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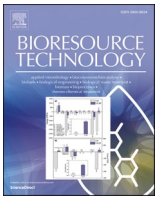

Biomining using microalgae to recover rare earth elements (REEs) from bauxite

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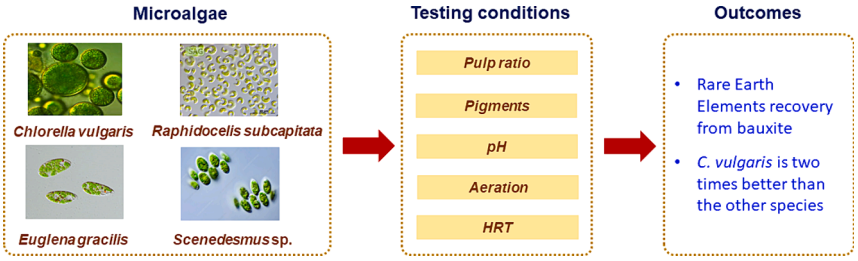
HIGHLIGHTS

- The optimal pulp ratio ranges from 0.2 % to 0.6 %.
- Pigments and Ca-Mg ATPase enzyme increased up to 10 % in the culture with bauxite.
- *Chlorella vulgaris* was the most promising one than other microalgae species.
- The optimal aeration rate ranged from 0.4 vvm to 1.2 vvm.
- HRT 4d recovered REEs two times higher than HRT 3d.

GRAPHICAL ABSTRACT



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ABSTRACT

Biomining using microalgae has emerged as a sustainable option to extract rare earth elements (REEs). This study aims to (i) explore the capability of REEs recovery from bauxite by microalgae, (ii) assess the change of biochemical function affected by bauxite, and (iii) investigate the effects of operating conditions (i.e., aeration rate, pH, hydraulic retention time) to REEs recovery. The results showed that increasing bauxite in microalgae culture increases REEs recovery in biomass and production of biochemical compounds (e.g., pigments and Ca-Mg ATPase enzyme) up to 10 %. The optimum pulp ratio of bauxite in the microalgae culture ranges from 0.2 % to 0.6 %. *Chlorella vulgaris* was the most promising, with two times higher in REEs recovery in biomass than the other species. REEs accumulated in microalgae biomass decreased with increasing pH in the culture. This study establishes a platform to make the scaling up of REEs biomining by microalgae plausible.

1. Introduction

Rare earth elements (REEs) are a group of 17 transition metals with unique chemical and physical properties (e.g., catalytic, metallurgical, magnetic and luminescent). They are essential for a wide range of applications in advanced manufacturing, such as electrical, electronic, optical, and magnetic production. As they play a critical role in modern industry, REEs have an increasing commercial value in the market. In 2023, the global production of REEs was estimated to 350,000 tons; of which China accounts for 66 % (Survey, 2024). Worldwide demand for REEs is projected to increase from 7 to 26 fold by 2025 (Mwewa et al., 2022). However, the supply of REEs is on the edge of shortage due to several reasons, including the uneven geographical distribution, uncertainty in global geopolitics, and challenges in the purification of REEs

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at industrial scale due to low concentrations of REEs in the ores (Vo et al., 2024).

Bauxite is a sedimentary rock formed by intense lateritic weathering of aluminosilicate protoliths. It contains substantial deposits of Al-hydroxides, Fe-hydroxides, and Ti-oxides. The current bauxite reserve in the world is estimated at 30 billion tons, while there is a much higher reserve under investigation (Mishra et al., 2022). In addition to aluminum, bauxite is also a rich source of REEs (Barnett et al., 2020). Specifically, scandium (Sc) and cerium (Ce) are REEs with the highest concentration found in bauxite (Barnett et al., 2020; Mishra et al., 2022). Hence, bauxite and its related products (e.g., red mud) are potential alternatives for the current crisis of REEs supply.

The existing technologies used for the extraction of REEs, such as hydro-, thermal- and electro-metallurgical are known to be associated with substantial consumption of chemicals and energy, as well as the generation of secondary pollutants (Čížková et al., 2019). Those technologies have also been reported to contribute to the loss of compounds of interest during the extraction process (Tezyapar Kara et al., 2023). Biomining is a solution to address the above issues. Biomining has exploited the nature of microorganisms through various forms of biogeochemical processes. Significant progress in biomining REEs has been reported with relevance to a wide range of microorganisms such as bacteria, fungi, microalgae, such as diatoms (Ayangbenro et al., 2018; Cao et al., 2021). Biomining has been performed at various scales, such as flasks, column bioreactor, and stirred bioreactors. Currently, biomining is only commercialized for low-grade copper and refractory gold ores. There is still a huge venue for biomining to recover REEs from tailing materials and low grade ores (Tezyapar Kara et al., 2023).

Microalgae are promising for phycoremediation and biomining as they are a highly diverse group of microorganisms with up to 300,000 species, which can grow in almost all environmental conditions (Vo Hoang Nhat et al., 2018; Zhou et al., 2023). Green microalgae (e.g., *Chlorella vulgaris*, *Desmodesmus quadricauda*, *Euglena gracilis*) and red microalgae (*Galdieria sulphuraria*) can uptake metals and REEs from the minerals (Cao et al., 2021; Kim et al., 2022; Siciliano et al., 2021b). Microalgae can recover metals and REEs by three main mechanisms: surface adsorption to cell walls, adsorption of extracellular polymeric substances (EPSs) excreted by microalgae, and intracellular accumulation into cells. The REEs after the recovery process are subsequently recovered via extraction processes (Heilmann et al., 2021). Though microalgae are promising agents for biomining, there are very limited number of studies on REEs recovery from bauxite. Other studies have not fully optimized the culturing and operating conditions such as aeration, hydraulic retention time (HRT), and pH (Čížková et al., 2019; Náhlík et al., 2022), which is critically necessary for upscaling the biomining technology. The insightful biochemical function and biomining conditions of microalgae affected by bauxite is also not fully investigated, with limited biochemical compounds (e.g., chlorophyll *a*, carotenoids, phycocyanin) and REEs studied (e.g., Ce, La, Nd).

The overarching aim of this work is to assess the feasibility of microalgae for REEs recovery from bauxite. The specific objectives are to (i) explore the REEs biomining capability of four microalgae strains, (ii) assess the change of biochemical composition of microalgae treated with bauxite, and (iii) investigate the effects of operating conditions (e.g., aeration rate, pH, HRT) to REEs recovery.

2. Materials and methods

2.1. Microalgae strains and culture

The microalgae strains were obtained from the culture collection of Climate Change Cluster (University of Technology Sydney (UTS)) (*C. vulgaris, Scenedesmus* sp.) and National Algae Supply Service (Tasmania, Australia) (*E. gracilis, Raphidocelis subcapitata*). These strains were selected for the REEs recovery experiments with bauxite as promising results were reported in the previous studies to recover REEs from acid mine drainage (Birungi and Chirwa, 2014; Kim et al., 2022; Siciliano et al., 2021b). Microalgae stocks were maintained in the appropriate media: MLA for *C. vulgaris, Scenedesmus* sp., *R. subcapitata*; and MBL for *E. gracilis*. The MLA medium was prepared using the concentrated media kit provided by AusAqua Pty Ltd (South Australia, Australia). The MBL medium was prepared in-house using modification formulation from CSIRO. The compositions of MLA and MBL are provided in supplementary material. The microalgae culture stock was maintained at room temperature (20 ± 1 °C) at illumination 15–30 μmol/m^2.s. The culture stocks were renewed fortnightly to ensure the culture was healthy.

2.2. Bauxite material

The bauxite mineral for the experiment came from Western Australia. The bauxite sample constituted of REEs including Scandium (Sc) – 5.87 μg/g, Yttrium (Y) – 0.94 μg/g, Cerium (Ce) – 32.29 μg/g, Praseodymium (Pr) – 0.32 μg/g, Neodymium (Nd) – 1.61 μg/g, Samarium (Sm) – 0.38 μg/g.

2.3. Experimental design

This study comprises four main experiments: (i) REEs accumulation by microalgae at different pulp ratios, (ii) effect of aeration rate on REEs accumulation by microalgae, (iii) effect of pH on REEs accumulation by microalgae, (iv) long-term monitoring of REEs recovery at different HRT.

2.3.1. Rare earth elements recovery by microalgae

This set of experiments was conducted using four microalgae species, as mentioned above, with seven pulp ratios (mass of bauxite vs mass of culture media) for each microalga species, including: 0 %, 0.05 %, 0.1 %, 0.2 %, 0.6 %, 1 %, 1.5 %. The experiments were conducted in a 60 ml tissue culture flask (polystyrene, sterile, filtered cap, Westlab). Appropriate media (50 mL) were used for the microalgae species. The initial optical density (OD) of the inoculum for the experiments was controlled at approximately 0.1. The light intensity and temperature were maintained at 100 μmol photons/m^2.s, 24 h constant light, and 25 °C for seven days. Samples were collected on day 0, day 2, day 4, day 6 and day 7 to track microalgae growth via cell counting. The biomass yield (mg/L) was also measured by harvesting, centrifuging and freeze drying the biomass. Two promising microalgae species (i.e., *C. vulgaris* and *E. gracilis*) were selected for the next experiments. The biochemical products of the selected microalgae (i.e., pigments, carbohydrate, protein, Ca-Mg ATPase enzyme) are chosen to assess the effect of bauxite on the biochemical functions of microalgae.

2.3.2. Effect of aeration rate

The effect of aeration rate on REEs recovery was conducted by using four different aeration schemes as volume of air (L) per volume of the culture (L) per minute (vvm) ratio: 0.4, 1, 1.2, 1.6. The photobioreactors were one litre Schott bottles (borosilicate glass, Duran®, diameter × heigh: 101 mm × 230 mm) with 500 mL effective media used for the experiment. The inlet air was sterilized by air filter (0.2 μm, Midisart, Sartorius). The optimal pulp ratio (0.2 %) was selected for this experiment, based on the results of the experiment described in 2.3.1. The experiment was conducted over seven days using the same light intensity (100 μmol photons/m^2.s, 24 h constant light), temperature (25 °C) and initial inoculum ratio (0.1).

2.3.3. Effect of pH

The effect of pH on REEs accumulation included a wide range of pH (e.g., 4.5, 5, 6, 6.5). The experimental conditions were similar to the previous experiments; however, the initial inoculum ratio was increased to 0.1–0.2 to reduce the shock load of low pH to microalgae. Throughout the experiment, pH was not maintained at its initial level to reflect the

real capacity of microalgae in REEs recovery.

2.3.4. Effect of hydraulic retention time

In this experiment, only one promising microalga specie was selected (*C. vulgaris*). Two HRTs were examined, including: 3d and 4d. The experimental conditions were similar to the previous experiments, with an aeration rate of 1 vvm. The initial optical density of the inoculum culture was enriched to 1 and then used for experiments.

2.4. REEs quantification by ICP-MS

Media and microalgae biomass samples were analysed for REEs using inductively coupled plasma mass spectrometry (ICP-MS), adapted from Čížková et al. (2019). 10 mL of aqueous media samples were collected and freeze-dried. The biomass was subject to washing by phosphor-buffer saline solution (PBS) after harvest and then freeze-dried. After freeze-drying, the biomass pellets were accurately weighed on an analytical balance and put into a new tube. Both biomass and freeze-dried samples were digested in concentrated HNO_3 at 100 °C for 2 h. After cooling to room temperature, all samples were diluted 1:40 with MilliQ water (Millipore).

The quantification of REEs was conducted using Agilent 7700 s-series ICP-MS (Agilent, USA). 200 ppb Rhodium in 1 % HNO_3 combined using a t-piece, was used as an internal standard. Quantification of REEs was performed using an eight-point calibration curve plotted using analytical standards. The limit of detections (LOD) and quantification (LOQ) of the seven REEs are provided in the supplementary material. The calibration curves of REEs are provided in the supplementary material, which were linear over six orders of magnitude.

2.5. Pigments, protein, carbohydrate, and enzyme quantification

The quantification of pigments, protein and carbohydrate was carried out by HPLC, Lowry, Phenol-sulphuric, and Ca-Mg-ATPase assay kit, respectively. The HPLC method was previously developed to profile pigments such as chlorophyll *a*, chlorophyll *b*, ß-carotene, lutein, diadinoxanthin, violoxanthin, and neoxanthin (Kuzhiumparambil et al., 2022; Windhagauer et al., 2024). The Lowry method for protein determination was based on a modified Lowry kit (Sigma, Australia). The Phenol-sulphuric method for carbohydrate determination was reported previously (Nielsen, 2017). The enzyme assay kit for Ca-Mg-ATPase was purchased from MyBioresource Inc. (San Diego, USA).

2.6. Statistical analysis

All the experiments in batch condition were conducted in duplication. The results were written as mean ± standard deviation. Data processing and plotting were conducted using Origin 2023b. The analyses of variance (ANOVA) were applied for statistical analysis. The repeated measures ANOVA was used to assess the significant difference in microalgae growth curve, REEs recovery kinetics, and effect of HRT. The factorial one-way ANOVA was used to evaluate the significant differences in REEs accumulation, production of biochemical compounds, effects of aeration and pH. The significant difference in statistics is considered with $p < 0.05$. The *post-hoc* test was also conducted using the Tukey test after one-way ANOVA to further assess the significant difference of pairs of groups means.

3. Results and discussion

3.1. Growth curve of microalgae in different pulp ratios

In this study, bauxite samples containing a mixture of REEs and other heavy metals (e.g., Pb, Co, and Ni) were used for extracting REEs. The growth profiles of four microalgae species at different pulp ratios (0, 0.05, 0.2, and 0.6 %) are shown in supplementary material. Higher bauxite concentration in the culture caused inhibition to the growth of all microalgae species. The control samples (0 % bauxite) showed the highest growth rate. *R. subcapitata* and *Scenedesmus* sp. possessed the highest growth rate compared to others. *E. gracilis* showed the highest inhibitory effect, with the cell count decreasing from day 4. The growth of *C. vulgaris* showed a spike in the first two days; however this slowed down over the subsequent days.

The inhibition of microalgal growth with increased pulp ratio was observed in this study that was consistent with other reported studies (Čížková et al., 2019; Náhlík et al., 2022). Two main reasons for the suppression of cell growth with increased bauxite in the solution are (i) the shading effect caused by insoluble particles, and (ii) the toxicity of REEs and other metals in bauxite. The increase of bauxite in the solution led to more insoluble particles, consequently decreasing the mean light intensity provided to microalgae cells. By increasing pulp density from 0 % to 0.1 %, the mean light intensity was reduced by five times (Čížková et al., 2019). This hindered cell growth, together with increasing REEs and metals in the solution; hence, the cell growth was significantly reducted compared to the control (5–75 %). To account for the effect of shading, Náhlík et al. (2022) extracted REEs from red mud and cultured the extraction with microalgae. It was found that increasing REEs also caused an inhibitory effect on cell growth. Cell division was delayed by approximately two hours (Náhlík et al., 2022). Therefore, it can be considered that the shading and toxicity of REEs (and other heavy metals) have synergistic effects.

Previous studies showed that low concentration of REEs can stimulate microalgae growth via activation of cell endocytosis (Kim et al., 2022). For example, at a concentration less than 100 μM, La^{3+} could improve cell growth by 16 % (Kim et al., 2022). For Ce^{3+}, the suitable concentration was less than 25 μg/L, otherwise biomass concentration and productivity of microalgae were reduced (Song et al., 2024). It suggests that different microalgae species had a particular range of REEs concentrations that can enhance their growth. High concentration of aluminium and iron which are substantially present in bauxite also restrains microalgae growth up to 5.5 times (Gebara et al., 2020; Polat et al., 2020).

3.2. Effect of pulp ratio to REEs recovery by microalgae

The REEs recovery by four microalgae species at different pulp ratios is shown in Fig. 1. Overall, increasing pulp ratio resulted in the elevation of REEs recovered in the biomass. The increase seems to reduce when the pulp ratio was higher than 0.6 %. The optimal pulp ratio ranges from 0.2 % to 0.6 %. Our preliminary experiment showed that microalgae can not survive at a pulp ratio higher than 1.5 %.

Amongst the four microalgae species, *C. vulgaris* was the most promising one. The recovery capacity of *C. vulgaris* is almost two times better than the others. *R. supcapitata*, exhibited poor recovery of REEs at low pulp ratios. In previous studies, *R. subcapitata* could recover Ce up to 240 μg/g (Siciliano et al., 2021b). Other microalgae such as red microalgae *Galdieria sulphuraria* can recover 25 μg/g (Náhlík et al., 2022), which is equivalent to *E. gracilis* in this study, but less than the other microalgae species. These findings suggest that the culturing and operating conditions significantly impact the REEs recovery capacity of microalgae.

Our results are congruent with Čížková et al. (2019), in which concentrations of REEs in biomass were found to increase proportionally with the increase of pulp ratio, in conjunction with a decrease in growth rate. Together with the three microalgae species studied by Čížková et al. (2019) (e.g., *P. kessleri*, *C. reinhardtii*, and *D.quadricauda*), it can be concluded that REEs recovery capacity is independent of the taxa.

There have been debates over the mechanism of REEs recovery by microalgae. Three main mechanisms have been identified: adsorption to cell walls of microalgae, adsorption to extracellular polymeric substances (EPS) excreted by microalgae, and intracellular accumulation. Song et al. (2024) found that adsorption via EPS played a minor role,

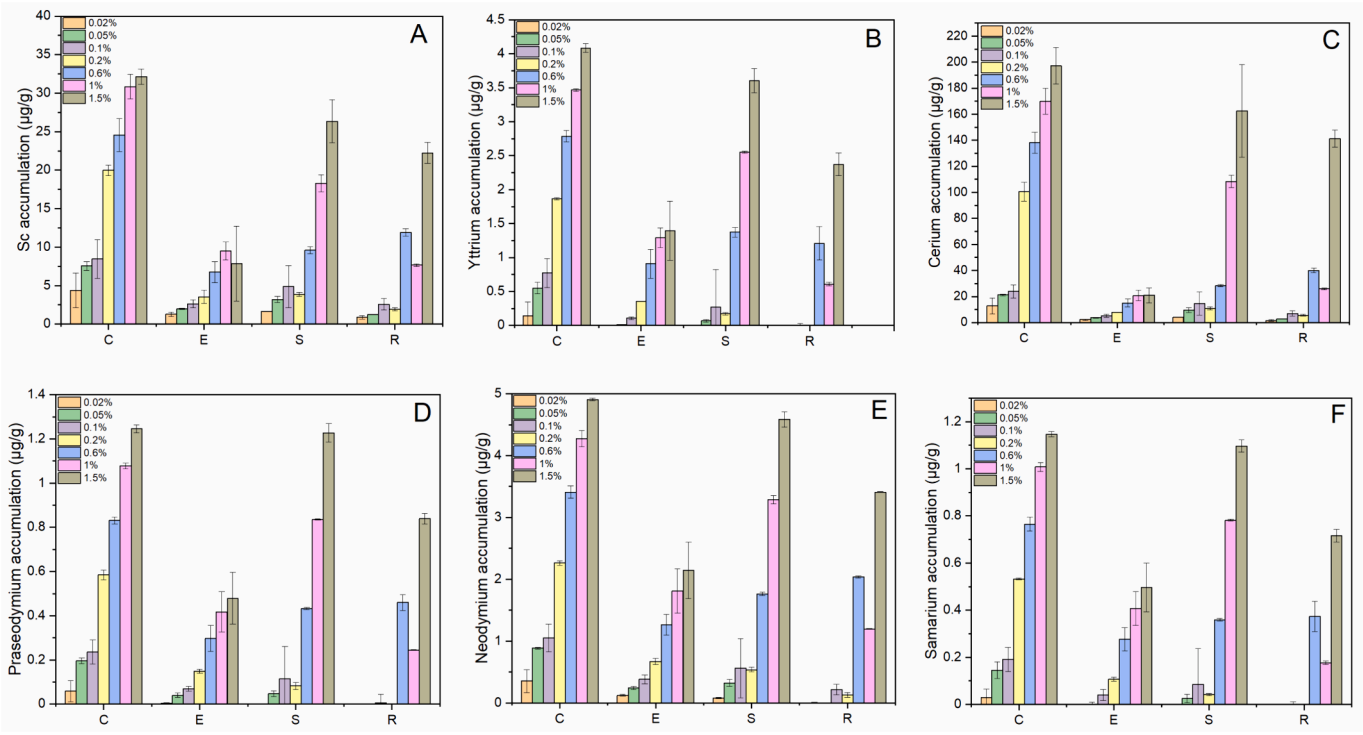

Fig. 1. REEs accumulation in four microalgae species at different pulp ratios. All measurements show significant differences ($p < 0.05$) amongst microalgae species. The x panels perform microalgae species. The abbreviations of microalgae species are C for *C. vulgaris*, E for *E. gracilis*, S for *Scenedesmus* sp., R for *R. subcapitata*). The y panel performs the specific REEs: Scandium in panel A, Yttrium in panel B, Cerium in panel C, Praseodymium in panel D, Neodymium in panel E, Samarium in panel E. The error bars represent two replications ($n = 2$). The accumulation of Sc ($p = 0.03$) and Ce ($p = 0.05$) are significantly different amongst four microalgae species. The post-hoc tests show a significant difference of *C. vulgaris* and *E. gracilis* for Sc accumulation ($p = 0.03$), and for Ce accumulation ($p = 0.04$).

which occupied less than 10 %. Cell wall adsorption and intracellular accumulation contributed significantly to REEs recovery, given the contribution of intracellular accumulation is usually less than cell wall adsorption by 20–40 %. In this study, we considered the cell wall adsorption and intracellular accumulation together in the form of total REEs recovery, which needs to be acknowledged for future works.

3.3. Production of pigments, carbohydrates, protein, and enzymes at different pulp ratios

Based on the above results, the 0.2 % pulp ratio is selected to investigate the influence of bauxite on the biochemical composition of algae, including pigments, carbohydrates, protein and Ca-Mg ATPase enzyme (Fig. 2). The results showed that these compounds showed different effects with bauxite. The total pigments and Ca-Mg ATPase enzyme exposed to bauxite increased by 10 % compared to the control. Chlorophyll *a* and chlorophyll *b* were the most sensitive ones and clearly showed increased concentration. Other pigments also slightly increased (see supplementary material). However, carbohydrate levels in biomass reduced up to two times while exposed to bauxite. Protein level seems to remain stable for most microalgae species, except *E. gracilis*, in which *E. gracilis* showed the highest effect on pigments, protein and carbohydrate concentration.

The results of the red microalgae (e.g., *G. sulphuraria*) were contradictory with this study, given chlorophyll *a* content was reduced by 10–20 % when exposed to REEs. However, carotenoids increased by up to 50 % (Náhlík et al., 2022; Song et al., 2024). REEs could cause stress and carotenoids are able to block ROS/oxidative stress (Zuluaga et al., 2017). This seems to suggest that pigments produced by microalgae are increased by bauxite stress up to a certain level, then after that, pigments started decreasing, likely due to an inhibitory effect on metabolism and physiology of microalgae. In terms of REEs, they act as a catalyst for chlorophyl formation and being stress factor to stimulate pigment content (Hong et al., 2002). This indicates that using an appropriate level environmental stressor, such as 0.2 % pulp ratio of bauxite in this case, can improve pigment production of microalgae. However, the pigment production can potentially be reduced at pulp ratio higher than 0.2 %.

The reduction of carbohydrate production observed in this study agreed well with Song et al. (2024) which is probably due to the shift of carbon towards lipid production, especially *C. vulgaris* and *E. gracilis*. Under high stress of REEs and other metals, protein may undergo misfolding in the endoplasm (Morel et al., 2021). Proteins and carbohydrates play a critical part in the biosorption process. Proteins were involved in the sorption of some of the REEs such as Pr^{3+}, Sm^{3+}, Eu^{3+}, Tb^{3+}, Dy^{3+}, while carbohydrates participated in Ce^{3+} sorption (Manfredi et al., 2023). The cell walls comprise various polysaccharides, proteins and lipids components, which are the main driving force for the adsorption of REEs. By increasing the pulp ratio, the production of carbohydrates and protein is reduced, which implied that the contribution of cell wall adsorption to REEs recovery was compromised.

An increase in the Ca-Mg ATPase enzyme on exposure to bauxite could be influenced by REEs being carried into microalgae cells via the Ca^{2+} channel (Homer and Mortimer, 1978; Zeng et al., 2003). For example, Eu^{3+} was carried into cells for the synthesis of amaranthin. Excess Eu^{3+} led to the replacement of Ca^{2+} in the calcium/calmodulin-dependent phytochrome signal transduction system (Zeng et al., 2003). Together with the increase of pigments, this suggests that intracellular accumulation of REEs in microalgae cells is subject to a proportional increase with pigments.

3.4. Kinetics of REEs recovery by microalgae

The kinetics of Sc recovery by *C. vulgaris* and *E. gracilis* in the media are shown in Fig. 3. This section only reports the kinetic of Sc as its concentrations are substantial. For *C. vulgaris*, the recovery kinetic

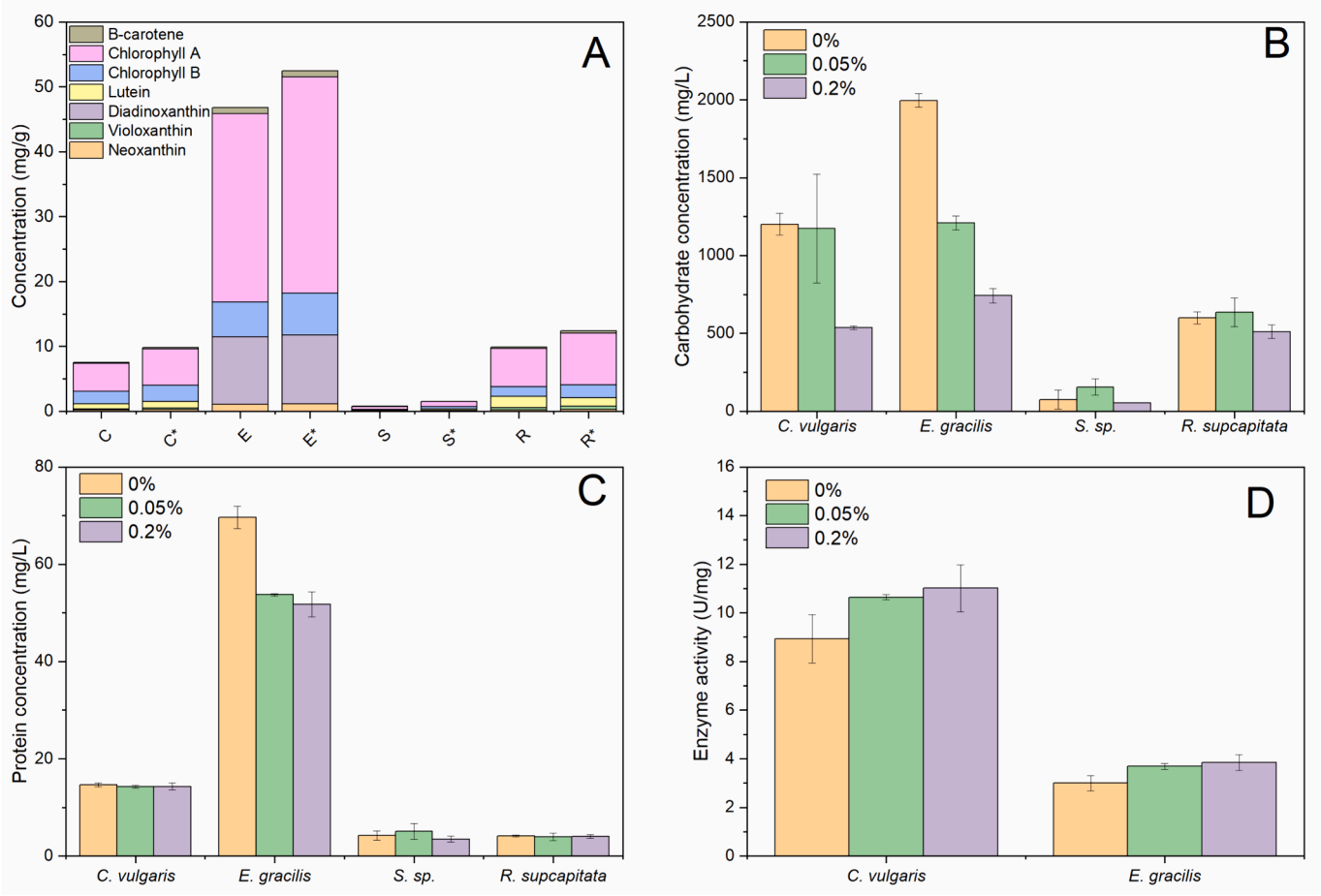

Fig. 2. The production of pigments (panel A), carbohydrates (panel B), protein (panel C), and Ca-Mg ATPase enzyme (panel D) by microalgae at 0.2 % pulp ratio. Panel A represents control (no asterisk) and bauxite-exposed (with asterisk) microalgae. The "*" is spiking bauxite mineral at 0.2 % pulp ratio. Only data of *C. vulgaris* and *E. gracilis* are shown in panel D due to the limited numbers of test kit. For pigment production between spiking and non-spiking bauxite, the post-hoc tests show a significant difference between *C. vulgaris* ($p = 0.05$) and *Scenedesmus* sp. ($p = 0.03$). The carbohydrate production of the four microalgae species is significantly different ($p = 0.005$). The protein production of the four microalgae species is significantly different ($p < 0.05$). The enzyme production of the four microalgae species is significantly different ($p < 0.05$).

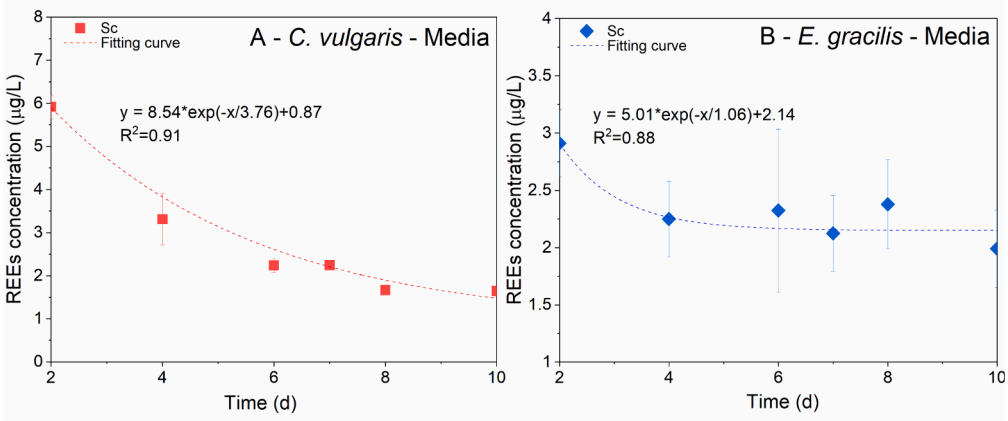

Fig. 3. Recovery kinetics of Sc by *C. vulgaris* (Panel A) and *E. gracilis* (Panel B) in media. The error bars represent two replications ($n = 2$). The non-linear curve is fitted to the curves.

decreased gradually until day 10 of the experiment. The recovery kinetics of *E. gracilis* was much faster given that the recovery happened rapidly in the first 2 d and then reached the equilibrium phase on day 4. In the previous studies, the kinetics of REEs sorption were faster, just within 20 min to 120 min (Kucuker et al., 2017; Sadovsky et al., 2016). However, those studies used the processed powder under ideal conditions by spiking pure REEs chemicals into the algal growth media. It is different from the living biomass using bauxite with a high level of biological interaction between biomass, REEs and other elements such as heavy metals. It is probably the reason for the slower sorption kinetic

in our study.

3.5. Effect of aeration rate to REEs recovery

The effect of the aeration rate on REEs recovery by microalgae is shown in Fig. 4. The optimal aeration rate ranges from 0.4 vvm to 1.2 vvm. Increasing the aeration rate led to increasing REEs concentration in the media. The remaining REEs in the liquid media after the experiment are less than 2 μg/L for *E. gracilis* and 1.5 μg/L for *C. vulgaris*. In the biomass, REEs were recovered in *E. gracilis* higher than in *C. vulgaris* from two to three times. The achieved biomass of *C. vulgaris* was two times higher than *E. gracilis*, which explains the lower REEs recovered in biomass of *C. vulgaris* (see supplementary material).

The increasing aeration has a certain effect on the REEs adsorption process. The rigorous mixing at high vvm ratio might cause desorption of REEs from microalgae cells, which explains the higher REEs in the media at a higher vvm ratio. In this air-sparging condition, the growth of *C. vulgaris* was favoured by the photoautotrophic mode using CO_2 as a nutrient. Although *E. gracilis* is adaptable to mixotrophic conditions, the biomass produced was less than *C. vulgaris* biomass.

3.6. Effect of pH to REEs recovery

The effect of pH on REEs recovery by microalgae is shown in Fig. 5 and Fig. S4. Generally, at lower pH, REEs were increasingly recovered into the algal biomass; however, the REEs residual in the media also increased. The reason is that at lower pH, REEs are mainly present in trivalent forms (86–88 %), which are more bio-available for microalgae (Siciliano et al., 2021a). However, the REEs adsorption capacity of microalgae cells is reduced at a lower pH range due to the competition of ion H^+ to REEs. The substantial presence of H^+ reduces the binding of REEs ions to ligands on the cell walls. At pH 4 to 6, the proportion of free REEs (e.g., In) decreased from 35 to 0.02 %, while the proportion of REEs hydroxide increased from 7.1 to 96 % (Yang et al., 2019). As a result, although REEs accumulation in biomass is higher at lower pH, REEs adsorption is compromised due to H^+ competition and therefore resulted in higher residual REEs. This also indicates that the biosorption of REEs is mostly about ionic attraction (Chang et al., 1997).

In the static culture condition without aeration, the REEs recovered in biomass by two microalgae species are not significantly different. Despite the lower biosorption capacity of microalgae, the overall REEs accumulated in the biomass at lower pH are more significant. This indicates that at a lower pH range, microalgae tend to accumulate REEs in the biomass increasingly. However, it is noted that the effect of pH is microalgae species-specific. For example, the optimal pH for *C. sorokiniana* is 6.0 (Qiu et al., 2017), while *Chlorella* sp. showed the best nutrient uptake at pH 7.0 (Zhang et al., 2014).

3.7. Effects of hydraulic retention time on REEs recovery

The effect of HRTs on REEs recovery is shown in Fig. 6. At HRT 4d, the concentration of REEs recovered by biomass was higher than HRT 3d by two times. However, at both HRT, the concentrations of REEs remaining in the media are insignificantly different ($p < 0.05$) due to the

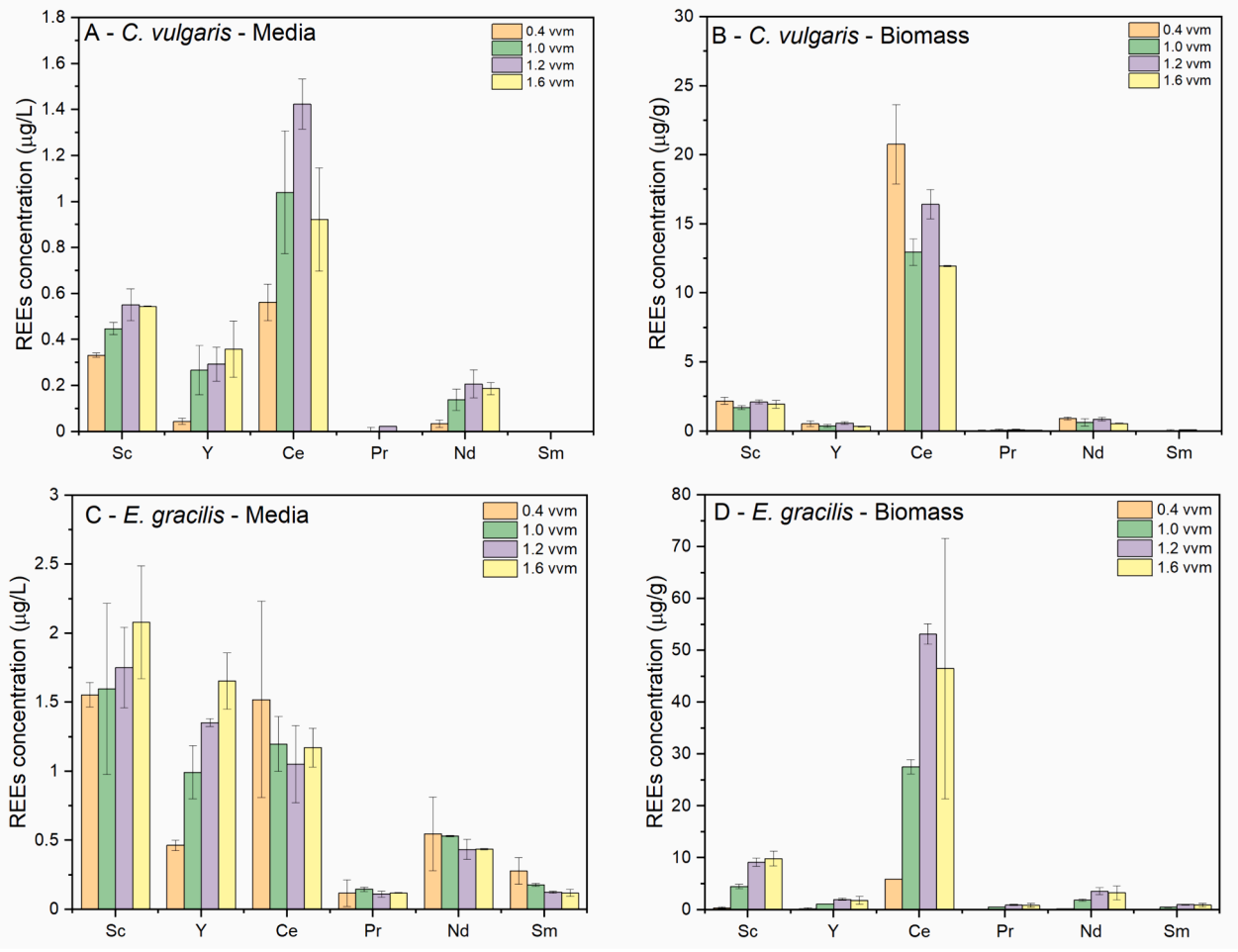

Fig. 4. Concentration of REEs in the media and biomass of microalgae at different aeration rates. The error bars represent two replications (n = 2). The effects of aeration rates on REEs recovery in both media and biomass of two microalgae species are insignificantly different ($p > 0.05$).

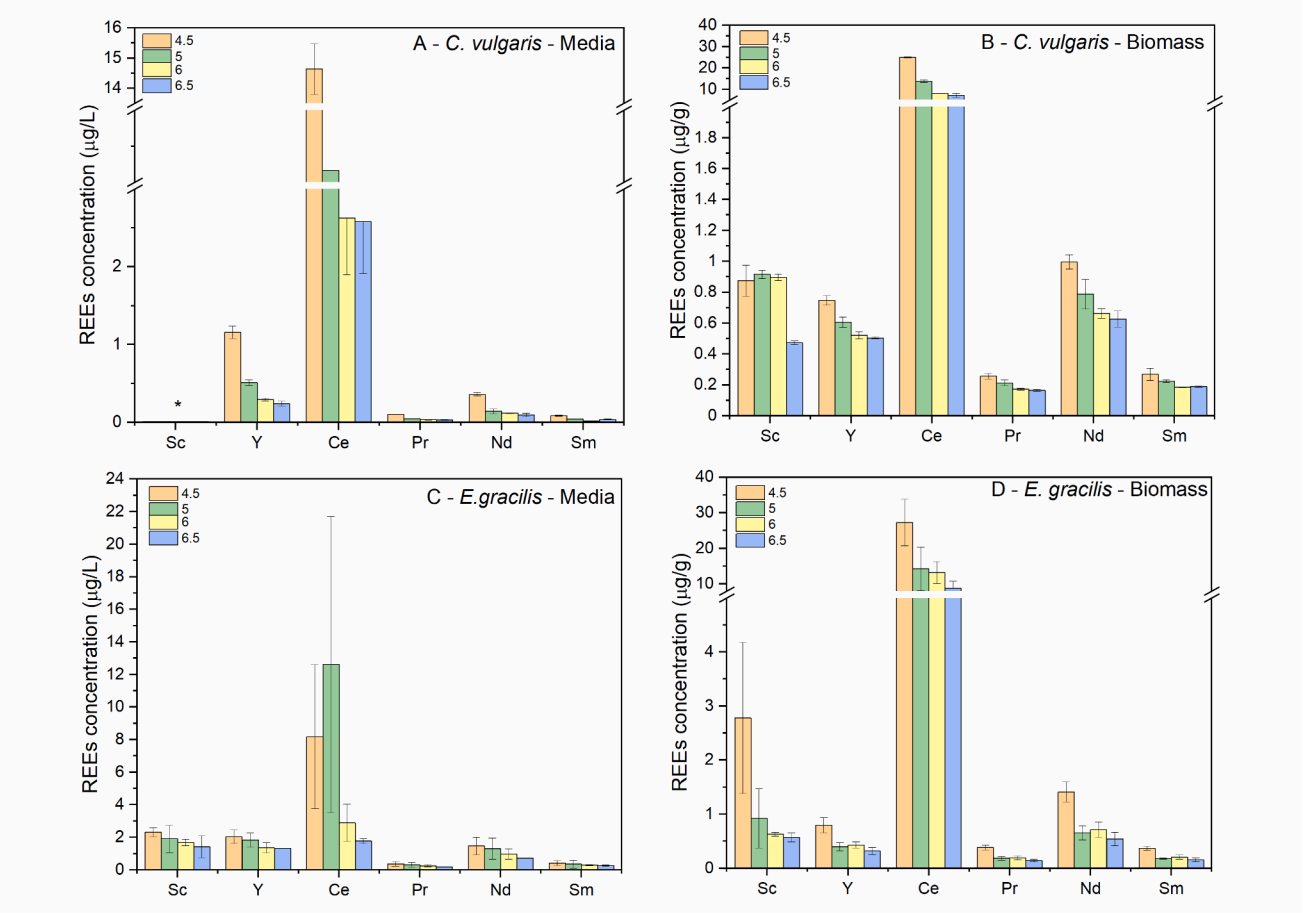

Fig. 5. Concentration of REEs in the media and biomass of microalgae at different pH. Data of Sc in Panel A is missing due to below LOD. The error bars represent two replications (n = 2). The effects of pH on REEs recovery in both media and biomass of two microalgae species are insignificantly different ($p > 0.05$).

difference in biomass generated in the two operational schemes (see supplementary material). In the continuous mode (4d HRT), REEs recovered by biomass were higher than in the batch condition (HRT 7d). For example, Ce in biomass at HRT 4d ranged from (40–100 μg/g), whereas it was 10–30 μg/g for the batch condition. However, at HRT 3d, biomass concentrations were quite equal to the batch condition. At HRT 3d, the biomass concentrations (800–1200 mg/L) were subject to a wider variation than HRT 4d (200–400 mg/L). This is probably due to the adaptation of microalgae at HRT 3d after more than a month of operating at HRT 4d. The biomass concentration at HRT 3d is in a good range with Gao et al. (2018) (1200 mg/L after 20 d). It is well known that HRT significantly affects biomass yield, directly affecting the REEs recovery. In a study investigating effect of HRT, Takabe et al. (2016) recommended HRT of 2–3 d for an optimal biomass yield in an outdoor algae photobioreactor which confirmed by a sensitivity analysis of a model. Sheng et al. (2017) also suggested the optimal HRT is 4 d for a sequencing batch membrane photobioreactor. Gao et al. (2018) found that HRT 2d achieved the highest biomass productivity for the system. The main reason is the optimal removal of nutrients and the production of pigments. The inclusion of solid retention time is recommended in future works as the ratio of SRT/HRT is an important parameter to maximize the performance of the system (Pastore et al., 2022; Xu et al., 2015). In practice, the use of HRT and SRT for system operation needs to be considered inclusively after the techno-economic assessment. However, shorter HRT would be preferentially used to increase the total volume of wastewater treated per unit of time.

4. Implications

The application of microalgae for wastewater treatment and heavy metals recovery was widespread; however, there is still very little for biomining REEs. This study addresses the knowledge gap of using microalgae as a biomining agent to recover REEs from bauxite and make upscaling feasible. This study also serves as a platform to calibrate REEs biomining for other resources such as mining wastewater and tailing. For instance, previous studies have shown that microalgae can recover high ammonia load, and low C/N ratio in acidic mining wastewater (Geng et al., 2022; Kim et al., 2022; Wang et al., 2023).

One technical challenge of biomining (e.g., microalgae) is extended extraction time, up to days and months, compared to other physical and chemical processes (Čížková et al., 2019; Jha et al., 2016). There are also limited full scale systems for biomining of REEs at this stage, so substantial trial and error works might be required. For example, the highest technology readiness level for REEs biomining in US is less than 4 (Brown et al., 2023). High cost of material feedstock is also an issue for upscaling biomining; hence, biomining is more suitable for low-grade REEs resource (Vo et al., 2024). Future works are suggested to delve into other REEs minerals such as mineral sand, clay, and coal tailing. To shorten the extraction time and improve extraction efficiency, the initial bioleaching step is recommend to liberate REEs into the leaching solution, then recover REEs by microalgae (Vo et al., 2024). Techno-Economic Assessment and Life Cycle Analysis are also needed before potential application at scale, especially for low-grade REEs resources.

Other biological research has been conducted to improve the recovery of REEs, such as mutagenesis and synthetic biology research (Macdonald Miller et al., 2023; Park et al., 2020; Xie et al., 2022a; Xie

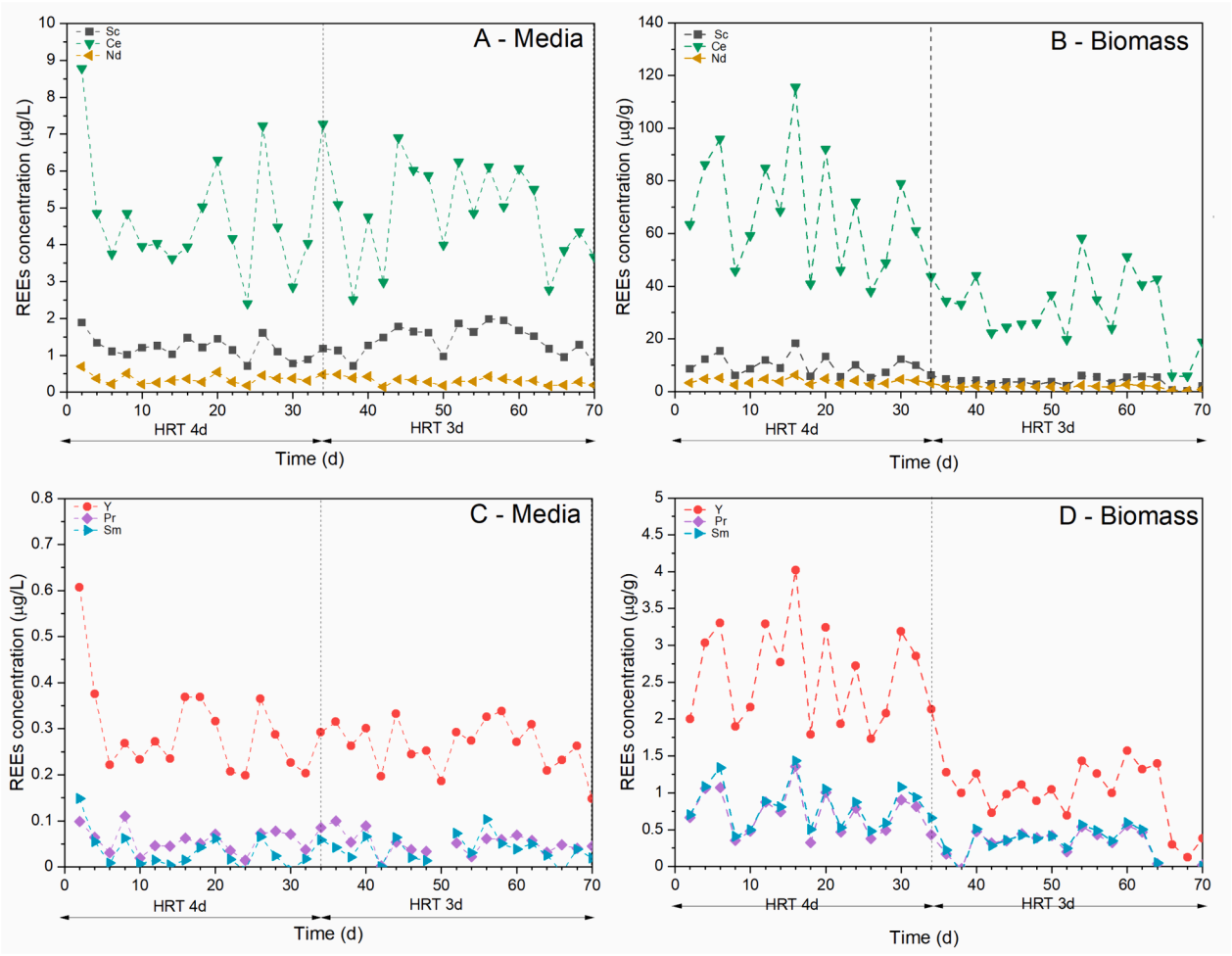

Fig. 6. Concentration of REEs in the media (Panel A, C) and biomass (Panel B, D) of microalgae at two HRTs (4d and 3d). Panel A and Panel B include Sc, Ce and Nd. Panel C and Panel D include Y, Pr, and Sm. The x-axis represents time from day 0 to day 70. HRT 4d includes day 0 to day 34. HRT 3d includes day 36 to day 70. The concentration of REEs in the media of two HRTs are insignificantly different ($p < 0.05$). The concentration of REEs in the biomass of two HRTs are significantly different ($p > 0.05$).

et al., 2022b). Special proteins like lanmodulin and lanthanide-binding tags are customized to adsorb a specific REEs selectively. Hence, the REEs of interest were selectively recovered from the mixture of REEs, which met the expectations of industries. However, these proteins were designed for REEs recovery from bacteria, not microalgae. Therefore, further research is needed in order to apply this technology to microalgae.

5. Conclusion

C. vulgaris is a promising species, recovering two times more REEs than the other three species in this study. The optimal pulp ratio of bauxite in microalgae culture ranged from 0.2 to 0.6 %. Increasing bauxite in the culture of microalgae increased the production of certain biochemical compounds up to 10 %. The optimal aeration rate to culture microalgae was from 0.4 to 1.2 vvm. REEs accumulated in microalgae biomass decreased with increasing pH in the culture. The recovery of REEs in microalgae biomass at HRT 4 d was higher than 3 d although REEs residuals in the culture are insignificantly different.

CRediT authorship contribution statement

Phong H.N. Vo: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Unnikrishnan Kuzhiumparambil:** Resources, Conceptualization. **Mikael Kim:** Writing – review & editing. **Cora Hinkley:** Resources. **Mathieu Pernice:** Writing – review & editing, Resources. **Long D. Nghiem:** Writing – review & editing, Supervision, Conceptualization. **Peter J. Ralph:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2024.131077.

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