CEST 2021

17th International Conference on Environmental Science and Technology

Athens, Greece, 1 to 4 September 2021

Red Mud as a Secondary Source of Scarce Metals - Recovery using Red Microalgae

NÁHLÍK V.[1,4], ČÍŽKOVÁ M.[1], SİNGH A.[1], BİŠOVÁ K.[1], MEZRİCKY D.[2], RUCKİ M.[3], VÍTOVÁ M.[1,*]

[1]Laboratory of Cell Cycles of Algae, Centre Algatech, Institute of Microbiology of the Czech Academy of Sciences, Novohradská 237, 379 81 Třeboň, Czech Republic

[2]Institute of Medical and Pharmaceutical Biotechnology, IMC FH Krems, Piaristengasse 1, A-3500 Krems, Austria

[3]Laboratory of Predictive Toxicology, National Institute of Public Health, Šrobárova 48, 100 42 Prague, Czech Republic

[4]Faculty of Science, University of South Bohemia, Branišovská 1760, 370 05 České Budějovice, Czech Republic

*corresponding author:
e-mail: vitova@alga.cz

Abstract Red mud is a by-product of the production of alumina from bauxite ore. Less than 2% of the red mud produced is currently being reused. The red mud contains a number of residual elements, some at a considerable concentration. The red microalga *Galdieria sulphuraria* was used to test the accumulation of scarce metals from red mud. Algal cells were cultured autotrophically and mixotrophically in a liquid medium with an alternative addition of glycerol as a source of carbon. Red mud was added into the growth medium as the acidic extract (in 10% HNO_3). The growth of the cultures was monitored. The content of single scarce metals in the red mud extract and the biomass, was determined using ICP-MS. The most abundant element in red mud was Fe followed by Na and Al (53%, 17% and 12% respectively). The most abundant lanthanides were Ce, Y and La. The growth of cultures grown in the presence of red mud was comparable with the control. The red alga *Galdieria sulphuraria* can grow in the presence of red mud and accumulate scarce metals from it. The accumulation is more effective under the mixotrophic regime, shoing Y as the most accumulated lanthanide.

Keywords: red mud, scarce metals, rare earth elements, red algae, *Galdieria sulphuraria*

1. Introduction

The unicellular red alga *Galdieria sulphuraria* belongs to Cyanidophyceae, the class of primitive rhodophytes. These extremophilic microalgae are adapted to thermo-acidophilic growth conditions inhabiting hot sulfur springs and geothermal habitats (Gross et al., 1998). They thrive wide range of temperature up to 56 °C and pH values bellow pH 1 (Ciniglia et al., 2004; Reeb and Bhattacharya, 2010). *G. sulphuraria* shows distinguished metabolic versatility which allows, apart from an autotrophic growth, a mixotrophic and heterotrophic growth on variety of carbon sources (Gross and Schnarreberger, 1995; Oesterhelt et al., 2007). The growth was increased under the mixotrophic and heterotrophic regimes compared to autotrophy (Čížková et al., 2020a; López et al., 2019; Liu et al., 2021). The resistance of *Galdieria* against numerous toxic metals, rare earth elements (REEs; lanthanides), high salt and the adaptation to high temperature and low pH is quite unique among other eukaryotic algae and make it a promising adept for biotechnologies (Vítová et al., 2016; Čížková et al., 2020b; Hirooka et al., 2020).

Red mud (RM) as a by-product of alumina production contains high amount of residual metals including REEs. The use of microalgae for recovery of REEs was studied already (Čížková et al., 2019, 2020a; Minoda et al., 2015; Ramasamy et al., 2019).

The aim of this study was to examine the ability of the red alga *G. sulphuraria* to accumulate scarce metals from RM applied as the acidic extract. In order to examine bio-absorption capacity, *G. sulphuraria* was cultivated auto- and mixotrophically. As a comprehensive determination of the content of scarce metals accumulated in algal biomass, inductively coupled plasma mass spectrometry (ICP-MS) was used. The potential to use red algae for bio-recovery of scarce metals from red mud was evaluated.

2. Methods

2.1 *Experimental organism and culturing*

The unicellular red alga *Galdieria sulphuraria* 002 was obtained from Algal Collection of University "Federico II" of Naples, Italy (http://www.acuf.net/index.php?lang=en). Cultures were grown in photobioreactors in modified Galdieria-nutrient medium pH 3 (Vítová et al., 2016) with or without addition of 1% glycerol (added every 48 hr) at 40°C and the light intensity 500 µmol photons m^{-2} s^{-1} for 72 hr. Starting concentration of the inoculum was 0.1 mg L^{-1}. Red mud (Envirotis Holding zrt., Hungary) was extracted with 10% HNO_3 in the concentration 50 ug RM mL^{-1}. Algae were treated with 5% RM extract (v/v). The pH of the cultures was adjusted by NH_4OH to pH 3. The biomass was harvested by centrifugation and freeze-dried.

2.2 *Dry matter evaluation*

Dry matter was determined from 5 mL of algal suspension centrifuged at 3000 rpm for 5 min in dried and pre-weighed 5-mL test tubes. The pellet was dried at 105°C for 12 h and weighed on the analytical balance Sartorius TE214S-0CE.

2.3 *Quantitative analysis of metal content by ICP-MS*

Samples of red mud and algal biomass were digested with 67% HNO_3 and 30% H_2O_2 in a PTFE microwave oven at 250–600 W for 20 min. ICP-MS measurements were performed using an Elan DRC-e equipped with a concentric PTFE nebuliser and cyclonic spray chamber. Values were expressed as microgram per gram of dry matter (ug g^{-1} DM) (Goecke et al., 2015).

3. Results

3.1 *Composition of red mud*

The composition of RM was analyzed using ICP-MS. The most abundant element in RM was Fe followed by Na and Al (53%, 17% and 12% respectively). The acidic extract of RM (see Methods) was evaluated for the levels of REEs. The most abundant lanthanide was Ce, followed by Y and La (6106, 3900 and 3334 µg L^{-1}, respectively) (Tab. 1, column 1).

Table 1. Content of single rare earth elements in the red mud extract (RM) (µg L^{-1}) and in the biomass of *Galdieria sulphuraria* cultivated auto- and mixotrophically and treated by RM extract (G.s.+RM Auto/ Mixo) (µg g^{-1} DM).

REEs	**RM extract**	**G.s.+RM Auto**	**G.s.+RM Mixo**
	µg L^{-1}	**µg g^{-1} DM**	**µg g^{-1} DM**
Y	**3900**	**5,4**	**94,3**
La	**3334**	**0,4**	**0,4**
Ce	**6106**	**0,5**	**0,2**
Eu	**185**	**0,4**	**9,8**
Gd	**794**	**0,1**	**0,1**

3.2 *Algal growth in the presence of red mud*

Cultures of *G. sulphuraria* of the same initial concentration (1x10^6 cells L^{-1}) were cultivated auto- and mixotrophically in the liquid mineral medium with addition of 1% glycerol at continuous light. They were harvested after 3 days to obtain the biomass for elemental analysis. The RM extract was added to the final concentration 5%. The amount of produced biomass was monitored by dry matter determination. When compared with the control cultures (no RM added) the growth of cultures grown in the presence RM extract was comparable (Fig. 1). The autotrophic control reached the DM value 3.50 mg mL^{-1}, while the maximal DM value of RM treated culture was 3.95 mg mL^{-1}. The mixotrophic control grew to 4.06 mg mL^{-1} while the RM treated culture to 3.00 mg mL^{-1} showing the negative effect of high metal levels accumulated in the cells.

3.3 *Accumulation of rare earth elements in biomass*

To follow the accumulation of REEs by algal cells, the harvested biomass from above described experiments was analyzed by ICP-MS for the REEs content. Total amount of accumulated REEs differed with the metabolic regime used for cultivation in behalf of mixotrophic one (Fig. 2). The algal biomass from cultures grown in the presence of RM extract, contained Y as most abundant element (5.4 and 94.3 µg g^{-1} DM in auto- and mixotrophic, respectively) followed by Eu (Tab. 1, column 2, 3; respectively). Surprisingly, the accumulation fo Ce and La was negligible.

To summarize, the red alga *G. sulphuraria* 002 was able to grow both auto- and mixotrophically in the presence of RM extract and accumulate REEs from the extract into the cells. The most successful accumulator was mixotrophic *G. sulphuraria* and the most abundant lanthanide accumulated in the biomass was yttrium.

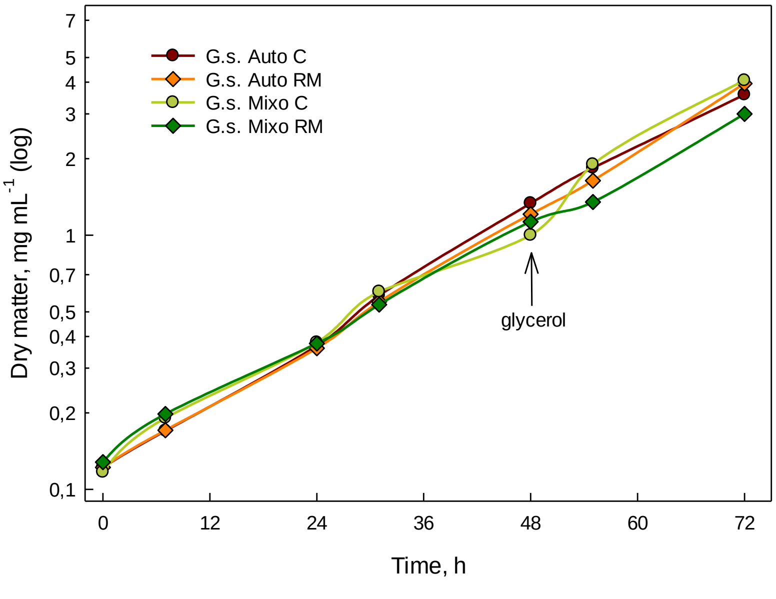

Figure 1. Dry matter of *Galdieria sulphuraria* 002 grown auto- and mixotrophically (mg mL^{-1}). C = control (no RM extract), RM = 5% RM extract treatment.

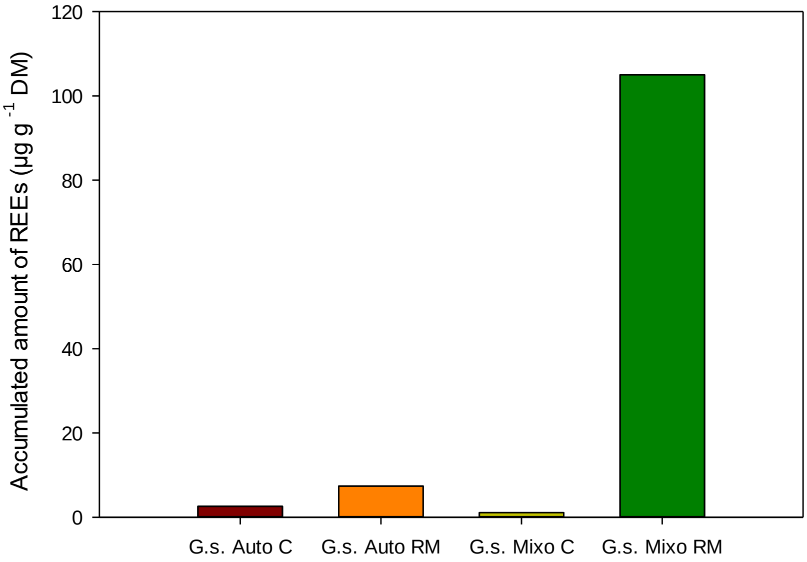

Figure 2. Total amount of REEs accumulated in the biomass of *Galdieria sulphuraria* 002 (µg g^{-1} DM).

Acknowledgements

Project was supported by the program Interreg V-A ATCZ172 REEgain and by Institutional Research Concept No. AVOZ61388971.

References

Ciniglia C., Yoon H. S., Pollio A., Pinto G. and Bhattacharya D. (2004), Hidden biodiversity of the extremophilic *Cyanidiales* red algae. *Molecular Ecology*, **13**, 1827-1838.

Čížková M., Mezricky D., Rucki M., Tóth T.M., Náhlík V., Lanta V., Bišová K., Zachleder V. and Vítová M. (2019), Bio-mining of lanthanides from red mud by green microalgae. *Molecules*, **24**, 1356, 1-19.

Čížková M., Mezricky P., Mezricky D., Rucki M., Zachleder V. and Vítová M. (2020a), Bioaccumulation of rare earth elements from waste luminophores in the red algae, *Galdieria phlegrea*. *Waste and Biomass Valorization*, https://doi.org/10.1007/s12649-020-01182-3.

Čížková M., Vítová M. and Zachleder V. (2020b), The red microalga *Galdieria* as a promising organism for applications in biotechnology. In Microalgae edited by Vítová M.,DOI:http://dx.doi.org/10.5772/intechopen.89810.

Goecke F., Jerez C., Zachleder V., Figueroa F.L., Bišová K., Řezanka T. and Vítová M. (2015), Use of lanthanides to alleviate the effects of metal ion-deficiency in *Desmodesmus quadricauda* (Sphaeropleales, Chlorophyta). *Frontiers in Microbiology*, 6, 2.

Gross W. and Schnarrenberger C. (1995), Heterotrophic growth of two strains of the acido-thermophilic red alga *Galdieria sulphuraria*. *Plant Cell Physiology*, **36**, 633–638.

Gross W., Küver J., Tischendorf G., Bouchaala N. and Büsch W. (1998), Cryptoendolithic growth of the red alga *Galdieria sulphuraria* in volcanic areas. *European Journal of Phycology*, **33**, 25-31.

Hirooka S., Tomita R., Fujiwara T., Ohnuma M., Kuroiwa H., Kuroiwa T. and Miyagishima S. (2020), Efficient open cultivation of cyanidialean red algae in acidified seawater. *Scentific Reports*, **10**, 13794.

Liu L., Sanchez-Arcos C., Pohnert G. and Wei D. (2021), Untargeted metabolomics unveil changes in autorophic and mixotrophic *Galdieria sulphuraria* exposed to high-light intesity. *International Journal of Molecular Sciences*, **22**, 1247.

López G., Yate C., Ramos F.R., Cala M.P., Silvia Restrepo S. and Baena S. (2019), Production of polyunsaturated fatty acids and lipids from autotrophic, mixotrophic and heterotrophic cultivation of *Galdieria* sp. strain USBA-GBX-832. *Scientific Reports*, **9**, 10791.

Minoda A., Sawada H., Suzuki S., Miyashita S., Inagaki K., Yamamoto T. and Tsuzuki M. (2015), Recovery of rare earth elements from the sulfothermophilic red alga *Galdieria sulphuraria* using aqueous acid. *Applied Microbiology and Biotechnology*, **99,** 1513-1519.

Oesterhelt C., Schmalzlin E., Schmitt J.M. and Lokstein H. (2007), Regulation of photosynthesis in the unicellular acidophilic red alga *Galdieria sulphuraria*. *The Plant Journal*, **51**, 500-511.

Ramasamy D.L., Porada S., Sillanpaa M. (2019), Marine algae: A promising resource for the selective recovery of scandium and rare earth elements from aqueous systems. *Chemical Engineering Journal*, **371**, 759–768.

Reeb V. and Bhattacharya D. (2010), The thermo-acidophilic *Cyanidiophyceae* (Cyanidiales). In: Red Algae in the Genomic Age, Seckbach J. and Chapman D.J. (eds.), Cellular Origin, Life in Extreme Habitats and Astrobiology **13**, 409–426. Springer Science+Business Media B.V.

Vítová M., Goecke F., Sigler K. and Řezanka T. (2016), Lipidomic analysis of the extremophilic red alga *Galdieria sulphuraria* in response to changes in pH. *Algal Research*, **13**, 218-226.