G. & Mc-Cabe, C., Predicting the phase equilibria of petroleum fluids with the SAFT-VR approach. *AIChE J.,* 53 (2007) 720-731.

40 Grobbelaar, J.U., Physiological and technological considerations for optimising mass algal cultures. *J. Appl. Phycol.,* 12 (2000) 201–206.
41 Martínez, M.E., Sánchez, S., Jiménez, J.M., Yousfi, E. & Munoz, L., Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*, *Bioresour. Technol*,. 73 (2000) 263–272.
42 Kumar, M.S., Miao, Z.H.H. & Wyatt, S.K., Influence of nutrient loads, feeding frequency and inoculum source on growth of *Chlorella vulgaris* in digested piggery effluent culture medium, *Bioresour. Technol.,* 101(2010) 6012–6018.
43 Dash, A.K., Mishra, P.C., Role of the blue green algae *Westiellopsis prolifica* in reducing pollution load from paper mill wastewater, *Indian J. Environ. Pro.,* 19 (1) (1998) 1-5.