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Biosorption of Neodymium by Selected Photoautotrophic and Heterotrophic Species

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Abstract

In a search for alternative methods for isolation of rare earth elements from diluted solutions, a possibility of their preconcentration on biomass of mainly phototrophic species, that can be easily cultivated in open or closed systems, was investigated in this study. A screening of 19 biological species, consisting of 17 microalgae, one yeast and one moss, in regard to the biosorption of neodymium (Nd) on their lyophilized biomass was performed. Maximum sorption capacity (q_M) was determined after incubation for 3 h at room temperature and centrifugation through measuring the Nd content in the supernatant via a spectrophotometric assay. The obtained q_M values ranged from 0.26 mmol*g^{-1} for *Chlamydomonas reinhardtii* to 0.48 mmol*g^{-1} for *Calothrix brevissima* and up to 0.74 mmol*g^{-1} for *Physcomitrella patens*. Kinetic measurements show the reaching of q_M after 30 min for *C. reinhardtii* and 180 min for *P. patens*. Based on titration studies the proton exchange capacity was found to be 0.39 mmol*g^{-1} for *C. reinhardtii* and 0.44 mmol*g^{-1} for *P. patens* leading to a conclusion that proton exchange was mainly responsible for the biosorption mechanism. To our knowledge it is the first report of screening regarding such a wide scope of microalgae for a sorption of Nd.

Keywords: Biosorption; Microalgae; Lyophilized biomass; Recovery of Nd from highly diluted solutions; Mechanism

Introduction

Rare earth elements (REEs) consist of the lanthanides plus yttrium and scandium. Global demand for REEs in 2015 was estimated to 210,000 tons [1]. These elements are chemically very similar and randomly distributed in nature. Hence, their production is difficult and challenging due to many byproducts such as thorium, uranium, heavy metals and acidic waste waters [2]. Moreover, they can be found together in bulky rocks, so their separation is sophisticated. The classical exploitation of REE sources currently pollutes nature heavily. Due to that and the fact that around 97% of the worldwide need of REEs are provided by China [3], making REE-consuming industries excessively dependent on rationing and the rising world market prizes, there is a great interest in alternative and environmentally friendly methods for delivering these elements. One important representative of the REEs is neodymium (Nd), with its applications ranging from auto catalysts, petroleum refining, hard drives in laptops, headphones, hybrid engines up to permanent magnets in wind turbines [1,4].

The idea of binding metal ions from aqueous solutions via biosorption is not new. There are several methods reported in literature and applications for biosorption of heavy metals by various microorganisms such as bacteria, yeasts, fungi and algae [5-8]. During the last decade, REE biosorption and bioaccumulation by plant biomaterial became of increased interest [9-11], where so called "hyperaccumulators" such as *Pronephrium simplex*, *Sargassum sp.* and *Dicropteris dichotoma* were employed. Also microalgae were involved into this research field [12-14] with special emphasis on biosorption of REEs from acidic aqueous solutions. Microalgae are an interesting group of organisms for such research since they have numerous members and most of them can be produced, due to their high productivity in comparison to land plants, in high amounts under relatively simple conditions.

Our work aims to develop a potent method for recovery of Nd from highly diluted sources. In order to reach this aim, primarily, species have to be identified, that can be produced easily and bind Nd with high efficiency and preferably selectively with respect to heavy metals. In this paper we present a broad algal screening concerning Nd uptake from defined solutions and the first studies aiming at the elucidation of the binding mechanism of Nd.

Materials and Methods

Instrumentation and chemicals

Ultraviolet/Visible (UV/Vis) absorption spectra were recorded using a spectrophotometer (SPECORD 205, Analytik Jena, Germany) and a multilabel reader (EnSpire 2300, Perkin Elmer, USA). For measuring of pH-values, a pH-meter (pH 510, EUTECH Instruments, Germany) was used in combination with a micro electrode (InLab Micro, Mettler Toledo, USA). Biomass was centrifuged from culture medium using a Contifuge (Stratos, Heraeus, Germany). Centrifugation steps during the experiments were performed utilizing a lab centrifuge (5810R, Eppendorf, Germany). Lyophilized biomass was prepared by using a freeze dryer (Alpha 1-2, Christ, Germany). Additional determination of neodymium contents was performed via inductively coupled plasma atomic emission spectroscopy (ICP-AES, CIROS CCD, Spectro, Germany) at the Institute of Chemical Reaction Engineering, Friedrich-Alexander University of Erlangen-Nürnberg, Germany.

Neodymium (III) was investigated as neodymium (III) nitrate hexahydrate (99.9%, Aldrich, USA) and xylenol orange disodium salt (XO; Fluka, Germany) was used for UV/Vis-spectroscopic assay. ICP-

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AES machine was calibrated using an Nd^{3+}-ICP standard solution with 1000 $mg^{*}l^{-1}$ in diluted nitric acid (VWR, USA).

Biomass material and cultivation

Nineteen species included in the screening were either available in the in-house algae library or ordered from the Culture Collection of Algae at Goettingen University (SAG), Germany. In-house algae library contains mostly algae strains originating from SAG, but also from Chlamydomonas Research Center at the University of Minnesota (CC), Microbial Culture Collection at National Institute of Environmental Studies (NIES), ATCC and International Moss Stock Center at Freiburg University (IMSC).

In order to obtain enough biomass for following experiments, different microalgae (*Arthronema africanum, Arthrospira platensis, Calothrix brevissima, Chlamydomonas reinhardtii, Chlorella sorokiniana, Euglena gracilis, Euglena mutabilis, Euglena stellata, Euglena viridis, Galdieria sulphuraria, Lyngbya taylorii, Nostoc ellipsosporum, Nostoc punctiform, Porphyridium purpureum, Prymnesium saltans, Schizochytrium limacinum, Tetraselmis chuii*), yeast (*Saccharomyces cerevisiae*) and moss (*Physcomitrella patens)* were cultivated either in a well-established photobioreactor screening module (PSM) [15], a 25 l loop photobioreactor, developed at the Institute of Biotechnology, Department of Bioprocess Engineering, TU Berlin [16] or in 2 l Erlenmeyer flasks. For investigation of the sorption mechanism, the green alga *Chlamydomonas reinhardtii* (CC-125) and the moss *Physcomitrella patens* (IMSC 40001) were cultivated according to Heining et al. [15]. All other species were obtained from other works performed at the Institute of Bioprocess Engineering at the University Erlangen-Nürnberg (for conditions see supporting information, SI).

Preparation of stock solutions

A Nd^{3+} stock solution was prepared by dissolving Nd $(NO_3)_3$ x 6 H_2O (4.82 g) in 1 l ultrapure water resulting in a final concentration of 10 mM of Nd^{3+}. The concentration of Nd^{3+} was monitored using ICP-AES. Working solutions used for sorption experiments and calibration were prepared from the stock solution by appropriate dilutions with ultrapure water. XO stock solution was prepared by dissolving XO (71.65 mg) in 50 ml ultrapure water reaching a final concentration of 2 mM of XO. The XO concentration for calibration and unknown solutions was held constant at 200 µM by dilution of 100 µl 2 mM XO stock solution in 1 ml.

Quantification of Nd^{3+}

The UV/Vis-spectroscopic assay employing XO as a complexing agent, as described previously, was used with slight modifications for determination of unknown Nd^{3+} concentrations in aqueous solutions obtained from all experiments described below [17]. Absorption spectra from 300 nm to 700 nm were recorded. Calibration solutions ranging from 10 µM to 100 µM Nd^{3+} with 200 µM XO were prepared in a 96-well microtiter plate made from quartz (Hellma, Germany) and the absorbance of the Nd-XO-complex was measured at 572 nm. Absorbance values were constant for at least 3 h when stored in closed reaction vessels. The influence of sodium chloride was investigated through repetition of the calibration in presence of NaCl (1.71 mM and 17.1 mM).

Biosorption screening

To harvest the biomass the cultivation medium was centrifuged, resuspended in little deionized water, freezed at -80°C and lyophilized (vacuum, 24 hr). The dried biomass was homogenized using mortar and pestle prior further use and each experiment was performed in triplicates. Next, 2 ml of ultrapure water were added to the dry biomass (10-40 mg) in polycarbonate tubes (12 ml, Thermo Scientific, USA) and stirred at room temperature (rt) for 1 hr. After centrifugation (10 min, 10000×g, rt), the supernatants were removed and the residual water into the biomass was determined by weighing. Sorption experiments were performed through incubation with 2 ml of the Nd^{3+} stock solution (10 mM) under stirring at rt for 3 hr. After the incubation the biomass samples again were centrifuged (10 min, 10000×g), biomass removed and the neodymium content in the supernatant measured using spectroscopic method described above. The supernatants were prediluted 1:49 (100 µl of the supernatant plus 4,900 µl ultrapure water) in order to fit the working range of the assay and 200 µl of the prediluted solution were added to a solution containing 100 µl XO (2 mM) and 700 µl ultrapure water to reach a final XO concentration of 200 µM. The maximum sorption capacity (q_M) was determined based on the difference in the amounts of substance of Nd^{3+} in solution before (n_i) and after (n_f) incubation with the biomass:

$$qM = \frac{n_i - n_f}{m} = \frac{\left(c_i - c_f\right) * \mathrm{V}}{m} \quad (1)$$

With c_i=initial Nd^{3+}-concentration, c_f=final Nd^{3+}-concentration, V=volume and m=biomass dry weight. In order to check if the maximum sorption capacity was reached, each species was investigated twice with the same concentration of Nd^{3+} but two different amounts of biomass. Additionally, pH-values of the biomass supernatant were determined after washing with ultrapure water and after sorption.

Temporal and pH-behavior

Kinetic investigations utilizing lyophilized biomass without further treatment were conducted by measuring the Nd^{3+} content in the supernatant after centrifugation over 4 hr in duplicates. For generation of one data point, the biomass was centrifuged (5 min, 10,000×g) and the concentration of Nd^{3+} in the supernatants were measured as describe above. Predilution was carried out through addition of 10 µl of the supernatants to 490 µl of ultrapure water. Additionally, pH-value of the supernatant was determined for every data point.

Titrations of biomass

In order to determine the pK_a values and maximum proton exchange capacity of the biomass species titration experiments with the biomass (50 mg) of moss (*P. patens)* and green alga (*C. reinhardtii*) were conducted in duplicates. The native lyophilized biomass was fully protonated before titration through stirring in 10 ml of 0.1 M HCl-solution at rt for 2 hr. After centrifugation (10 min, 10,000×g) the biomass was washed with ultrapure water until the conductivity was lower than 20 $\mu S^{*}cm^{-1}$ to guarantee a defined starting point. Finally, the biomass was suspended in 10 ml of 1 mM NaCl-solution and titrated with 0.1 M NaOH or HCl by sequential addition of 500 µl of respective acid/base solution.

The pK_a values were determined using ProtoFit 2.1. Fit parameters were adjusted as 4 discrete binding sites, all amphoteric in a non-electrostatic adsorption behavior, calculated with the extended Debye-Huckel equation.

Swelling behavior

For assessing the swelling behavior of the lyophilized algal biomass, 50 mg of *C. reinhardtii* and *P. patens* in duplicates were incubated in

ultrapure water (10 ml) for 1 hr, centrifuged (10,000×g, 10 min), the supernatant was removed and the residual wet biomass was weighed.

Results and Discussion

Quantification of Nd^{3+}

For an easy quantification of unknown Nd concentrations, a spectrophotometric assay employing XO was established. Optimization of the XO-assay is shown here, where absorption spectra (300 nm to 700 nm) of different XO-concentrations (100 µM, 200 µM, 1000 µM; Figures 1-3 SI) in presence of Nd^{3+} (from 10 µm to 100 µM) were recorded. The free dye shows an absorption maximum at 430 nm, whereas the maximum of the Nd^{3+}-XO-complex lays at 580 nm, hence they are separated adequately for quantifying Nd^{3+} in solution. When measuring with 100 µM XO, the upper limit of the Nd^{3+} concentration is limited to approximately 70 µM due to the fact, that the Nd^{3+}-XO-complex exists at a 1 : 1 ratio of both molecules [18] and XO should be in excess. Therefore, XO was employed in concentrations of 200 µM and 1000 µM for Nd^{3+}-concentrations between 10 µM and 100 µM. The use of 1000 µM XO (Figure 2) shows decreased absorbances for the XO-Nd^{3+}-complex at 580 nm when compared to XO of 100 µM due to broadening of the absorption peak of XO. For this reason a XO-concentration of 200 µM (Figure 3) was selected for the assay.

The calibration curve (Figure 1) prepared with Nd^{3+} concentrations ranging from 10 µM up to 100 µM in presence of 200 µM XO results in two different linear slopes indicating possible formation of two different complexes. This interpretation is supported by findings of Budesinsky et al. [18] who reported various extinction coefficient for different metal-to-XO-ratio resulting in higher signals for a 1 : 1 ratio of metal ions to XO that could explain a higher slope for a concentration between 60 µM and 100 µM Nd^{3+} and 200 µM XO. For quantifications of Nd^{3+}-concentrations in sorption experiments the calibration between 10 µM and 50 µM Nd^{3+} was used and samples to be analyzed were diluted adequately to fit the working range.

Biosorption screening

The maximum Nd^{3+}-uptake of 19 species including: 9 green, 2 red and one heterotrophic microalgae, 5 cyanobacteria, one moss and one yeast, were investigated in this study. The results are shown in Figure 2. The species showed very different maximum sorption capacities

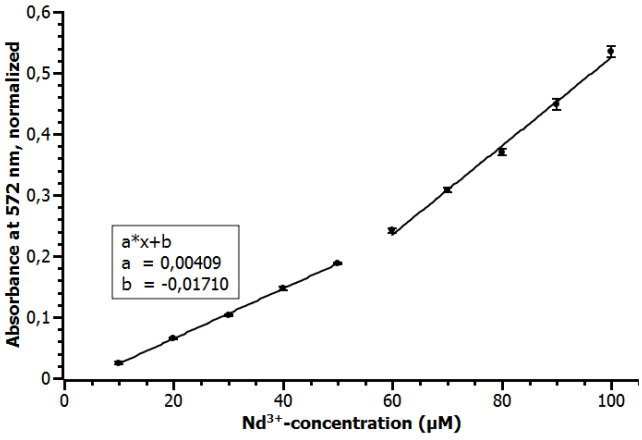

Figure 1: Calibration curve of Nd^{3+} with XO (200 µM), normalized against XO-blank. Each concentration was performed in triplicates. Slope was calculated for Nd^{3+}-concentrations between 10 µM and 50 µM.

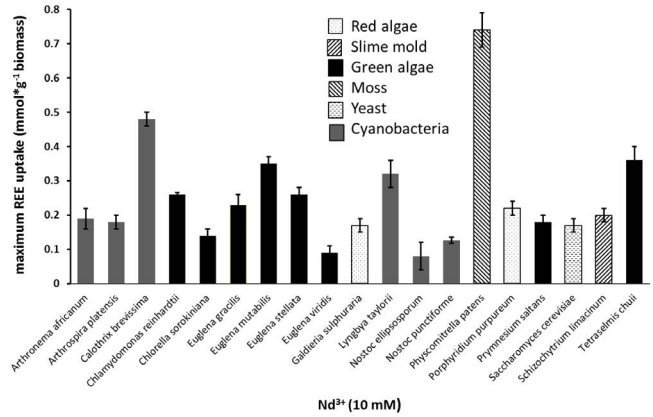

Figure 2: Biosorption screening regarding Nd^{3+} (initial concentration: 10 mM) including different microalgae, but also a moss and a yeast.

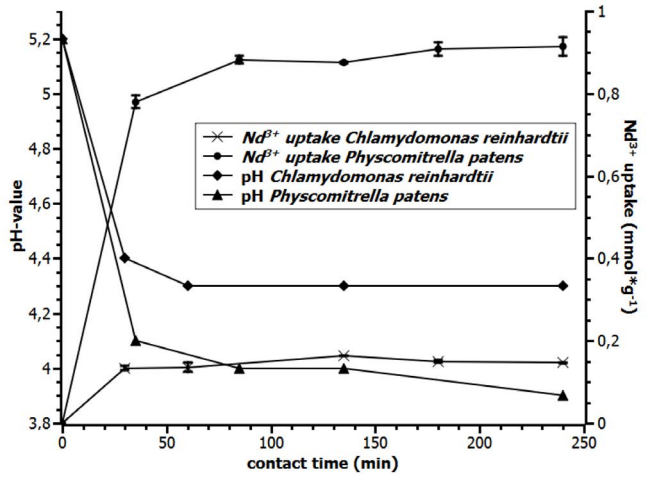

Figure 3: Temporal and pH-behavior of *C. reinhardtii* and *P. patens* over 4 hr. Starting pH-value is that of Nd^{3+} stock solution (pH 5.2).

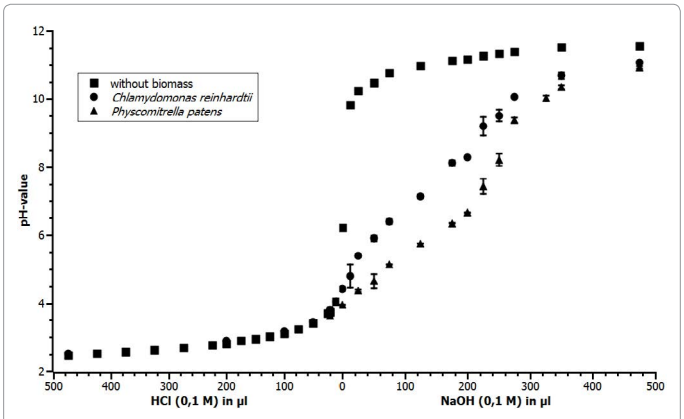

Figure 4: Titration curve of protonated biomass (50 mg respectively) of *C. reinhardtii* and *P. patens*. Each experiment was performed twice.

of Nd^{3+} ranging from 0.08 mmol*g^{-1} dry biomass (*N. ellipsosporum*) to 0.74 mmol*g^{-1} dry biomass (*P. patens*). Within the microalgae the highest metal-uptake was registered for *C. brevissima* (0.48 mmol*g^{-1} dry biomass), *T. chuii* (0.36 mmol*g^{-1} dry biomass), *E. mutabilis* (0.35

mmol*g^{-1} dry biomass) and *L. taylorii* (0.32 mmol*g^{-1} dry biomass). These results correspond well with previously published biosorption experiments with the brown macroalga *Sargassum sp.*, where the found sorption capacities ranged between 0.60 mmol*g^{-1} and 0.70 mmol*g^{-1} dry biomass for La, Nd, Eu and Gd [10].

Examination of sorption mechanisms

In order to elucidate the mechanism of the REE-uptake, kinetic, pH- and titration measurements with the according biomass were performed. Results of the temporal sorption and pH-behavior can be seen in Figure 3. Starting from the pH-value of the Nd^{3+} stock solution (pH 5.2) a pH drop to 4.3 for *C. reinhardtii* and to 3.9 for *P. patens* were observed after 4 h. When repeated without Nd^{3+} in the solution (incubation of 30 mg dry biomass in 2 ml ultrapure water) no pH change for *C. reinhardtii* could be registered and an increase from pH 6.0 to 7.0 was observed for *P. patens*. Hence, it could be followed that pH drops for *C. reinhardtii* and *P. patens* were induced exclusively by the metal-uptake resulting in a proton release into the medium suggesting the proton exchange mechanism.

During the same experiments the maximum Nd^{3+}-uptake was reached already after 30 min for *C. reinhardtii* whereas for *P. patens* it could be reached only after around 90 min. Simultaneously, the pH drop correspond well with the time of reaching the maximum sorption capacity of Nd^{3+} also supporting the thesis that ion exchange was a predominant mechanism of the metal-uptake. In comparison, reaching a steady state of the REE sorption was previously reported to range from 0.5 hr up to 24 hr for various species [8,19,20]. REE sorption at moss biomass has not been reported previously in peer reviewed literature, however Sari et al. [21] investigated biosorption behavior of Cd (II) and Cr (III) at other moss species - *Hylocomium splendens* - where they found that the maximum of biosorption was reached after 90 min. This fits well with results reported here.

Titration studies were performed with *C. reinhardtii* and *P. patens* (Figure 4). Proton exchange capacities (PEC), maximum Nd^{3+}-uptake capacities (MUC) and pK_a values for *C. reinhardtii* and *P. patens*, calculated from titration curves, are shown in Table 1. Taking the last titration data point PEC could be calculated as 0.39 mmol*g^{-1} for *C. reinhardtii* and 0.44 mmol*g^{-1} for *P. patens*. These PK_a values can be compared with those of functional groups of glycoproteins. For *C. reinhardtii* pK_a=4.4 can be aligned to carboxyl groups in asparagine or glutamine and pK_a=8.9 corresponds to α-amino groups [22].

The cell wall of *C. reinhardtii* contains several layers of hydroxyproline-rich glycoproteins [23] bearing many free hydroxyl groups that are potential candidates for metal ion binding. Nieboer and Richardson [24] classified metal ions due to their covalent or ionic binding character in metal-ion/ligand complexes. Following their definition, REEs are added to Class A metals, which prefer O-bearing donor molecules. Hence, it can be concluded that a main binding mechanism is proton-to-Nd^{3+}-exchange at hydroxyl groups. Taking into account that Nd^{3+} possesses 3 positive charges, it can be proposed, that one Nd^{3+} occupies more than one binding site. The proton-to-Nd^{3+} ratio for *C. reinhardtii* is 1.5, meaning 2 Nd^{3+} ions releases 6 protons in the hypothesis of an exclusive proton exchange mechanism. For *P. patens* proton-to-Nd^{3+} ratio is 0.6 indicating a combination of more sorption mechanisms such as electrostatic interactions, precipitation and complexation. Martins et al. [25] postulated a competing process between protons and metal ions while sorption of Cd (II) and Zn (II) among the aquatic moss *Fontinalis antipyretica*.

	C. reinhardtii	*P. patens*
proton exchange capacity (mmol*g^{-1})	0.39	0.44
maximum Nd^{3+} sorption capacity (mmol*g^{-1})	0.26	0.74
pK_a1	4.4	4.0
pK_a2	7.5	5.4
pK_a3	8.9	6.8
pK_a4	11.0	9.5

Table 1: Chemical properties of biomass binding sites, generated by titration of dry biomass.

Outlook

Experiments shown here will be adapted to Europium, Cerium and Dysprosium in the near future. Furthermore, we will investigate selective binding of REEs with respect to heavy metals. With the best applicable microalgal species, we want to build up a minor cartridge for REE production from high diluted aqueous solutions.

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References

1. Humphries M (2013) Rare Earth Elements: The Global Supply Chain. CRS Report for Congress R41347: 1-27.
2. Öko-Institut e.V. (2011) Hintergrundpapier Seltene Erden.
3. Deutscher Bundestag (2011) Fairen Rohstoffhandel sichern - Handel mit Seltenen Erden offenhalten.
4. Du X, Graedel TE (2011) Global in-use stocks of the rare Earth elements: a first estimate. Environ Sci Technol 45: 4096-4101.
5. Davis TA, Volesky B, Mucci A (2003) A review of the biochemistry of heavy metal biosorption by brown algae. Water Res 37: 4311-4330.
6. Veglio' F, Beolchini F (1997) Removal of metals by biosorption: a review. Hydrometallurgy 44: 301-316.
7. Volesky B, Holan ZR (1995) Biosorption of heavy metals. Biotechnol Prog 11: 235-250.
8. Wilke A, Buchholz R, Bunke G (2006) Selective biosorption of heavy metals by algae. Environmental Biotechnology 2: 47-56.
9. Lai Y, Wang Q, Yang L, Huang B (2006) Subcellular distribution of rare earth elements and characterization of their binding species in a newly discovered hyperaccumulator Pronephrium simplex. Talanta 70: 26-31.
10. Oliveira R C, Garcia Jr. O (2009) Study of Biosorption of Rare Earth Metals (La, Nd, Eu, Gd) by Sargassum sp. Biomass in Batch Systems: Physicochemical Evaluation of Kinetics and Adsorption Models. Advanced Materials Research 71-73: 605-608.
11. Shan X, Wang H, Zhang S, Zhou H, Zheng Y, et al. (2003) Accumulation and uptake of light rare earth elements in a hyperaccumulator Dicropteris dichotoma. Plant Science 165: 1343-1353.
12. Birungi ZS, Chirwa EM (2014) The kinetics of uptake and recovery of lanthanum using freshwater algae as biosorbents: comparative analysis. Bioresour Technol 160: 43-51.
13. Minoda A, Sawada H, Suzuki S, Miyashita S, Inagaki K, et al. (2015) Recovery of rare earth elements from the sulfothermophilic red alga Galdieria sulphuraria using aqueous acid. Appl Microbiol Biotechnol 99: 1513-1519.
14. Palmieri M C, Garcia O, Melnikov P (2000) Neodymium biosorption from acidic solutions in batch system. Process Biochemistry 36: 441-444.
15. Heining M, Sutor A, Stute S C, Lindenberger C P, Buchholz R (2015) Internal illumination of photobioreactors via wireless light emitters: a proof of concept. Journal of Applied Phycology 27: 59-66.
16. Walter C, Steinau T, Gerbsch N, Buchholz R (2003) Monoseptic cultivation of phototrophic microorganisms-development and scale-up of a photobioreactor system with thermal sterilization. Biomol Eng 20: 261-271.

17. Schmidt A (1967) Eine spektrophotometrische Simultanbestimmung von Thorium und Seltenen Erden mit Xylenolorange. Fresenius Zeitschrift für analytische Chemie 229: 293.

18. Budesinsky B, Bezdekova A (1962) Spektralphotometrische Studie der Reaktion von Gd 3+, Sm 3+, Dy 3+ und Yb 3+ mit Xylenolorange. Z analyt Chem 196: 172-177.

19. Diniz V, Volesky B (2005) Biosorption of La, Eu and Yb using Sargassum biomass. Water Res 39: 239-247.

20. Vijayaraghavan K, Sathishkumar M, Balasubramanian R (2010) Biosorption of Lanthanum, Cerium, Europium, and Ytterbium by a Brown Marine Alga, Turbinaria Conoides. Industrial & Engineering Chemistry Research 49: 4405-4411.

21. Sari A, Mendil D, Tuzen M, Soylak M (2008) Biosorption of Cd(II) and Cr(III) from aqueous solution by moss (Hylocomium splendens) biomass: Equilibrium, kinetic and thermodynamic studies. Chemical Engineering Journal 144: 1-9.

22. Kiefer E, Sigg L, Schosseler P (1997) Chemical and Spectroscopic Characterization of Algae Surfaces. Environmental science & technology 31: 759-764.

23. Voigt J (1988) The lithium-chloride-soluble cell-wall layers of Chlamydomonas reinhardii contain several immunologically related glycoproteins. Planta 173: 373-384.

24. Nieboer E, Richardson DH (1980) The replacement of the nondescript term 'heavy metals' by a biologically and chemically significant classification of metal ions. Environmental Pollution Series B, Chemical and Physical 1: 3-26.

25. Martins RJ, Pardo R, Boaventura RA (2004) Cadmium(II) and zinc(II) adsorption by the aquatic moss Fontinalis antipyretica: effect of temperature, pH and water hardness. Water Res 38: 693-699.