EXPLOITING *GALDIERIA SULPHURARIA* FOR THE BIO-RECOVERY OF RARE EARTH ELEMENTS (REES) FROM ELECTRONIC WASTE

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ABSTRACT: Modern technologies are often constituted by Rare Earth Elements, which are metallic elements belonging to the group of lanthanides, plus scandium and yttrium. These indispensable constituents are often challenging to acquire from natural resources and there is a clear need to develop efficient and eco-friendly recycling methods. In the present paper, the extremophilic microalga *G. sulphuraria* was used to explore its potentiality in recovering Rare Earth Elements (REEs). Living cells of *G. sulphuraria*, strain SAG 107.79, were first used to evaluate the response of the microalga to Cerium; data demonstrated the absence of significant metal effects on the physiology of the microalgae. Freeze-dried cells of *G. sulphuraria*, strain ACUF 427, were then used to recover REEs from spent fluorescent lamps (FL) luminophores, evaluating the effect of biosorbent dosage (0.5–5 mg/ml) and biosorption time (5–60 min). The best biosorption performances were achieved after 5 min with the lowest biosorbent dosage (0.5 mg/mL). The rapidity of the biosorption process and the low biosorbent dosage required confirmed this microalga as a promising material for creating an eco-sustainable protocol REEs reycling.

Keywords: Galdieria sulphuraria, recycling, REEs, biosorption

1. INTRODUCTION

Rare Earth Elements (REEs) are a group of metallic elements that include the lanthanides, plus scandium and yttrium. They are irreplaceable materials in numerous technologies, such as solar panels, batteries, fluorescent lamps, etc. The high popularity of technologies requiring REEs is causing these metals' increasing demand and price and most countries need to rely their REEs economy on the recycling of End-of-Life products (Binnemans et al., 2013).

Biosorption and bioaccumulation are new biological approaches, which exploit the potential of biological matter for extracting resources from e-waste (Lo et al., 2014). Bioaccumulation occurs when cells actively transport the metals inside the protoplast (Lo et al., 2014). Biosorption approaches regard the absorption of metal particles on the cell's surface through physiochemical interactions. Important roles are played by the functional groups on the cell surface, which can bind the metal ion based on their protonation state. The optimisation of physicochemical parameters, such as temperature, pH, biosorbent dosage, and contact time, is fundamental for obtaining the highest metal recovery (Lo et al., 2014).

The polyextremophilic *Galdieria sulphuraria* represents a good candidate for recovering heavy, precious and REEs from metal artificial solutions (Iovinella et al., 2023; Jalali et al., 2018; Sirakov et al., 2021). *G. sulphuraria* is an unicellular red algae thriving in geothermal sites, characterised by low pH

(0.5–3.0), high temperature (50°C–55°C), and vast amounts of heavy, precious, and rare earth metals.

The present paper aims to understand the potential application of *G. sulphuraria* for the biorecovery of REEs. The microalga physiological and transcriptomic response was first evaluated, using living cells of *G. sulphuraria*, strain SAG 107.79, and the rare earth element Cerium (Ce^{3+}). Because of a scale-up industrial process, *G. sulphuraria*, strain ACUF 427, freeze-dried biomass was used to recover REEs from spent fluorescent lamps (FL) luminophores. The biosorption efficiency of the biomass was evaluated by testing different contact times and biosorbent dosages to optimise the biorecovery activity.

2. MATERIAL AND METHODS

2.1 Microalgal stock culture and exposition tests

G. sulphuraria, strains SAG 107.79 and ACUF 427, were grown on the orbital shaker at 37ºC, and weekly refreshed with new medium until they reached the late logarithmic phase. Strain ACUF 427 biomass was harvested by centrifugation and the algal pellet was washed with Milli-Q water and freeze-dried (SP VirTis Benchmark).

A Ce^{3+} stock solution was prepared by dissolving 2 grams of $CeCl_3.H_2O$ (Alfa Aesar, USA) in 1 liter of Milli-Q water. The microalga biomass with a final concentration of 6 x 10^6 cells/mL was incubated in 50 mL flasks with Allen medium, pH 2.5, enriched with increasing concentrations of Ce^{3+} (10, 25, 50, 75, 100, 125 and 150 mg/L) and cultivated at 37º C for 14 days.

For the following tests, leachate from fluorescent lamps was prepared suspending 250 mg of fluorescent lamp powder in 500 mL sulfuric acid 2M (CARLO ERBA, 96%), and adjusting pH at 5.5. The leachate was then centrifuged and the supernatant was filtered and stored at 4°C for further chemical characterisation using ICP-MS. Biosorption tests were carried out at different times (5, 15, 30, and 60 min) and biosorbent dosages (0.5, 1, 2.5 and 5 mg/mL).

2.2 Growth rate and cell vitality monitoring

Samples were collected from each flask (Day 0, 3, 6, 10 and 14) to measure the cells/µl using a haemocytometer and a light microscope (Nikon Optiphot-2). The percentage of the viable cells was assessed via Trypan Blue (0.4% w/v) assay. After 5 minutes at room temperature, cultures were washed twice with Allen pH 2.5, and dead cells were measured using the haemocytometer and the optical microscope. The vitality of the cultures was assessed by applying the following formula:

$$\% \text{ of viable cells} = \frac{Number\ of\ viable\ cells}{Number\ of\ total\ cells} \times 100 \quad (1)$$

2.3 Transcriptomic analysis

Total RNA was isolated RNeasy Kit (Qiagen). RNA library preparation was performed at Novogene (UK) Company Limited (Cambridge). The barcoded fragments from the library preparation were run on the Illumina Novaseq 6000 (s4 flow cell). Transcripts quantifications were done using the software Salmon v 1.4.0, and the differential expression analysis were performed with R Sleuth package and transcripts with a FDR < 0.01 were considered as differentially expressed. The annotated genes were associated to the Gene Ontology (GO) terms and mapped to the main functional categories, biological process, molecular function and cellular component using using OmicsBox software v 1.4.11. Annotated and mapped transcripts were used for GO enrichment analysis and the GO term with an FDR value < 0.01 was considered significantly enriched.

2.4 Metals quantification by ICP-MS

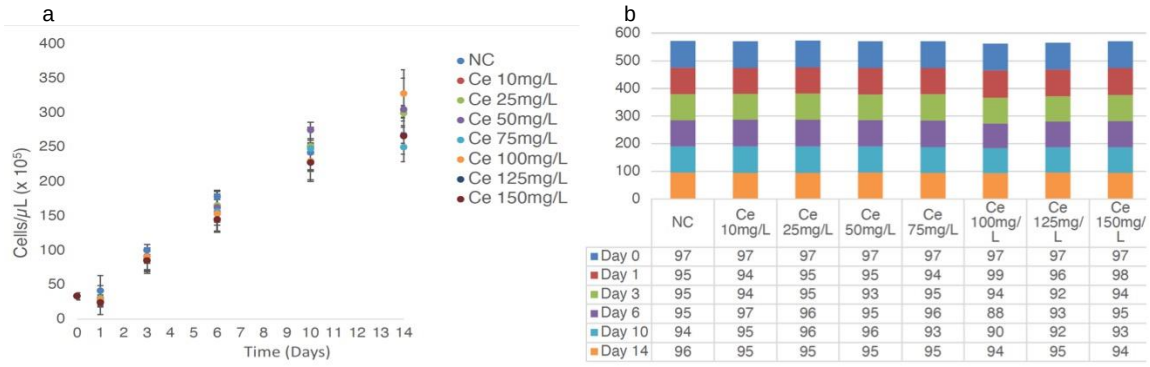
Samples were centrifuged and the pellets were mineralised with acqua regia (HNO_3:HCl = 1:3) and the metal concentrations were measured through ICP-MS (Aurora Bruker M90, Bremen, Germany). The evaluation of the metal uptake was conducted by measuring the total metal removed using the following formula:

$$\text{Total metal removed (µmol/g dm)} = (C_{biomass} \times V/M)/\text{metal molecolar weight} \quad (2)$$

where $C_{biomass}$ is the metal concentration measured in the biomass fraction; V is the volume of the test solutions; and M is microalgal biomass (g, dry matter).

3. RESULTS AND DISCUSSION

Growth rate is one of the parameters used to understand the effects of external factors on the state of health of microorganisms. *G. sulphuraria,* strain SAG107.79, could tolerate treatments with $Ce^{3+,}$ since no negative or positive growth rates were registered throughout the experiments, suggesting a null influence of Ce^{3+} on the growth performance of *G. sulphuraria* (Figure 1a). Data were confirmed by the cell vitality tests ($p < 0.05$; Figure 1b). These results were the first evidence of such a strong capacity of a microorganism to tolerate concentrations of Ce^{3+} ions up to 150mg/L, which counteracts the inhibitory effects demonstrated on Chlorophyceae and Cyanophyceae species (Goecke et al., 2017).



	NC	Ce 10mg/L	Ce 25mg/L	Ce 50mg/L	Ce 75mg/L	Ce 100mg/L	Ce 125mg/L	Ce 150mg/L
Day 0	97	97	97	97	97	97	97	97
Day 1	95	94	95	95	94	99	96	98
Day 3	95	94	95	93	95	94	92	94
Day 6	95	97	96	95	96	88	93	95
Day 10	94	95	96	96	93	90	92	93
Day 14	96	95	95	95	95	94	95	94

Figure 1. Growth rate (a) and percentage of *G. sulphuraria* viable cells (b), strain SAG 107.79, treated with increasing concentrations of $Ce3^{+}$. Data are shown as mean value (± S.D.; n = 3). Days marked with asterisk are significantly different from the control (Tukey test; p-value < 0.05).

To understand the physiological response of *G. sulphuraria* to Ce^{3+} and understand the molecular mechanisms involved in metal homeostasis, differentially expressed genes were characterised in response to acute metal application. As a result of the Ce^{3+} treatment, 887 transcripts were identified as differentially expressed (DE; FDR < 0.01), 459 of which were increased and 428 were decreased (Figure 2). An Enrichment analysis (Fisher's Exact Test) was performed to identify transcripts that were over-represented, identifying 44 GO terms belonging to the gene categories of cellular energy boost. The identified gene ontology terms belong to the three functional categories, biological process (BP), molecular function (MF), and cellular component (CC), in metal homeostasis (FDR < 0.05). Among the biological processes, the highest number of sequences was recorded for the oxidation-reduction process (GO:0055114), carboxylic acid (GO:0019752), carbohydrate (GO:0005975) and purine ribonucleotide metabolic processes (GO:0009150; Figure 2). Genes involved in carbohydrate metabolism were often identified in previous studies on the effects of heavy metals on microalgae (Olsson et al., 2015). The

activation of this metabolic pathway, regardless of the treatment analysed, suggests it as an occurring response of microorganisms to metal ions.

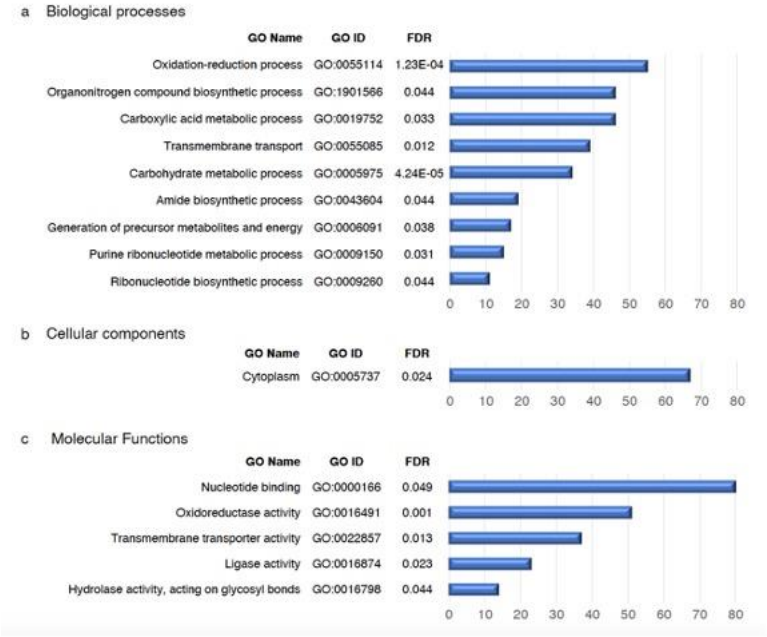


Figure 2. Distribution of enriched Gene Ontology (GO) terms belonging to the functional categories biological processes (a), cellular component (b) and molecular function (c) (FDR < 0.05).

To optimise the process because of a scale-up industrial application, different contact times and biosorbent dosages were applied to the process. From an economic perspective, these parameters must be carefully evaluated to significantly increase REE biorecovery (Balaram, 2019). When investigating the contact time as a variable parameter, the biosorbent was able to absorb the highest total amount of REEs after 5 min from the beginning of the experiment (Figure 3a). For the following assays, where variable biosorbent dosages were tested, the highest total metal amount was measured in samples where the lowest biosorbent concentration was applied (41.61 mg/g dm; Figure 3b). Our data demonstrated that this biosorbent could be successfully employed as the highest removal rate was already achieved after 5 min of contact time. The constant concentration of REEs measured in the biosorbent over the time points could be explained by the saturation of the binding sites on the cell wall (Sadovsky et al., 2016).

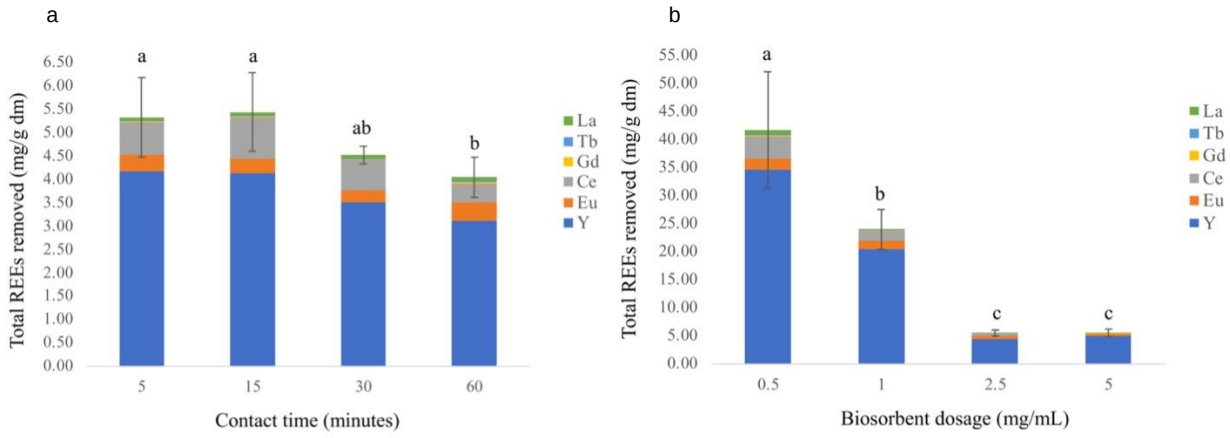


Figure 3. Total content of REEs in biosorbent samples at 5, 15, 30 and 60 of contact time (a) and 0.5, 1, 2.5 and 5 mg/mL of biosorbent dosage (b). Different colours indicate the individual REEs. Different letters indicate a

significant difference between the samples ($p < 0.05$).

4. CONCLUSIONS

G. sulphuraria is largely employed in a great variety of biotechnological applications. In the present paper, we first evaluated the physiological and transcriptomic response of living cells of *G. sulphuraria* to increasing concentrations of the rare earth element Ce^{3+}; then we employed freeze-dried *G. sulphuraria* biomass for the adsorption of luminophores-derived rare earth elements to establish it as new biosorbent material for the recycling of these metals. The first data in the present paper confirmed the natural capacity of *G. sulphuraria* to live in extremely metal-rich environments without suffering any toxic effects. The maximum ability to adsorb REEs was quickly achieved (5 min) by applying the lowest biosorbent dosage (0.5 mg/mL). The application of a few quantities of biosorbent and the rapid process demonstrated in this study make this microalga one of the best candidates for creating an eco-sustainable protocol for recycling REEs. The high capacity of *G. sulphuraria* freeze-dried biomass to recover a significant quantity of metal ions from the culture solution in a very short time makes this extremophilic microorganism one of the best candidates to achieve this purpose.

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