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Selenium recovery from wastewater by the green microalgae *Chlorella vulgaris* and *Scenedesmus* sp.

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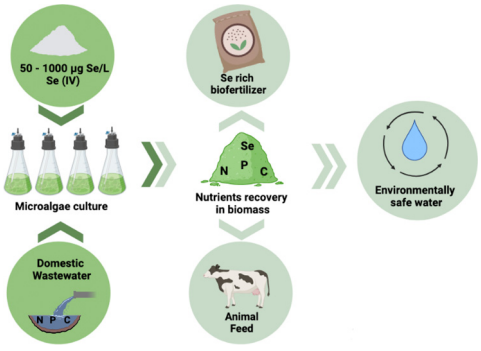
HIGHLIGHTS

- *Scenedesmus* and *Chlorella* strains were tested for Se uptake in wastewater and tolerate concentrations up to 1000 μg Se/L.
- *Chlorella* showed better growth and Se removal than *Scenedesmus* treating wastewater.
- *Chlorella vulgaris* was able to incorporate up to 323 mg Se/kg DW in biomass.

GRAPHICAL ABSTRACT



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ABSTRACT

Selenium (Se) is an important element for many living organisms and its supplementation may be needed in food, feed, and soil to make up for its deficiency. At the same time, high selenium concentrations can harm the environment, thus its management in sewage and the study of its removal from waste streams are important. Microalgae-based systems may be used for wastewater treatment and nutrients recovery, while producing biomass for bioproducts or bioenergy. In this study, *Chlorella vulgaris* and *Scenedesmus* sp. grown in urban wastewater with different selenium concentrations (50–1000 μg Se/L) were evaluated for their resistance and selenium removal/recovery efficiency. *Chlorella vulgaris* and *Scenedesmus* sp. were able to remove up to 43 and 52 % of Se from wastewater, respectively. *Chlorella vulgaris* accumulated up to 323 mgSe/kg DW (in urban wastewater with 1000 μg Se/L). The Se-rich biomass produced may be applied to the supplementation of animal feed or used for biofortification of crops.

1. Introduction

Almost all European countries are classified as low-selenium regions, and their dietary intake is still insufficient due to the low concentration in soils, water, food, and feed (Lintschinger et al., 2000). Selenium (Se) being the active center of numerous selenoproteins is an essential micronutrient for many living organisms playing a crucial role in enzyme function for humans and animals (Hatfield et al., 2014). The deficiency of this element causes oxidative stress and, in turn, health disorders (Gómez-Jacinto et al., 2020). Therefore, Se supplementation has received much attention, mainly from microorganisms like microalgae for food, feed, and soil application (Chen et al., 2021; Guimarães et al., 2021).

Selenium was reported as required for the growth of 33 microalgae species, mainly green algae. Microalgae can take up inorganic Se in the form of selenate or selenite, which are the dominant soluble forms of Se in aquatic

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bodies and are assimilated by incorporation into amino acids through the sulfur assimilation metabolic pathway (Schiavon et al., 2017).

Selenium enrichment in microalgae was studied for several microalgal strains. *Haematococcus pluvialis* was able to bioaccumulate 380 μg Se/gDW of organic Se when cultivated with concentrations from 3 to 13 mg/L of selenite; higher concentrations of the compound increased the pigments content but were toxic for the microalga (Zheng et al., 2017). *Microcystis aeruginosa* cultured with 2.6 mg/L of selenium was able to remove 97 % through accumulation and 3 % through volatilization (Zhou et al., 2017). The low volatilization rate was justified by the absence of SeMet (SelenoMethionine, e.g. dimethylselenide), precursors of volatile Se, in the algal biomass. Li et al. (2021b) studied a mixed microalgae consortium for selenium removal to be applied as a potential feed supplement. They found that 49–63 % of Se in the Se-enriched microalgae was bioaccessible for animals.

Besides its importance, there is a narrow margin between nutritional essentiality and toxicity of Se (Zhou et al., 2017). Selenium contamination in an aqueous environment is a topic of concern as anthropogenic activities like mining, agricultural, petrochemical, and industrial manufacturing operations can release the compound into water (Lemly, 2004). Selenium is considered as a contaminant of potential concern in North America, Australia and New Zeland (Tan et al., 2016). Urban/domestic wastewater can be a mixture of waste generated by households, industry and rainwater outflows and it can also be a source of Se contamination. Indeed, a concentration of 5–50 μg Se/L was reported for municipal wastewater (Santos et al., 2015). Selenium contamination due to anthropogenic activities and the effect on the environment has been well documented in the literature. For instance, an agriculture irrigation drainage (140–1400 μg Se/L) in the United States, which caused local fish extinction and deformities in waterfowls; and Se contamination of a Lake in Australia by fly ashes of a powerplant (50–300 mg Se/kg), leading to bioaccumulation in aquatic species exceeding allowable selenium intake values for human consumption (Tan et al., 2016).

Some methods for removing Se from wastewater involve high cost and process complexity. The ion-exchange process is mainly used for selenate removal, which is limited for treating large wastewater volumes because of the need for resin generation. Bacterial reduction of Se is a widely applied biological alternative. However, its operational complexity and cost of bioreactors are a challenge. Moreover, the process produces a large Se-containing sludge requiring post-treatment. The sludge production problem is also observed for direct precipitation of Se by reducing selenium oxyanions to an elemental form (Okonji et al., 2020). Alternatives of environmentally friendly, effective methods for Se removal and recovery are needed, as this Se source can close the cycle and be used for Se-enriched materials production.

In our previous study (Li et al., 2021b), we evaluated the selenium removal/recovery capacity of a spontaneous mixed culture of microalgae naturally growing and treating domestic wastewater, as a first attempt to elucidate the potential of Se-enriched microalgae biomass as feed supplement. However, Se-enriched microalgae commercial valorization would eventually require the specification of microalgae strains present in the product. Indeed, spontaneous mixed culture reproduction in wastewater is not feasible at large scale. Thus, depending on the final product targeted from harvested biomass, it is important to study unialgal cultures for wastewater treatment coupled with selenium enrichment, which is approached for the first time in this manuscript.

Therefore, the aim of the present study is to assess the selenium removal/recovery capacity of two green microalgae capable of growing in wastewater (i.e. *Chlorella vulgaris* and *Scenedesmus* sp.). Real urban wastewater was spiked with different selenium concentrations, in order to evaluate their resistance, and removal/recovery capacity. The use of cultures unialgal cultures may lead to a stable biomass composition for target applications.

2. Material and methods

2.1. Microalgae inoculum and adaptation to domestic wastewater

Experiments were carried out with two green microalgae, *Chlorella vulgaris* and *Scenedesmus* sp. The first one was obtained from a microalgae production plant of Global Iberican Entity (GIE) S. L. (Barcelona, Spain), while *Scenedesmus* sp. was obtained from a pilot high-rate algae ponds (HRAPs) system treating domestic wastewater from a residential area close to the Universitat Politècnica de Catalunya·BarcelonaTech (UPC) (Barcelona, Spain).

Firstly, both microalgae were cultivated in two 3 L Erlenmeyer flasks containing BG11 medium (Liu et al., 2016). The flasks were maintained at room temperature (25 °C) in natural light and continuously mixed using an air pump to avoid sedimentation. The composition per L of BG11 medium was as follow: $NaNO_3$, 1500 mg; $CaCl_2{\cdot}2H_2O$, 36 mg; $MgSO_4{\cdot}7H_2O$, 75 mg; $K_2HPO_4{\cdot}3H_2O$, 40 mg; Na_2CO_3, 20 mg; $Fe(NH_4)_3(C_6H_5O_7)_2$, 6 mg; EDTA-2Na, 1 mg; $C_6H_8O_7{\cdot}H_2O$, 6 mg; H_3BO_3, 2.86 mg; $MnCl_2{\cdot}4H_2O$, 1.81 mg; $ZnSO_4{\cdot}7H_2O$, 0.222 mg; $NaMoO_4{\cdot}2H_2O$, 0.39 mg; $CuSO_4{\cdot}5H_2O$, 0.079 mg; and $Co(N0O_3)_2{\cdot}6H_2O$, 0.0494 mg. The pH of the medium was adjusted to 7.5 daily with 0.1 M HCl or 0.1 M NaOH.

Next, another two 3 L Erlenmeyer flasks were used for the adaptation of microalgae to wastewater over a period of two weeks. For this, 1.5 L of each culture were transferred to the new 3 L Erlenmeyer flasks and filled with wastewater. Every 4 days, 1.5 L of each culture were withdrawn and Erlenmeyer flasks were filled with wastewater. A light intensity range of 4330 to 5150 lx was provided to the flasks by one blue-red LED light (model G90, 90 W) with a 12 h/12 h of light/darkness photoperiod at 25 °C. The microalgae biomass was continuously agitated (350 rpm) with a magnetic stirrer and an air pump with diffusers. The pH was adjusted to 7.8 by adding 0.1 M HCl or 0.1 M NaOH daily and after wastewater feeding.

2.2. Batch experiments

Batch experiments were conducted in Erlenmeyer flasks with a working volume of 320 mL. Domestic wastewater was used as culture medium, supplemented with 0, 50, 100, 500, 1000 μg/L (as Se) of selenite in triplicates. The microalgae adapted to domestic wastewater were thickened by centrifugation at 4200 rpm (centrifuge Unicen 21, Orto Alresa, Spain). Then the cell number of the thickened biomass was counted by optical microscopy (microscope BA310, Motic, China), and volatile suspended solids (VSS) were quantified according to Standard Methods (APHA-AWWA-WEF, 2012). When thickened biomass reached 10^7 cells/mL, the biomass was added to Erlenmeyer flasks to reach 78 mg VSS/L in the mixed liquor. A light intensity range of 4330 to 5150 lx was provided to the flasks by one blue-red LED light (model G90, 90 W) with a 12 h/12 h of light/darkness photoperiod at 25 °C.

Microalgae growth was monitored by measuring the pH (portable pH-meter 506, Crison Instruments, Spain) and optical density (OD) at 680 nm using UV/Visible spectrophotometer (Spectronic Genesys 8, ThermoFisher Scientific, USA). For Se concentration quantification, 5 mL of the mixed liquor were collected every two or three days, acidified (0.10 μL of concentrated HNO_3 at 65 %), filtered over a 0.20 μm syringe PVDF membrane filter, and stored in 10 mL closed plastic tubes at 4 °C until analyzed.

Once the microalgae reached the stationary growth phase, 15 days for *C. vulgaris* and 24 days for *Scenedesmus* sp., magnetic stirring was stopped to let the biomass settle and separate it from treated wastewater. The biomass of each flask was centrifuged at 4200 rpm (Orto Alresa centrifuge, Unicen 21, Spain), rinsed with distilled water, stored in 10 mL closed plastic tubes, frozen, and lyophilized for total Se measurement.

The maximum specific growth rates (μ_{max}) for the two microalgae were determined from experimental data following Eq. (1) (Uggetti et al., 2014):

$$\mu_{max}\left(d^{-1}\right) = \frac{\ln\left(Abs_2\right) - \ln\left(Abs_1\right)}{t_2 - t_1} \quad (1)$$

where *the* Abs_1 and Abs_2 correspond to the absorbance measured in each culture (λ = 680 nm) at the beginning ($t = t_1$) and the end ($t = t_2$) of the exponential phase, respectively. The exponential phase of each microalga was determined from the logarithmic growth curve of the optical density, and μ_{max} values were found from days 1 to 3.

2.3. Analytical methods

2.3.1. Physico-chemical characterization of domestic wastewater and treated wastewater

Domestic wastewater used in each batch test was collected from the primary settler of a HRAPs system treating domestic wastewater from a residential area close to the Universitat Politècnica de Catalunya·BarcelonaTech (UPC) (Barcelona, Spain) and characterized before the start-up of each batch experiment. Wastewater quality parameters analyzed were pH (portable pH-meter 506, Crison Instruments, Spain), turbidity (turbidimeter HI 93703, Hanna Instruments, USA), total suspended solids (TSS), volatile suspended solids (VSS), ammonium nitrogen (NH_4^+-N), total and soluble chemical oxygen demand (COD_t and COD_s). All analyses were carried out in accordance with the Standard Methods (APHA-AWWA-WEF, 2012), except for ammonia nitrogen which was measured according to the Solórzano method (Solórzano, 1969). The COD removal was determined based on the initial COD_t in the primary effluent and the final COD_s in the treated wastewater, after removing the microalgae grown in the culture by filtration through a 0.45 μm filter.

As the main compounds of the studied wastewater are COD and ammonia nitrogen (Li et al., 2021a), these two parameters were selected to analyse the influence of the culture conditions on the treatment capacity of both microalgae cultures.

2.3.2. Total selenium determination in treated domestic wastewater

The total selenium concentration was measured at the end of each batch test from the mixed liquor of each flask after filtration over a 0.20 μm syringe PVDF membrane and in the microalgae culture by inductively coupled plasma-mass spectrometry (7800 ICP-MS, Agilent Scientific Instruments, Santa Clara, USA). The ICP-MS operating conditions and measurement parameters were: radiofrequency power, 27.2 MHz; plasma power, 1550 W; plasma gas flow, 15 L min^{-1}; sampling depth, 10 mm; nebulizer pump, 0.1 rps. The recovery of Se in the microalgae samples was assessed using reference material BCR-402 White Clover (certified value: 6.70 ± 0.25 mg Se kg^{-1}) in each digestion batch.

The removal of Se every two or three days was determined with the equation:

$$Se\ removal = \frac{C_i - C_w}{C_i} \times 100\% \quad (2)$$

where C_i and C_w are the Se concentration in the liquid phase (μg Se/L) at the beginning and at the end of the experiment, respectively.

2.3.3. Total selenium determination in microalgae biomass

For the determination of the total Se concentration in the microalgae, 0.2 g freeze-dried sample was weighed into a microwave digestion vessel followed by the addition of 9 mL concentrated HNO_3 (65 %) and 1 mL of H_2O_2 (30 %). After one-hour of incubation at room temperature, the samples were digested in closed vessels using a microwave digestion system (START D microwave digestor, Milestone Srl, Milan, Italy). This was conducted at 1200 W, 210 °C, 20 min of ramp time and 15 min of hold time. After digestion, the content of each cooled vessels was poured into 50 mL plastic tubes. The volume of the digested samples was then adjusted to 50 mL with distilled water, and the tubes were capped and shaken by hand to ensure sample homogeneity.

To investigate the possible mechanisms of Se removal of both microalgae, a Se mass balance was determined at the end of each batch test. Firstly, the Se amount (μg) remaining in the wastewater (Se_{water}) was calculated by the following equation:

$$Se_{water} = C_w \times V_E \quad (3)$$

where C_w is the Se concentration in the liquid phase (μg Se/L) at the end of the experiment and V_E is the initial volume (L) of wastewater in the Erlenmeyer flasks.

Secondly, the Se amount (μg) in the microalgae biomass ($Se_{biomass}$) was calculated at the end of the batch test using the following equation:

$$Se_{biomass} = C_B \times M_D \quad (4)$$

where C_B is the Se concentration in the microalgae biomass (mg Se/kg dry biomass) at the end of the experiment and M_D is the dry weight of biomass (kg).

Finally, the Se mass balance was calculated using the Eq. (5), where $Se_{no\ measured}$ (μg) was the selenium amount not measured in the biomass and neither present in the water after the culturing, determined by the difference between the initial Se amount (μg) in the wastewater (Se_{total}) and the sum of Se present in the medium at the end of the culturing (Se_{water}) and accumulated on the biomass ($Se_{biomass}$):

$$Se_{total} = Se_{water} + Se_{biomass} + Se_{no\ detected} \quad (5)$$

The initial selenium concentration in microalgae biomass before the treatment with Se was not considered in the calculation (~1.7 mg/kg DW).

2.4. Statistics

All the data obtained in this study were presented as mean ± standard error and analyzed using one way analysis of variance (ANOVA) with SPSS Statistics 26.0 (IBM, USA). Significant differences of the means were confirmed by pairwise post hoc analyses which is generated by using the Tukey HSD test and the result was considered statistically significant when $p < 0.10$.

3. Results and discussion

3.1. Microalgae growth on Se enriched wastewater

Both *Chlorella vulgaris* and *Scenedesmus* sp. showed tolerance to the tested selenite concentrations (0–1000 μg Se/L) as shown by the growth curves that reached the stationary phase after 15 and 24 days, respectively. For both species, the applied selenite concentrations did not show toxicity even at the higher concentration tested (1000 μg Se/L), with no significant difference in growth compared to the control cultures (Fig. 1a, c).

Li et al. (2021b) also reported a high tolerance from a microalgae consortium grown in selenite enriched wastewater in concentrations up to 500 μg Se/L, demonstrating that microalgae growing on domestic wastewater could tolerate this high Se concentration. Geoffroy et al. (2007) reported symptoms of stress or toxicity after an exposure of the microalgae *Chlamydomonas reinhardtii* to concentrations of 734 μg Se/L or above. However, in our study we were able to show that even higher concentrations of up to 1000 μg Se/L were tolerated by both *Chlorella vulgaris* and *Scenedesmus* sp. Miranda et al. (2017) also demonstrated the resistance of a mixed microalgae biofilm to high selenium concentrations (800 μg Se/L).

Sun et al. (2014) cultivated *Chlorella vulgaris* strain on BG-11 medium and found the best growth condition at 75 mg Se/L, with an improvement in growth compared to the control culture. For *Scenedesmus* strains, not many works have reported the selenium effect on growth. Vitová et al. (2011) compared a selenite resistant strain (SeIV) developed and patented by their own group to a wild-type *Scenedesmus quadricauda*. In their study, 100 mg Se/L applied to the wild-type *Scenedesmus quadricauda* was lethal and caused cell malformations, and abnormal spine number and position; while the SeIV strain grew normally and divided similarly to the untreated wild-type (Vítová et al., 2011).

3.2. Microalgae specific growth rate

Specific growth rates obtained for *Chlorella vulgaris* in all selenium concentrations tested had no significant difference from the control (maximum of 0.32 d^{-1} at 50 μg Se/L), except for the highest concentration applied which was lower than the previous one (0.22 d^{-1}, 1000 g Se/L). For

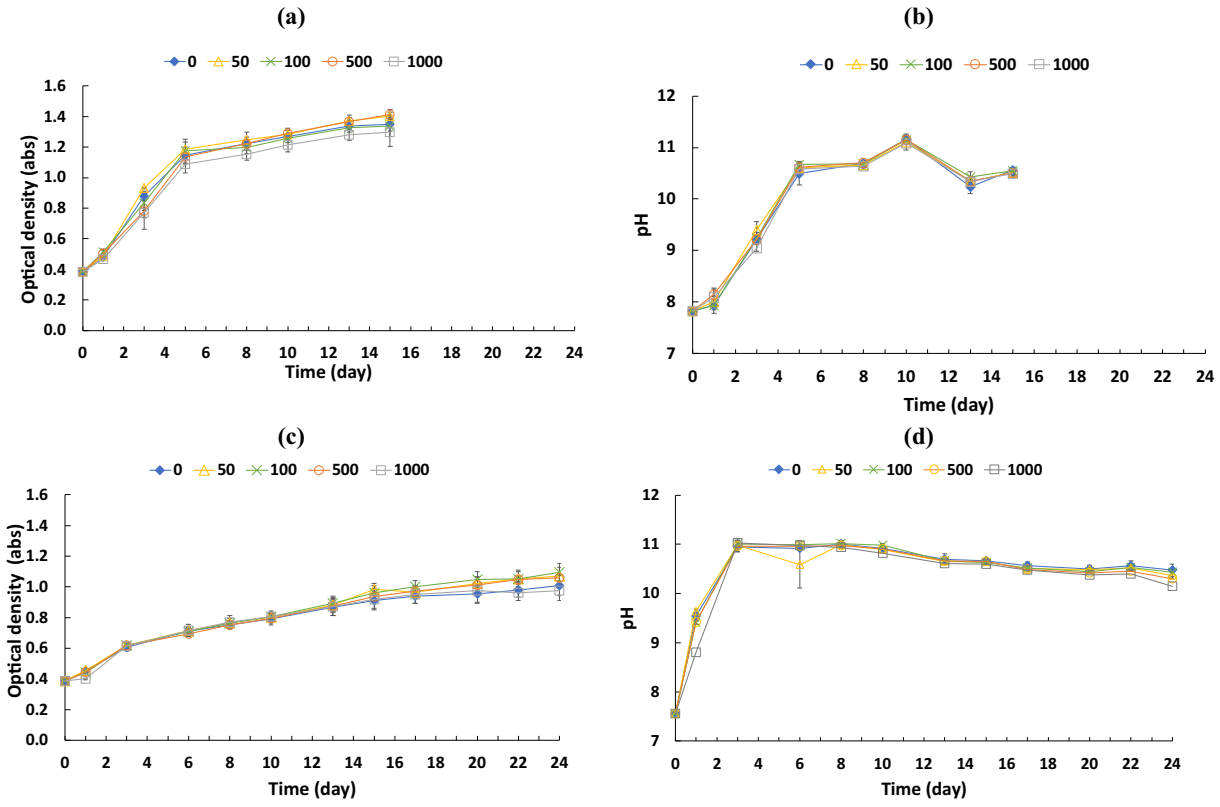

Fig. 1. Cell growth measured as OD and pH evolution during (a, b) *Chlorella vulgaris* and (c, d) *Scenedesmus* sp. growth in domestic wastewater supplemented with different Se concentrations (0–1000 μg Se/L). Values are mean ± SD (n = 3).

Scenedesmus sp., a significantly higher specific growth rate was observed at 1000 μg Se/L (0.21 d^{-1}) with respect to the other conditions including the control (Table 1).

Several studies have shown that Se is essential for the growth of some microalgae in the synthesis of proteins and lipids (Schiavon et al., 2017), but there is a narrow margin between nutritional essentiality and toxicity of Se (Zhou et al., 2017). Depending on the concentration in the media and culture conditions, Se can also stimulate growth in microalgae and this can be related to its antioxidative function (Sun et al., 2014). In this way, the highest Se concentration applied was beneficial for the *Scenedesmus* sp. growth rate, but may have had a negative effect on the *Chlorella vulgaris* specific growth rate. Nonetheless, comparing both species, no statistical difference was observed with 1000 μg Se/L.

Chlamydomonas reinhardtii exposed to different Se concentrations in the media showed a decrease in the specific growth rate as the concentration of selenium increased (Geoffroy et al., 2007). *Haematococcus pluvialis* grown on a medium enriched with selenate showed normal growth in concentrations up to 3 mg/L and levels higher than 13 mg/L restrained microalgae growth (Zheng et al., 2017). Chloroplasts are the main target for growth inhibition in microalgae cells in Se-enriched medium. The toxic concentration of Se can lead to inhibition of photosynthesis, chlorophyll biosynthesis, structural damage, and interference with other metabolic activities, such as phosphorus uptake and substitution of sulfur during the synthesis of cystine and methionine (Gojkovic et al., 2015).

Strategies for increasing the selenium concentration applied in the microalgae growth media were explored by Chen and Zheng (2006). The authors tested a stepwise addition of selenium on *Spirulina platensis* mixotrophic growth, avoiding an inhibitory effect up to an accumulative concentration of 250 mg Se/L, at which highest biomass Se concentration of 460 μg/g, and Se yield of 1033 μg/L was reached (Chen and Zheng, 2006).

There is a significant difference in the growth rates of *Chlorella vulgaris* and *Scenedesmus* sp. with Se concentrations up to 500 μg Se/L. *Chlorella vulgaris* also achieved higher biomass concentrations measured as VSS in all Se-enriched medium cultures (629 mg/L at 50 μg Se/L) even with fewer days of duration than the *Scenedesmus* sp. culture (535 mg/L at 500 μg Se/L) (Table 1). Gupta et al. (2016) also observed a better growth for a *Chlorella sorokiniana* strain than *Scenedesmus obliquus* grown in wastewater,

Table 1
Maximum growth rate and final biomass concentration for *Chlorella vulgaris* and *Scenedesmus* sp. grown in domestic wastewater supplemented with different Se concentrations (μg Se/L). Values are mean ± SD (n = 3).

Treatment (μg Se/L)	*C. vulgaris*		*Scenedesmus* sp.	
	μ_{max} (d^{-1})	Final VSS (mg/L)	μ_{max} (d^{-1})	Final VSS (mg/L)
0	$0.30 \pm 0.02^{a,b,A}$	$631 \pm 10^{a,A}$	$0.15 \pm 0.01^{b,B}$	$529 \pm 16^{a,B}$
50	$0.32 \pm 0.01^{a,A}$	$629 \pm 13^{a,A}$	$0.15 \pm 0.01^{b,B}$	$513 \pm 30^{a,B}$
100	$0.25 \pm 0.02^{a,b,A}$	$611 \pm 4^{a;A}$	$0.17 \pm 0.01^{b,B}$	$518 \pm 40^{a,B}$
500	$0.24 \pm 0.05^{a,b,A}$	$622 \pm 17^{a,A}$	$0.16 \pm 0.01^{b,B}$	$535 \pm 27^{a,B}$
1000	$0.22 \pm 0.06^{b,A}$	$604 \pm 17^{a,A}$	$0.21 \pm 0.02^{a,A}$	$518 \pm 11^{a,B}$

Different lower-case letters in columns indicate a statistical difference.
Different capital letters in rows indicate a statistical difference between *Chlorella vulgaris* and *Scenedesmus* sp. for the same concentration of Se.

showing a better adaptability to physiological stresses when cultured in raw sewage.

3.3. pH of the culture

The pH variation in microalgae cultures is mainly responsible for the dissolution of nutrients in the media. Comparing both species, there was no significant difference in the pH variation. In the *Chlorella vulgaris* culture, the pH varied from 7.83 to 11.08 (Fig. 2b) and in *Scenedesmus* sp. from 7.55 to 11 (Fig. 2d). Both microalgae showed an increase in the first 4 and 3 days of culturing, respectively, from 7.83 to 10.5 (*Chlorella vulgaris*) and from 7.5 to 11.5 (*Scenedesmus* sp.). This increase in the first days is consistent with the stage identified as the exponential growth phase of microalgae growth, where cells start their division process, reaching a constant generation time and maximum growth rate. The increase in the pH of the culture can be attributed to the consumption of inorganic carbon releasing basic bioreaction metabolites. In this process, rubisco fixes carbon during the photosynthesis. In the photosynthesis, intracellular OH^- ions need to be neutralized by H^+ ions, which are absorbed from the extracellular medium, thus increasing the pH. This increase in pH by green microalgae growth has been widely observed, and other authors like Yang et al. (2022) and Xue et al. (2021), both culturing *Chlorella sorokiniana* strain in wastewater, also identified this rapid increase in pH during the first days of culturing after inoculation.

Microalgae are capable of using CO_2 and bicarbonate (HCO_3^-) as carbon source, but not carbonate (CO_3^{2-}) (García et al., 2000; Su, 2021). The main inorganic carbon source in domestic wastewater is typically bicarbonate. But if the pH increases as a result of microalgae growth, then the main form of inorganic carbon becomes CO_3^{2-}, which can explain the reduction in the growth rate of microalgae observed when the pH increased to values close to 11.

The increase in pH also has an influence on the nitrogen forms present in the culture medium (García et al., 2000). Absorption of nitrogen by microalgae increases the pH of the medium, as every nitrate ion reduced to ammonia produces one OH^-. In the studied wastewater, the main form of nitrogen was ammonia. However, at pH higher than 9.2, alkaline conditions can promote the formation of NH_3 from NH_4^+, which can be volatilized. Indeed, when the pH increase to values where ammonia could have been volatilized a decrease in the growth of *Chlorella* (day 5) and *Scenedesmus* (day 3) were observed (García et al., 2000; Su, 2021).

The pH values found in these experiments can also indicate microalgae predominance in the cultures. The pH increase can be considered one of the removal mechanisms of pathogens by microalgae in wastewater treatment as most of them do not resist a pH higher than 9 (Dar et al., 2019). Values between 9 and 11 indicate a predominance of microalgae in the wastewater, mainly due to the transformation of CO_2 in carbonates through photosynthesis. The decrease in bacterial proliferation and increase in microalgal proliferation can lead to an increase in pH (to values above 9) in the medium due to the reduction of dissolved CO_2.

3.4. COD and ammonium removal from domestic wastewater

Domestic wastewater can contain human excreta, detergents, pharmaceutical drugs, pathogenic bacteria, household wastes, solid garbage wastes, plants or animal tissues, heavy metals, and salts. These compounds contribute to the total organic (e.g. COD) and nutrient (e.g. nitrogen as ammonium) load in the wastewater. The wastewater composition is variable according to environmental and seasonal changes. In this study, domestic wastewater after primary treatment was used (Table 2).

For *Chlorella vulgaris*, no significant difference was observed for COD removal with Se concentrations up to 50 μg Se/L compared with the control (64 % removal). In these conditions (control and 50 μg Se/L), COD levels were above the 125 mg/L value defined by the European wastewater treatment directive (Directive 91/271/CEE). From 100 μg Se/L, an increase of 17 % in the removal efficiency was observed, reaching 81 % in this condition, meeting the limits for treated wastewater discharge in the environment (Fig. 3a). For the *Scenedesmus* sp. culture, the COD removal varied from 78 to 87 %, reaching a maximum value at 500 μg Se/L, but with no difference among treatments. Gupta et al. (2016) found a 76.1 % COD

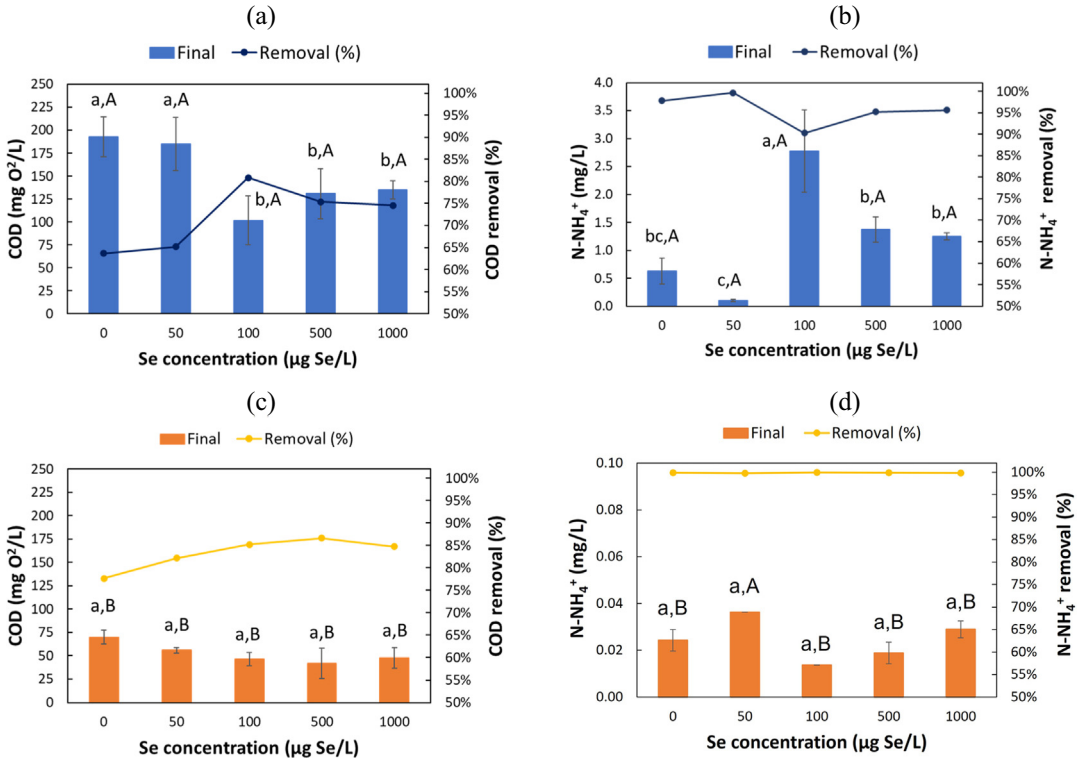

Fig. 2. Final effluent concentration and removed COD and ammonium in microalgae culture in domestic wastewater supplemented with different Se concentrations (μg Se/L) of (a, b) *Chlorella vulgaris* and (c, d) *Scenedesmus* sp. Different lower-case letters indicate a statistical difference between the Se concentration for each microalga for COD and $N\text{-}NH_4^+$. Different capital letters indicate a difference between *C. vulgaris* and *Scenedesmus* sp. for each Se concentration.

Table 2
Average characteristics of the domestic wastewater used in this study.

Parameter	Value
pH	7.69 ± 0.20
Turbidity (NTU)	144 ± 77
COD_t (mg/L)	423 ± 153
COD_s (mg/L)	158 ± 69
N-NH_4^+ (mg/L)	22.5 ± 9.2
TSS (mg/L)	165 ± 88
VSS (mg/L)	144 ± 64

removal for *Scenedesmus obliquus*, a higher value than the one found for *Chlorella sorokiniana* that reached 69.4 %, which is in agreement with this study. Gupta et al. (2016) attributed this difference to the higher mixotrophic growth potential of *Scenedesmus* as compared to *Chlorella*.

The higher removal of COD for *Scenedesmus* sp. compared to *Chlorella vulgaris* may also be related to the fact that the removal can be affected by the release of organic compounds by microalgae, mainly during the stationary phase in a batch process (Lee et al., 2019). As *Chlorella vulgaris* has a higher final biomass production than *Scenedesmus*, maybe this biomass accumulation harmed the COD removal by microalgae because of the greater generation of exudates or cell breakdown products from *Chlorella* leading to less COD removal. Lee et al. (2019) followed the daily COD removal for *Coelastrum microporum* and found an increase in COD on day 3 of incubation and also that different consortia composition can affect this parameter. Several studies have reported that the interaction between microalgae and bacteria can play an important role in the wastewater COD removal (Wang et al., 2016; Zhu et al., 2013). Indeed, microalgae photosynthesis produces oxygen for aerobic heterotrophic bacteria to biodegrade organic matter, while in turn, bacteria break down organic molecules releasing carbon dioxide utilized by microalgae (Xu et al., 2019). Moreover, each microalgae species produces specific metabolites that can alter the wastewater's microbial population, resulting in different COD removal rates (Lee et al., 2016).

For ammonia, the removal efficiencies varied from 90 to 96 %, decreasing at 100 μg Se/L (Fig. 3b) for *Chlorella vulgaris*, but with no significant difference from the other conditions. The *Scenedesmus* sp. removal efficiency was up to 99 %. The primary mechanism of ammonia removal by microalgae is biomass assimilation, as they use it as a nitrogen source mainly for protein synthesis. According to Chai et al. (2021), strains like *Chlorella vulgaris* were able to uptake nitrogen from ammonium ions through the cell membrane via ammonium assimilation. The high removal efficiencies of NH_4^+ can also be partially attributed to volatilization/stripping (Li et al., 2021a). This is one of the most important mechanisms of ammonia removal in microalgae cultures with high growth rates which occurs under the influence of a pH above 9.2 by the formation of NH_3. Gupta et al. (2016) also found a better ammonium removal by *Scenedesmus* compared to a *Chlorella* strain when culturing these microalgae in raw sewage. Still, in our study, both *Chlorella* and *Scenedesmus* were able to reduce the ammonium content from 50 mg N-NH_4^+/L to values below 3 mg N-NH_4^+/L (0.10–2.78 and 0.02–0.01 mg N-NH_4^+/L in the case of *Chlorella* and *Scenedesmus*, respectively).

3.5. Selenium removal and accumulation in biomass

The Se metabolism in microalgae is considered analogous to that in higher plants, and Se is incorporated in amino acids and proteins through the sulfur assimilation metabolic pathway (Bottino et al., 1984). For both microalgae, the increase in the selenium concentration in the cultures reduced their capacity to remove selenium from the wastewater (Fig. 3). Morlon et al. (2006) reported that the selenite transport for a green microalga (*Chlamydomonas reinhardtii*) was conducted via two different transport systems depending on the selenite concentration in the culture medium. According to the author, at low concentrations, the selenite is transported by a specific but rapidly saturated transport system and at higher concentrations by a non-specific transport system that depends on the extracellular selenite concentration (Morlon et al., 2006). The same behavior was already reported to other microalgae like *Haematococcus*

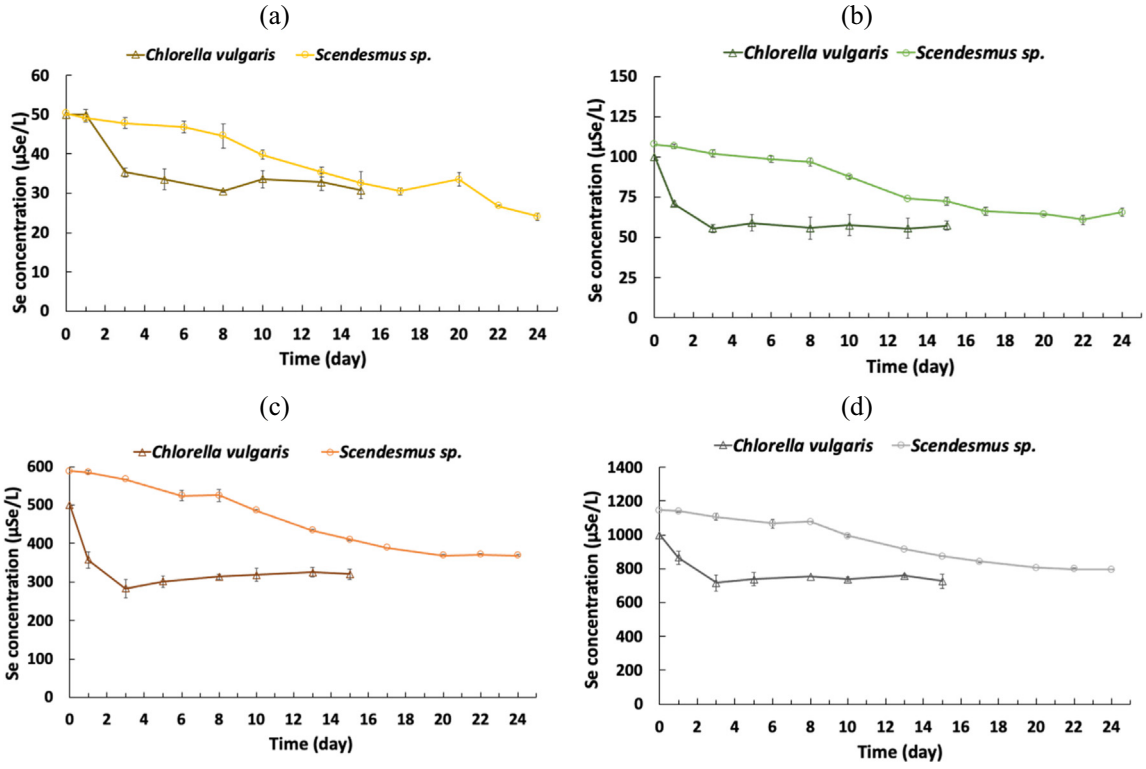

Fig. 3. Selenium concentration on wastewater for *Chlorella vulgaris* and *Scenedesmus* sp. culture in wastewater enriched with different selenium concentrations: (a) 50 μg Se/L (b) 100 μg Se/L (c) 500 μg Se/L and (d) 1000 μg Se/L.

pluvialis (Zheng et al., 2017). Li et al. (2021b) also observed the same behavior on selenium uptake by a microalgae consortium cultured in Se-enriched wastewater.

Chlorella vulgaris was able to remove up to 43 % of selenium from wastewater (100 μg Se/L, day 15) and *Scenedesmus* sp. up to 52 % (50 μg Se/L, day 24) (Fig. 3). Comparing the two microalgae, even with fewer days of cultivation, *Chlorella vulgaris* removed more Se from wastewater than *Scenedesmus* sp. at concentrations exceeding 100 μg Se/L. This higher removal value for *Scenedesmus* at 50 μg Se/L was mainly due to the longer exposure time to the Se in the culture. When comparing the uptake of the two microalgae at 15 days with the same Se concentration (50 μg Se/L), *Chlorella vulgaris* had no significant difference from *Scenedesmus* sp. on the Se removal.

In concentrations up to 500 μg Se/L, *Chlorella vulgaris* (15 days of culture) and *Scenedesmus* sp. (24 days of culture) showed no significant difference in the accumulation of Se in the biomass (190 mg Se/kg DW and 175 mg Se/kg DW, respectively at 500 μg Se/L) (Fig. 4). At 1000 μg Se/L, *Chlorella vulgaris* reached a value of 323 mg Se/kg DW, almost 100 mg of Se more than *Scenedesmus* sp. per kg of dry biomass.

Chlamydomonas reinhardtii cultured in a medium supplemented with 1580 μg Se/L (selenite form) accumulated 396.1 mg Se/kg DW in a period of 4 days, corresponding to the complete algal growth cycle (from inoculation to stationary growth phase) (Morlon et al., 2005). Similarly to *Chlorella vulgaris* and *Scenedesmus* sp., in addition to the reduction of the removal rate proportional to the Se concentration increase in the media, the accumulation in biomass increased with the increase of the Se compound. According to the authors, the *C. reinhardtii* culture reached a value of up to 1507.6 1 mg Se/kg DW for the culture with 3950 μg Se/L (Morlon et al., 2005).

The Se accumulation and the transformation of this element in organic compounds is considered a mechanism of detoxification and adaptation of algae to Se present in aquatic environments and a storage method in cells (Bodnar et al., 2015). Se removal by microalgae depends on cell uptake and detoxification mechanisms that varies according to the Se chemical form, concentration, exposure time, media composition, and algae species (Schiavon et al., 2017). According to Schiavon et al. (2017) the main mechanisms are reduction and accumulation, volatilization, and incorporation into proteins. The adsorption of Se to the microalgae cell wall was not significant compared to the uptake (Schiavon et al., 2017).

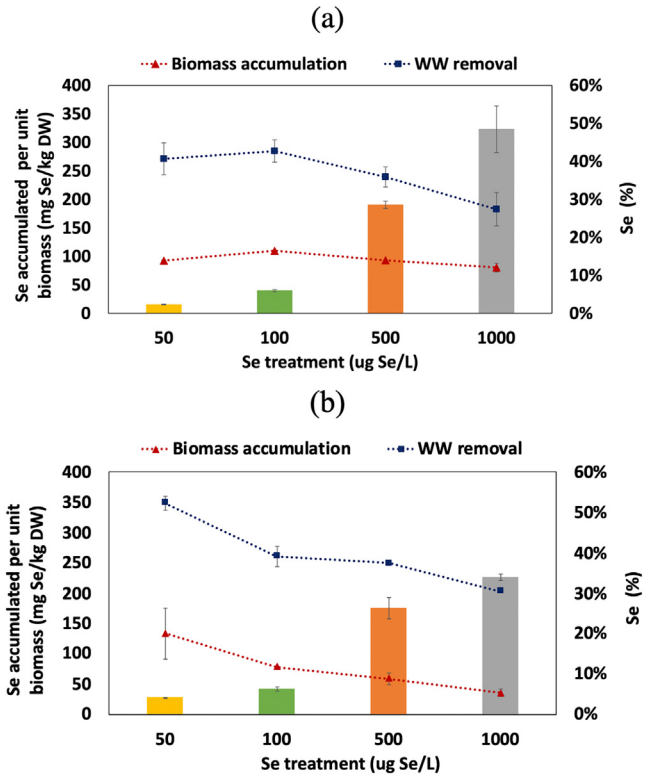

Fig. 4. Se concentration removed from the wastewater (%) and accumulated in the biomass (%) of (a) *Chlorella vulgaris* and (b) *Scenedesmus* sp. The bars indicate the Se accumulated per unit biomass (mg Se/kg DW).

Analyzing the mass balance of Se in the cultures (Fig. 5), it was possible to observe that most of the Se removed was not in the biomass. This can be due to volatilization mechanisms of removal by microalgae as green microalgae can accumulate inorganic Se and metabolize it to organic compounds like selenomethionine and selenocysteine, and volatilization of Se may present the detoxification response of microalgal cells (Neumann et al., 2003). Upon uptake, selenite can be gradually reduced to selenide (Se^{2-}) that can be incorporated specifically into proteins via the selenocysteine insertion mechanism or gradually metabolized as selenium proteins selenomethionine (SeMet) and selenocysteine (SeCys). These Se-amino acids can be further incorporated non-specifically into proteins instead of Cys and Met, causing significant alteration in protein structure with function disruption. To excrete excess intracellular Se and alleviate Se toxicity, algae tend to convert SeMet and SeCys into the volatile compounds dimethylselenide (DMSe) and dimethyldiselenide (DMDSe), respectively (Zhou et al., 2017).

3.6. Potential valorization of Se-enriched biomass

The Se-rich microalgae biomass produced in the cultures of *Chlorella vulgaris* and *Scenedesmus* sp. has the potential for application as biofertilizer or animal feed, and even dietary supplement depending on the source and composition of the wastewater. Indeed, the so-called "waste" streams may have different composition depending on their origin, and regulations ought to refer to the contaminants that are actually present rather than the origin of the "wastewater" being treated or used as growth media. The recovery of nutrients from waste streams in the microalgae biomass and its further valorization would contribute to the deployment of the circular economy (López-Pacheco et al., 2021).

Daily Se requirements are approximately 40 μg for adults, whereas doses of 200 μg/day are considered therapeutic, and levels above 800 μg/day are considered toxic (Gojkovic et al., 2015). In selenium biofortified microalgae biomass, in addition to the Se incorporated into the biomass; microalgae can have a rich composition of micro and macronutrients such as proteins, vitamins, essential fatty acids and amino acids that are important for the human diet and animal feed.

Generally recognized as safe for consumption, strains like *Spirulina* (Chen et al., 2021) and *Chlorella* (Gómez-Jacinto et al., 2020) enriched with Se have already been suggested as a food supplement. *Scenedesmus quadricauda* was already used as a supplement for chicken feed and was compared with a sodium selenite enriched diet. Se concentrations in breast muscle were more pronounced in the diet with Se-rich microalgae biomass (Skřivan et al., 2010). Li et al. (2021b) evaluated Se-enriched microalgae of a natural consortium grown on domestic wastewater as animal feed. They found that 49–63 % of Se in the microalgae was bioaccessible for animals.

Microalgae biomass grown in abundance of nutrients may be rich in nitrogen and growth promoters and may be used as biofertilizer and biostimulant for crops (Morais et al., 2021). Shaaban (2001) reported an increase in the dry weight of shoots and roots of maize grown on a soil with *Chlorella vulgaris* using 150 and 200 kg microalgae per 0.42 ha. Li et al. (2021a) studied the application of a microalgae consortium dominated by *Chlorella* sp. grown on domestic wastewater enriched with Se as biofertilizer for beans. They studied both the soil drench and foliar application of the extract. The authors observed that the Se-enriched microalgae extract was beneficial for seed germination and seedling growth of beans. Moreover, selenium was accumulated in all tissues of the bean plant raising gradually with the increasing dosage of Se-enriched microalgae amendment. The Se content of the beans increased stepwise from 1.05 to 4.15 mg Se kg^{-1} in the roots, from 0.12 to 0.34 mg Se kg^{-1} in the leaves, from 0.09 to 0.42 mg Se kg^{-1} in the stems, and from 0.10 to 0.28 mg Se kg^{-1} in the seeds (Li et al., 2021a).

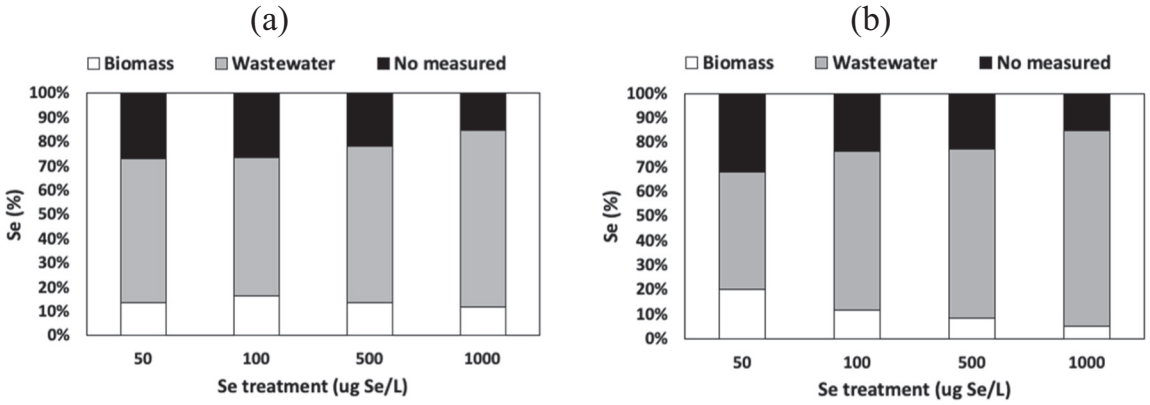

Fig. 5. Se mass balance for (a) *Chlorella vulgaris* and (b) *Scenedesmus* sp. at the end of batch experiments using domestic wastewater supplied with different Se concentrations (μg Se/L).

4. Conclusion

In this study, two green microalgae species, *Chlorella vulgaris* and *Scenedesmus* sp., were evaluated for their capacity remove/recover Se from urban wastewater and incorporate it into biomass. Both of them were able to grow with Se concentrations up to 1000 μg Se/L, showing Se removal efficiencies of 43–52 %. *Chlorella vulgaris* showed higher potential for growth and removal of Se from wastewater, incorporating up to 323 mg Se/kg DW in biomass (1000 μg Se/L). The Se-rich biomass produced may be applied to the supplementation of animal feed or used as biofertilizer for biofortification of crops.

CRediT authorship contribution statement

Methodology, Formal analysis and investigation, Data curation, Writing original draft preparation: Murillo, A.M., Uggetti, E., Ferrer, I., Lens, P. Writing and draft preparation, Conceptualization, Data curation, review and editing: Morais, E.G. - Review and editing, Conceptualization, Funding acquisition, Resources and Supervision: Uggetti, E.; Lens, P.N.L. and Ferrer, I.

Data availability

The data that has been used is confidential.

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.

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