Recycling and treatment of herbal pharmaceutical wastewater using *Scenedesmus quadricuada*

Atul P. Vanerkar[1], Abhay B. Fulke[2], Satish K. Lokhande[2,*], Manisha D. Giripunje[3] and Shanta Satyanarayan[3]

[1]National Institute of Miners' Health, JNARDDC Campus, Wadi, Nagpur 440 023, India
[2]CSIR-National Environmental Engineering Research Institute, Nagpur 440 020, India
[3]Department of Environmental Science, Sevadal Mahila Mahavidyalaya, Nagpur 440 015, India

Globally, herbal pharmaceutical industries are among the leading pharmaceutical industries. They generate large volume of wastewater during processing and production, which is highly biodegradable in nature and cannot be discharged into environment as such. Hence efforts are being made to evaluate the toxicity of herbal pharmaceutical effluents using green algae *Scenedesmus quadricauda*. Physico-chemically treated effluents (PCTEs) as well as biologically treated effluents (BTEs) were observed after the application of *S. quadricauda*. Also, *S. quadricauda* showed higher growth rate after the addition of PCTE and BTE. The highest yield of algae was observed in BTE up to 15 days of incubation by synthesis of chlorophyll and cell metabolites, even with 10–100% dilution of effluents. The present study also discusses the evaluation of biotoxicity and recycling on herbal pharmaceutical wastewater along with heavy metal removal.

Keywords: Biotoxicity, herbal pharmaceutical industry, *Scenedesmus quadricauda*, wastewater.

HERBAL pharmaceutical industries are among the leading pharmaceutical industries worldwide. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain active ingredients from plants or other plant materials[1]. The countrywide scenario of herbal pharmaceutical industries reveals that, the industrial process involves the cleaning of herbs (like flowers, stem, bark, nuts, fruits, resins, seeds, roots, leaves, etc.) to remove dust and soil adhered to the material. The manufacturing of herbal medicines involves several processes like drying, passage through cutters and or ball mills as required, and further processes such extraction, fermentation, distillation, decoction preparation, percolation, etc. Though the product is based on herbs, synthetic chemicals are also used in the manufacturing process. Chemicals that are used include alcohol, sugar, gelatin, lactose, mineral salts, clays and different organic solvents. Figure 1 shows the wastewater sources during the manufacturing process[2]. Herbal pharmaceutical industries generate a large volume of wastewater, which is highly biodegradable in nature. However, it cannot be discharged directly into the surface water, as it putrefies very fast. Herbal pharmaceutical waste is a complex constituent of alkaloids, plant extracts, heavy metal ions and toxic solutes, which contribute to environmental pollution. Untreated pharmaceutical wastewater discharge into the natural environment causes health hazards to existing flora and fauna and therefore, treatment of the effluents is necessary to bring down the concentration of toxic substances to desired limits, before they are finally discharged into the natural systems[3]. Physico-chemical characteristics like pH, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total solids, sodium, potassium and heavy metals were analysed for the evaluation of toxicity of herbal pharmaceutical wastewater after its treatment with micro green algae *Scenedesmus quadricauda*. Treatment of wastewater using algae has been studied for over 50 years[4,5]. Microalgae have a potential to reduce the metal contamination in aquatic systems[6–11]. At first, the metal ions are physically adsorbed over the algal cell surfaces rapidly. Then, these metal ions move slowly into the cytoplasm of the algae, which is known as aschemi-sorption[12]. The biomass of microalgae rises during wastewater treatment, and has the potential to remove nitrogen and phosphorus from wastewater generated by different sources. However, nutrients are removed from wastewater through a direct uptake by the algal cells[9]. Algal treatment of wastewater, mediated through a combination of nutrient uptake, elevated pH and high dissolved oxygen concentration, can offer an ecologically secure, cheap and efficient means to remove nutrients and metals than conventional tertiary treatment procedures[7,13–18]. Several researchers have established that metals such as Ti, Pb, Mg, Zn, Cd, Sr, Co, Hg, Ni and Cu are sequestered in polyphosphate bodies in green algae. The polyphase bodies serve as a storage pool for metals and also act as 'detoxification agents'. Studies have revealed that the alga *Scenedesmus obliquus* can accumulate Cd and Zn by increasing the amount of phosphorus in the media[19].

The aim of the present study is to evaluate the treatment and recycling of herbal pharmaceutical wastewater using freshwater green algae *S. quadricauda*. Tests for growth, survival rate and synthesis of metabolites in physico-chemically treated effluent (PCTE) and biologically treated effluent (BTE) of herbal pharmaceutical industry using *S. quadricauda* were conducted.

Samples of untreated (raw) effluent (UTE), PCTE and BTE are collected from a herbal pharmaceutical industry in Nagpur. The samples were subjected to physicochemical analysis following the standard methods[20,21]. pH, colour, total acidity/alkalinity, total SS, total solids, COD, BOD (5 day at 20°C), sulphide as S^{-2}, sulphates as SO_4^{-2}, total phosphates as PO_4^{-2}, total nitrogen as N, oil and

*For correspondence. (e-mail: s_lokhande@neeri.res.in)

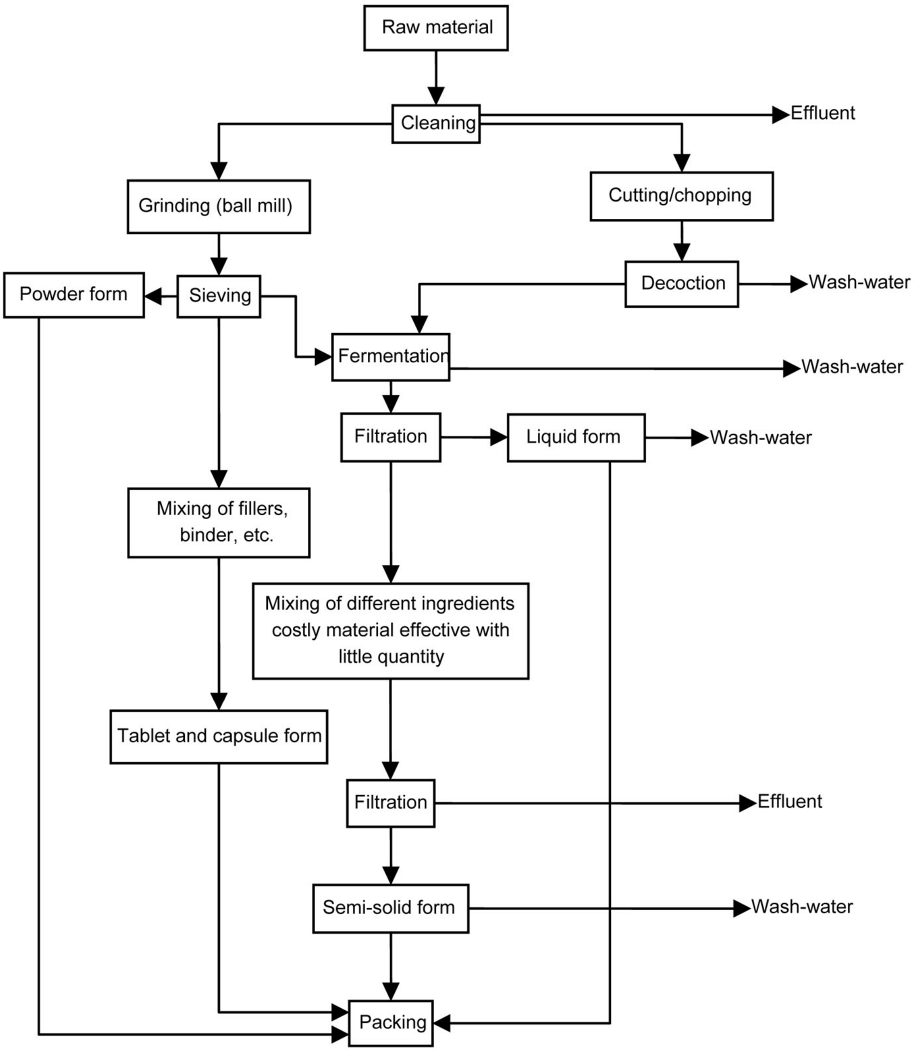

Figure 1. Flow sheet of general manufacturing process of herbal medicines and wastewater sources.

grease, sodium, potassium, and heavy metals were analysed using standard methods.

Unfiltered lake algal samples were also collected in sterilized sampling bottles. Algal samples were taken from 10 to 20 cm below the surface from a local tank in Nagpur[21]. They were immediately preserved in the cooler at 4°C.

S. quadricauda is a common freshwater species of the chlorophycean group, widely distributed in subtropical parts of India. It is a good pollution indicator species occurring in canals, rivers, lakes, reservoirs and other watersheds. The species is sensitive to polluted wastewater, and hence is selected for experimentation. The experimental alga (*S. quadricauda*) was identified using an Olympus microscope BX51 (Olympus, Japan), at a magnification of 100 and 400×. The identification was confirmed by referring to the keys reported earlier[21–23]. *S. quadricauda* was identified and separated.

Isolated species of microalgae were enriched by inoculating 10 ml of algal sample in 50 ml sterilized standard BG-11 medium[24] and microalgae culture maintained axenically for four generations. The sub-culture was identified under microscope and culture was used for core analysis. The culture conditions and growth of organism were followed according to the method of Rana and Kumar[25].

Chlorophyll was estimated by dissolving the algal pellets in 80% acetone and was measured using a spectrophotometer[26]. Protein estimation was carried out using the method of Lowry *et al.*[27] and carbohydrate by anthrone reagent method[8].

Detoxification test was performed for wastewater concentrations (20%, 40%, 60%, 80% and 100%) of untreated, physically treated and biologically treated wastewater. Cultured *S. quadricauda* algae were added into different concentrations and incubated for 21 days. After completion of the incubation period, optical density (OD) was measured at 660 nm using a spectrophotometer to find biomass.

The physico-chemical characteristics of UTE, PCTE and BTE before application of *S. quadricauda* are shown

Table 1. Physico-chemical characteristics of herbal pharmaceutical wastewater before application of *Scenedesmus quadricauda*

Parameters*	Untreated effluent	Physico-chemically treated effluent	Biologically treated effluent
pH	3.9 to 4.0	6.4	6.9
Colour	Dark yellow	Light hay	Colourless
Total acidity/alkalinity	3000	326	161
Total SS	4915	637	20
Total solids	8512	1140	30
Chemical oxygen demand (COD)	23980	6266	190
Biochemical oxygen demand (BOD) (5 day at 20°C)	13430	2867	28
Sulphide as S^{-2}	48	22	05
Sulphates as SO_4^{-2}	85	40	12
Total phosphates as PO_4^{-2}	270	110	65
Total nitrogen as N	444	136	-
Oil and grease	161	34	06
Sodium	210	118	08
Potassium	134	59	04
Heavy metals			
Iron	65.6500	16.2000	01.200
Copper	01.1595	00.5400	00.2400
Manganese	07.4400	01.4600	00.4612
Nickel	01.6210	00.6370	00.4420
Zinc	00.5955	00.2600	00.1200
Chromium	00.5835	00.1280	00.0186
Lead	03.5445	01.6730	00.0558
Cadmium	00.2600	00.1190	Nil
Selenium	00.5470	00.1390	Nil
Arsenic	00.0062	00.0018	Nil

*All values are expressed in mg l^{-1}, except pH and colour.

Table 2. Effect of pharmaceutical effluent on pigments and metabolites of *S. quadricauda*

Effluent concentration (%)	Chlorophyll	Protein	Carbohydrate
Control (no effluent)	0.010	20	30
PCTE			
10	0.112	185	235
20	0.080	130	180
40	–	–	–
60	–	–	–
80	–	–	–
100	–	–	–
BTE			
10	0.245	178	224
20	0.330	210	335
40	0.412	260	390
60	0.470	310	432
80	0.518	360	457
100	0.580	410	518

Protein and carbohydrate measured in (μg/100 mg fresh weight); chlorophyll measured in mg/g.

in Table 1. pH values were recorded as 3.9 to 4.0, 6.4 and 6.9 in UTE, PCTE and BTE respectively. The effect of different concentrations of herbal pharmaceutical wastewater on the synthesis of pigment and metabolites in terms of chlorophyll, proteins and carbohydrates is shown in Table 2. Values of these pigments and metabolites were reduced in PCTE. However, chlorophyll, proteins and carbohydrates were increased in BTE up to 15 days.

The results indicate that *S. quadricauda* is able to tolerate and sustain PCTE at 10% and 20% concentrations by synthesis of chlorophyll and cell metabolites. The degree of inhibition/death of algae starts at 40% concentration onwards. Likewise, for BTE, the synthesis of chlorophyll and cell metabolite production were uniformly high. In order to promote growth in higher concentration of PCTE (40%, 60%, 80% and 100%) and untreated wastewater, a detoxification test was performed and results are shown in Table 3. The growth characteristics of algae after 3, 6, 9, 12, 15, 18 and 21 days of incubation in PCTE and BTE are shown in Figure 2 *a* and *b* respectively.

The growth rate of *S. quadricauda* in control sample of PCTE and BTE was found to be low throughout 21 days of incubation. Similar findings were reported by Chevalier and de la Noue[28]. In their study, growth rates of algae were found to be lower in corresponding free-cell cultures. The growth rate of *S. quadricauda* in PCTE gradually increased throughout 21 days of incubation in 10%, 20% and 30% dilution. No growth of *S. quadricauda* was found in 40–100% dilution of PCTE (Figure 3). In 40% concentrated PCTE effluent, marginal but linear increase in growth of *S. quadricauda* was noticed. Whereas, in

Table 3. Growth rate of *S. quadricauda* in PCTE and BTE

	Days of incubation						
PCTE (%)	3	6	9	12	15	18	21
10	0.6	0.7	1	1.17	1.18	1.2	1.2
20	0.4	0.5	0.7	0.8	0.9	0.97	0.86
40–100	0	0	0	0	0	0	0
Control	0.26	0.28	0.3	0.29	0.28	0.27	0.26
BTE (%)	3	6	9	12	15	18	21
10	0.6	0.7	0.85	1.0	1.1	0.9	0.8
20	0.7	1.0	1.1	1.2	1.25	1.15	1.0
40	1.0	1.3	1.5	1.58	1.6	1.55	1.45
60	1.18	1.45	1.62	1.65	1.8	1.63	1.6
80	1.55	1.65	1.8	2.0	2.1	1.9	1.7
100	1.95	2.15	2.35	2.45	2.45	2.35	2.4
Control	0.1	0.15	0.2	0.25	0.1	0.1	0.1

*Growth was measured at 660 nm; initial OD of culture was between 0.1 and 0.2.

Table 4. Physico-chemical characteristics of herbal pharmaceutical wastewater after application of *S. quadricauda*

Parameters*	PCTE	BTE
pH	6.7	7.0
Total acidity/alkalinity	200	152
Total SS	234	9
Total solids	867	17
COD	2078	98
BOD (5 day at 20°C)	1345	13
Sulphide as S^{-2}	18	01
Sulphates as SO_4^{-2}	21	6
Total phosphates as PO_4^{-2}	93	50
Total nitrogen as N	136	–
Oil and grease	11	01
Sodium	123	05
Potassium	44	01
Heavy metals		
Iron	10.1300	00.9200
Copper	00.3640	00.1278
Manganese	00.9785	00.1942
Nickel	00.3561	00.2546
Zinc	00.1821	00.0548
Chromium	00.09445	00.0067
Lead	00.5312	00.0139
Cadmium	00.0190	Nil
Selenium	00.0098	Nil
Arsenic	00.0004	Nil

*All values are expressed in mg l^{-1}, except pH.

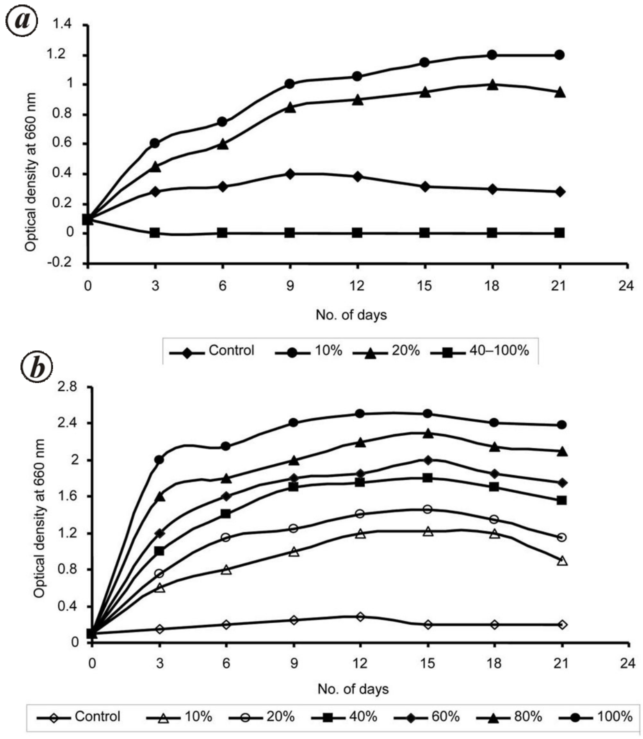

Figure 2. Growth rate of *Scenedesmus quadricauda* at different concentrations of (***a***) physico-chemically treated wastewater and (***b***) biologically treated wastewater.

earlier studies PCTE reduced the toxicity of herbal pharmaceutical wastewater by 25% using zooplankton *Ceriodaphnia dubia*[29]. The maximum growth rate of *S. quadricauda* in BTE increased up to 15 days of incubation. On 18th and 21st day of incubation, the growth rate of algae decreased slowly. The physico-chemical characteristics of PCTE and BTE-treated wastewater after application of *S. quadricauda* are shown in Table 4. pH values of 6.7 and 7.0 were recorded for PCTE and BTE respectively. The physical–chemical characteristics were reduced after the application of *S. quadricauda* in PCTE and BTE. Toxicity of PCTE and BTE was minimized on using *S. quadricauda.* Some workers have found similar results of reducing values of nitrogen and phosphorus in wastewater using *Scenedesmus* algae[28]. In the present study, the survival rate of *S. quadricauda* increased due to utilization of nutrients from the PCTE and BTE. In a similar study, Awasthi and Rai[30] also reported that *S. quadricauda* was used to remove toxicity of nickel, zinc and cadmium on major nutrient nitrate (NO_3^-) uptake.

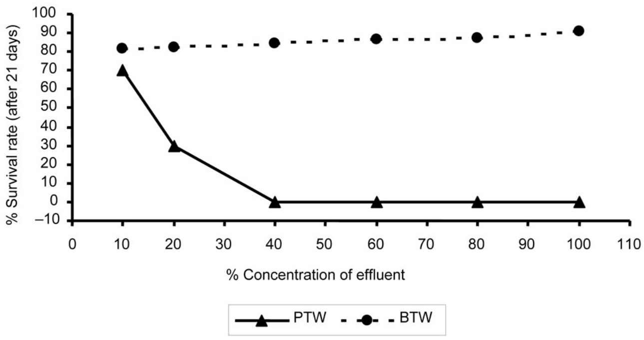

Figure 3. Percentage survival rate of *S. quadricauda* to different concentrations of herbal pharmaceutical wastewater. PTW, Physico-chemically treated wastewater; BTW, Biologically treated wastewater.

Further, the authors studied comparative metal–nutrient interaction in free and immobilized states of *Scenedesmus quadricauda* cells.

The present study leads to the conclusion that algae can remove toxic substances by absorption, accumulation, extracellular secretion and enzymatic degradation, and can slowly utilize the herbal pharmaceutical wastewater as nutrient source and make it available for reuse. If a suitable algae like *S. quadricauda* is applied to natural discharge of pharmaceutical wastewater, it will help in reducing the toxicity and facilitate recycling and reutilization of polluted water. Thus it will help in conserving our precious water. Differential tolerance of this microalga to the effluents shows that there is great scope for this algal treatment of industrial wastewater.

1. Rajashekharan, P. E., World of science. In *Herbal Medicine; Employment News*, Ministry of Information and Broadcasting, Government of India, 21–27 September 2002.
2. Pushpangadan, P., Ravi, K. and Santosh, V., *Conservation and Economic Evaluation of Biodiversity*, Oxford and IBH Publishing Co Pvt Ltd, New Delhi, 1997, 1st edn.
3. UNESCO, Water resources systems planning and management, 2005.
4. Nandy, T., Kaul, S. N. and Szpyrcowicz, L., Treatment of herbal pharmaceutical wastewater with energy recovery. *Int. J. Environ. Stud*., 1998, **54**, 83–105.
5. Oswald, W. J. and Gotaas, H. B., Photosynthesis in sewage treatment. *Trans. Am. Soc. Civ. Eng*., 1957, **122**, 73–105.
6. Fulke, A. B. *et al.*, Bio-mitigation of CO_2, calcite formation and simultaneous biodiesel precursors production using *Chlorella* sp. *Bioresour. Technol*., 2010, **101**, 8473–8476.
7. Fulke, A. B., Chambhare, K. Y., Sangolkar, L. N., Giripunje, M. D., Krishnamurthi, K., Juwarkar, A. A. and Chakrabarti, T., Potential of wastewater grown algae for biodiesel production and CO_2 sequestration. *Afr. J. Biotechnol*., 2013, **20**, 2939–2948.
8. Herbert, D., Phillips, P. J. and Strange, R. E., Chemical analysis of microbial cells. In *Methods in Microbiology* (eds Norris, J. R. and Ribbons, D. W.), Academic Press, New York, 1977, pp. 210–344.
9. Hoffman, J. P., Wastewater treatment with suspended and non suspended algae. *J. Phycol.*, 1998, **34**, 757–763.
10. Wang, H., Zhang, W., Chen, L., Wang, J. and Liu, T., The contamination and control of biological pollutants in mass cultivation of microalgae; doi.org/10.1016/j.biortech.2012.10.158.
11. Park, J. B. K., Craggs, R. J. and Shilton, A. N., Wastewater treatment high rate algal ponds for biofuel production. *Bioresour. Technol*., 2011, **102**, 35–42.
12. Omar, H. H., Bioremoval of zinc ions by *Scenedesmus obliquus* and *Scenedesmus quadricauda* and its effect on growth and metabolism. *Int. Biodeter. Biodegr*., 2002, **50**, 95–100.
13. Brennan, L. and Owende, P., Biofuels from microalgae – a review of technologies for production, processing, and extractions of biofuels and co-products. *Renew. Sustain. Energy Rev*., 2010, **14**, 557–577.
14. de la Nouë, J., Laliberte, G. and Proulx, D., Algae and wastewater. *J. Appl. Phycol*., 1992, **4**, 247–254.
15. Huntle, M. E., Nonomura, A. M. and de la Nouë, J., Algal culture systems. In *Biotreatment of Agricultural Wastewater* (ed. Huntley, M. E.), CRC Press, Boca Raton, Florida, 1989, pp. 111–130.
16. Lincoln, E. P. and Earle, J. F. K., Wastewater treatment with microalgae. In *Introduction to Applied Phycology* (ed. Akatsuka, I.), Academic Publishing, The Hague, The Netherlands, 1990, pp. 429–446.
17. Nijhawan, A., Labhasetwar, P., Jain, P. and Rahate, M., Public consultation on artificial aquifer recharge using treated municipal wastewater. *Resour., Conserv. Recycling*, 2013, **70**, 20–24.
18. USEPA, Algal assay procedure: bottle tests, National Eutrophication Research Programme, 1974.
19. Yu, R. Q. and Wang, W. X., Biokinetics of cadmium, selenium and zinc in freshwater alga *Scenedesmus obliquus* under different phosphorus and nitrogen conditions and metal. *Environ. Pollut*., 2004, **129**, 443–456.
20. Franson, M. A. H., Eaton, A. D., Clesceri, L. S. and Greenberg, A. E., Standard methods for the examination of water and wastewater. APHA, AWWA, WEF, Washington, DC, USA, 1998, 20th edn.
21. Standard methods for the examination of water and wastewater. APHA, AWWA and WEF, USA, 2005, 21st edn.
22. Prescott, G. W., *How to Know Freshwater Algae*, Wm. C. Brown Company Publishers, Dubuque, Iowa, 1978.
23. Ward, H. B. and Whipple, G. C., *Freshwater Biology*, John Wiley, USA, 1966.
24. Rippka, R., Deruelles, J., Waterbury, J., Herdman, M. and Stanier, R., Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol*., 1979, **111**, 1–61.
25. Rana, B. C. and Kumar, H. D., US Environmental Protection Agency, Oregon, 1974, vol. 13, pp. 67–83.
26. Mackinney, G., Absorption of light by chlorophyll solution. *J. Biol. Chem*., 1941, **140**, 315–322.
27. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein measurement with folin phenol reagent. *J. Biol. Chem*., 1951, **193**, 265–275.
28. Chevalier, P. and de la Nouë, J., Wastewater nutrient removal with microalgae immobilized in carrageenan. *Enzyme Microb. Technol*., 1985, **7**, 621–624.
29. Sitre, S., Dhadse, S. and Satyanarayan, S., Toxicity of herbal pharmaceutical wastewater to a freshwater crustacean *Ceriodaphnia dubia*. *Bull. Environ. Contam. Toxicol.*, 2009, **82**, 275–279.
30. Awasthi, M. and Rai, C., Toxicity of nickel, zinc and cadmium to nitrate uptake in free and immobilized cells of *Scenedesmus quadricauda*. *Ecotoxicol. Environ. Safety*, 2005, **61**, 268–272.

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