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Biosorption of Cd(II) by Nordic microalgae: Tolerance, kinetics and equilibrium studies

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ABSTRACT

The amount of heavy metals released into the environment has significantly increased. Industrial wastewaters, e.g. from mining or battery manufacturing, are often polluted with heavy metals such as Cd, Cr or Pb. These metals threat the environment and can cause health problems even at low concentrations. Therefore, their proper removal from industrial wastewater before its disposal is of paramount importance (Javanbakht et al. [1]). Here the ability of fourteen wild Nordic microalgal strains to remove cadmium (Cd(II)) from aqueous solutions has been studied. Three of the chosen strains, namely *Chlorella vulgaris* (13-1), *Coelastrella* sp. (3-4) and *Scenedesmus obliquus* (13-8), demonstrated high tolerance towards Cd(II) concentrations up to 2.5 mg L^{-1} and their sorption kinetics and equilibrium were studied. Metal sorption by *Chlorella vulgaris* (13-1) and *Coelastrella* sp. (3-4) was described best by pseudo-second order kinetics, whereas the removal kinetics of *Scenedesmus obliquus* (13-8) was best fitted by the intraparticle diffusion model. Starting from an initial concentration of 2.5 mg L^{-1} *Chlorella vulgaris* (13-1) and *Coelastrella* sp. (3-4) removed 72% and 82%, respectively, of the Cd(II) within only 24 h. Modeling their Cd(II) sorption equilibria revealed that the SIPS- and Dubinin-Radushkevich models were best suited for living microalgae, and the maximum adsorption capacity (q_{max}) was calculated. While *Chlorella vulgaris* (13-1) and *Coelastrella* sp. (3-4) were able to remove about 49 mg g^{-1} and 65 mg g^{-1} Cd(II), respectively, *Scenedesmus obliquus* (13-8) only removed around 25 mg g^{-1}. Fourier-Transform Infrared Spectroscopy (FTIR) analyses of the biomass revealed the carboxylic moieties of the cell wall to be the key player in Cd(II) removal. This study demonstrates the high potential of Nordic microalgae to remove heavy metals at conditions relevant for an industrial tertiary wastewater treatment unit and will support the development of new, biobased, innovative technologies for the bioremediation of heavy metal polluted streams.

1. Introduction

Within the last years Nordic microalgal species have been investigated regarding their potential to remediate different types of wastewater [2–4]. Their ability to grow very densely and fast in quite harsh conditions together with their large capacity to uptake nitrogen and phosphorous has made of these microorganisms excellent candidates to perform biological water treatment. While the removal capacities of pharmaceutical compounds from urban wastewater by Nordic microalgae have been investigated previously [5–7], their potential to adsorb heavy metals has so far been neglected.

Heavy metals cause dramatic problems when released into the environment, since they cannot be biodegraded; instead many of them tend to bio-accumulate in the trophic chain and end up causing very severe health problems in humans [1]. Cadmium (Cd(II)) is one of the metals exhibiting high bioaccumulation capacity and severe toxicity. Cd(II) intoxication can lead to kidney or liver damage, or even have impact on the development of melanoma [8].

Conventional methods to clean wastewaters from heavy metals are based on chemical precipitation, coagulation/flocculation, adsorption on chelating or ion exchange resins and membrane filtration. These methods are regularly very expensive or ineffective, especially at low (but still hazardous) concentrations [9–11]. Thus, there is a need to develop new technologies for efficient removal of these contaminants with the aim of improving the water treatment performance and reducing the acquisition and operational costs, while minimizing the carbon-footprint of large-scale treatment plants [12]. Less conventional and more recently developed techniques rely on the use of biomass to

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Table 1
Swedish microalgal strains and their assigned strain ID.

Family	Strain name	Strain ID	Water source
Chlamydomonadales i. s.	*Ettlia pseudoalveolaris*	FNY-2	Umeå - Lake Nydala (FW)
Chlorellaceae	*Chlorella sorokiniana*	B1-1	Dåva (MWW)
Chlorellaceae	*Chlorella sorokiniana*	2-21-1	Örnsköldsvik (IWW)
Chlorellaceae	*Chlorella vulgaris*	13-1	Umeå (MWW)
Chlorellaceae	*Micractinium* sp.	P9-1	Borås (FW)
Scenedesmaceae	*Coelastrella* sp.	3-4	Umeå (MWW)
Scenedesmaceae	*Coelastrum microporum*	FNY-1	Umeå - Lake Nydala (FW)
Scenedesmaceae	*Desmodesmus opoliensis*	SQ2	Bäckhammar (FW)
Scenedesmaceae	*Desmodesmus* sp.	2-6	Skåne - Lake Ringsjön (FW)
Scenedesmaceae	*Desmodesmus* sp.	RUC-2	Umeå - River Campus (FW)
Scenedesmaceae	*Scenedesmus obliquus*	13-8	Umeå (MWW)
Scenedesmaceae	*Scenedesmus obliquus* (UTEX 417)	RISE	Bäckhammar (FW)
Scenedesmaceae	*Scenedesmus* sp.	B2-2	Dåva (MWW)
Scenedesmaceae	*Scotiellopsis reticulata*	UFA-2	Dåva (MWW)

Water source: FW = fresh water; IWW = industrial wastewater; MWW = municipal wastewater [16].

remove pollutants [13–15]. Microalgae have become highly attractive in current wastewater treatment applications. Due to their multilayered cell walls with its functional groups as well as their mass to solution volume ratio they can remove high amounts of metals [9,11,15].

This study focused on the selection of Cd(II) tolerant microalgae strains from the Nordic environment and used them to remove this heavy metal from aqueous effluents in concentrations relevant for a tertiary treatment of an industrial process. Fourteen natural strains were screened for their ability to grow in the presence of cadmium, and the sorption kinetics and equilibrium of the most tolerant strains were characterized. Both, kinetics and equilibrium data were modeled to provide a mathematical description of the Cd(II) removal process and retrieve the characteristic constants that can be further employed in the design of future water treatment schemes. Three of these 14 strains showed a remarkably higher tolerance and therefore were further investigated. In order to get further insights into the mechanisms that govern the uptake of Cd(II) by these microalgae, Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) was employed, and the involvement of functional groups involved in the interaction Cd(II)-microalgae was investigated.

2. Materials and methods

2.1. Algal cultivation

Nordic strains had been collected from different water bodies in Sweden [16]; *Scenedesmus obliquus* – UTEX 417 (RISE), originating from the UTEX culture collection (University of Texas, USA) was used as reference strain. This strain had been cultured in a pond in Bäckhammar, Southern Sweden, for several years and therefore had been able to adapt to the Nordic climate. However, it is not cold tolerant and easily outcompeted by native strains [2,17]. Among the 13 Nordic strains, 8 belonged to the *Scenedesmaceae* family, 4 to the *Chlorellaceae* family and 1 to the family of *Chlamydomonadales incertae sedis* (see Table 1). *Chlorellaceae* and *Scenedesmaceae* have been investigated in the past regarding their ability to remove heavy metals [18,19]. The Nordic strains showed fast growth during previous experiments in municipal wastewater [2,16], however, studies on the potential of Nordic strains to tolerate and remove heavy metals are missing.

Cultures of the 14 selected microalgal strains were maintained on BG 11 Agar plates at 20 °C under continuous light with an intensity of 50 μmol m^{-2} s^{-1}. A pre-inoculum of each strain was grown under sterile conditions in TC flasks T75 (Sarstedt), filled with 20 mL BG11, pH 7.2, in a thermostatic orbital shaker at 120 rpm, 20 °C and 100 μmol m^{-2} s^{-1} [20].

To screen the tolerance towards heavy metals, algal growth was monitored in the presence of the Cd(II) at different concentrations (0 to 50 mg L^{-1}). Concentrations between 0.1 and 100 mg L^{-1} can be expected in different types of industrial wastewater [21]. 6–8-day old pre-inoculum of each strain was re-inoculated and diluted with fresh culture medium to achieve an optical density (OD_{750}) of 0.1 to allow full tracking of the microalgal growth phases. Four milliliters of diluted culture were transferred into a well of a 6-well plate (Sarstedt) and maintained in a thermostatic orbital shaker as described above. Optical density was measured daily at 680 nm and 750 nm until the end of the experiment. After 72 h the 6-well plates were spiked with Cd(II) (except the control) and kept in the shaker.

2.2. Analysis of optical density

Optical densities of the cultures were determined at 680 nm and 750 nm using an UV/VIS spectrophotometer (T90+ PG Instruments Ltd) and 10 mm light path polystyrene cuvettes. When growing in 6-well plates, optical densities were measured at the same wavelength in a Spectramax® 190 plate reader (Molecular Devices LLC) after sample transfer into 96 well plates.

2.3. Cd(II) sorption kinetics and equilibrium

To monitor Cd(II) removal in time-course experiments, the microalgal strains were grown in 0.75 L BG11, pH 7.2, bubbled with air at a flow rate of 0.5 L min^{-1} at 20 °C and 100 μmol m^{-2} s^{-1}. [6,16,22]. At the late log phase of growth, triplicates of the cultures were diluted with fresh BG11 culture medium to achieve 0.4 mg biomass mL^{-1}. The pH of the triplicates (controls & microalgae) was adjusted to 5.5 using a 0.1 M hydrochloric acid solution with the purpose to provide a representative pH of a tertiary treatment while avoiding the precipitation of cadmium hydroxides. Right after the pH adjustment, the solutions were spiked with a concentrated Cd(II) solution (1000 mg L^{-1}) to achieve a metal concentration in the microalgae photobioreactor of 2.5 mg L^{-1}.

Afterwards, the cultures were placed in an orbital shaker at room temperature illuminated with white light at an intensity of 100 μmol m^{-2} s^{-1}. Samples (1 mL) were taken after 0, 5, 10, 15, 30, 60, 120, 240, 480 and 1440 min, respectively. The samples were centrifuged immediately at 25,000*g* for 2 min at 4 °C to separate the solid biomass from the medium. The biomass pellet was removed, and the supernatant was stored at −20 °C until further analysis. Cd(II) removal by Nordic microalgae was determined by measuring the remaining cadmium concentration in the supernatant at given time points.

The characterization of the remaining Cd(II) concentration in solution in all the experiments was performed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (7700 Series ICP-MS, Agilent Technologies). Prior to analysis, each sample of the supernatant was diluted with Milli-Q water to 10 mL and acidified with concentrated nitric acid (Suprapur®, Merck Millipore, Germany) to reach a 1% of acid in the sample. The ICP-MS methodology employed allowed achieving a limit of detection (LOD) of 0.007 μg L^{-1}. Data acquisition and processing was performed using the Agilent Mass Hunter software.

2.4. Modeling Cd (II) sorption kinetics and equilibrium

Different models were used to describe the Cd(II) sorption kinetics and equilibrium. Pseudo-first- and pseudo-second order models were used to describe biosorption under non-equilibrium conditions. The pseudo-first order equation [23] expresses the idea of biosorption from the liquid phase according to the differential Eq. (1):

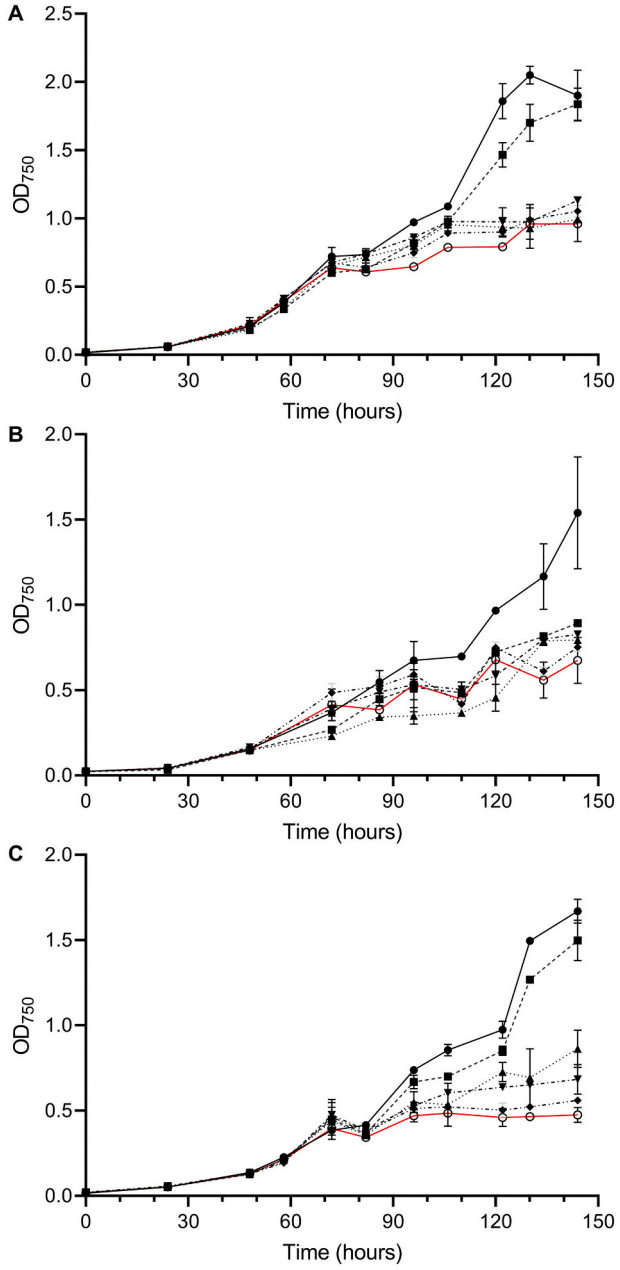

Fig. 1. Growth curves of *Chlorella vulgaris* (13-1) (A), Coelastrella sp. (3-4) (B) and *Scenedesmus obliquus* (13-8) (C) in the absence or presence of Cd^{2+} (● 0 mg L^{-1}; ■ 2.5 mg L^{-1}; ▲ 5.0 mg L^{-1}; ▼ 10 mg L^{-1}; ◆ 20 mg L^{-1}; ○ 50 mg L^{-1} (red)). Mean ± SD of three biological replicates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$$\frac{dq}{dt} = k_1(q_e - q_t) \quad (1)$$

where k_1 (h^{-1})) is the pseudo-first order constant, q_t (mg g^{-1}) the amount of heavy metal adsorbed at time t and q_e (mg g^{-1}) the maximal amount of heavy metal adsorbed at equilibrium. In the pseudo-second order Eq. (2) [24,25] the presence of two surface sites is essential. In this equation, k_2 (g mg^{-1} h^{-1}) is the pseudo-second order constant.

$$\frac{dq}{dt} = k_2(q_e - q_t)^2 \quad (2)$$

In addition, the intraparticle diffusion model, or Weber-Morris equation, has been used to describe the adsorption at the microalgal surface:

$$q_t = k_i t^{0.5} \quad (3)$$

with q_t (mg g^{-1}) being the amount of cadmium adsorbed at time t (h) and k_i (mg g^{-1} $h^{-0.5}$) being the intraparticle diffusion rate constant. This model assumes the intraparticle diffusion itself being the rate-limiting step in the biosorption process [26].

Four different isotherm models were compared to describe the removal of cadmium by Nordic microalgae, the equations of Langmuir (4) [27], Freundlich (5) [28], Sips (6) [29] and Dubinin-Radushkevich (7) [30]:

$$q_e = \frac{q_{max} * K_L * C_e}{(1 + K_L * C_e)} \quad (4)$$

$$q_e = K_F * C_e^{\left(\frac{1}{nF}\right)} \quad (5)$$

$$q_e = q_{max} * \left(\frac{K_S * C_e^{nS}}{1 + K_S * C_e^{nS}}\right) \quad (6)$$

$$q_e = q_{max} * e^{\left(-K_{DR} * \varepsilon^2\right)} \text{ being } \varepsilon = RT * ln\left(1 + \frac{1}{C_e}\right) \quad (7)$$

where q_{max} (mg g^{-1}) is the maximum amount of cadmium sorbed by the microalgae, C_e (mg L^{-1}) is the cadmium concentration at equilibrium, K_L, K_F, K_S, K_{DR}, n_S and n_F are constants related to the isotherm model, R is the kinetic gas constant (8.314 J mol^{-1} K^{-1}) and T (K) is the temperature of the system. Statistical analyses and curve fitting were performed in GraphPad Prism (Version 8.4.0) using Non-Linear Least Square regression (NLLS).

2.5. FTIR characterization of the biomass

Fourier Transform Infrared Spectroscopy (FTIR) analyses were performed on microalgae biomass in order to identify the functional groups involved in the adsorption of Cd(II). For this purpose, microalgal strains were grown in a 1 L bubbling culture of BG11, pH 7.2 at 20 °C and 100 µmol m^{-2} s^{-1}, supplied with 5% CO_2 at a volumetric flow rate of 0.5 L min^{-1} to provide sufficient CO_2 to support fast growth of the microalgae without acidifying excessively the growth media. This rate in the feeding gas stream was verified channeling through tubing the CO_2 into a water-filled inverted measuring cylinder and measuring the volume of water displaced from the cylinder by the incoming gas.

In the late log phase of growth, the cultures were diluted with fresh BG11 to achieve a microalgal concentration of 0.4 mg mL^{-1} and a final volume of 25 mL. After pH adjustment to pH 5.5 to avoid precipitation of cadmium hydroxides, triplicate samples were spiked with either 0, 50, 100 or 500 mg L^{-1} Cd(II) and shaken in a thermostatic orbital shaker at 120 rpm, 20 °C and 100 µmol m^{-2} s^{-1}. Those levels were chosen after modeling the maximum amount of adsorbed cadmium by the microalgae. While 50 mg L^{-1} and 100 mg L^{-1} cover the maximum adsorption capacity of the microalgae based on the modeling, the 500 mg L^{-1} were chosen to ensure a complete occupation of the functional groups that act as active sorption sites. After 4 and 24 h of exposure, the biomass was harvested by centrifugation at 15 °C and 2000*g* for 15 min. The biomass was freeze-dried, mixed with KBr and analyzed by Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS). Spectra were recorded in the range of 400–4000 cm^{-1} (128 scans per sample with a spectral resolution of 4 cm^{-1}) using OPUS (version 6.5). After importing the data into MATLAB, the infrared spectra were processed using a free open-source MATLAB-based script provided by the Vibrational Spectroscopy Core Facility at Umeå University. The spectra were baseline corrected by asymmetric least squares (λ = 10.000.000, p = 0.001), normalized to the amide I band (Region Min-Max 1600–1760 cm^{-1}) and

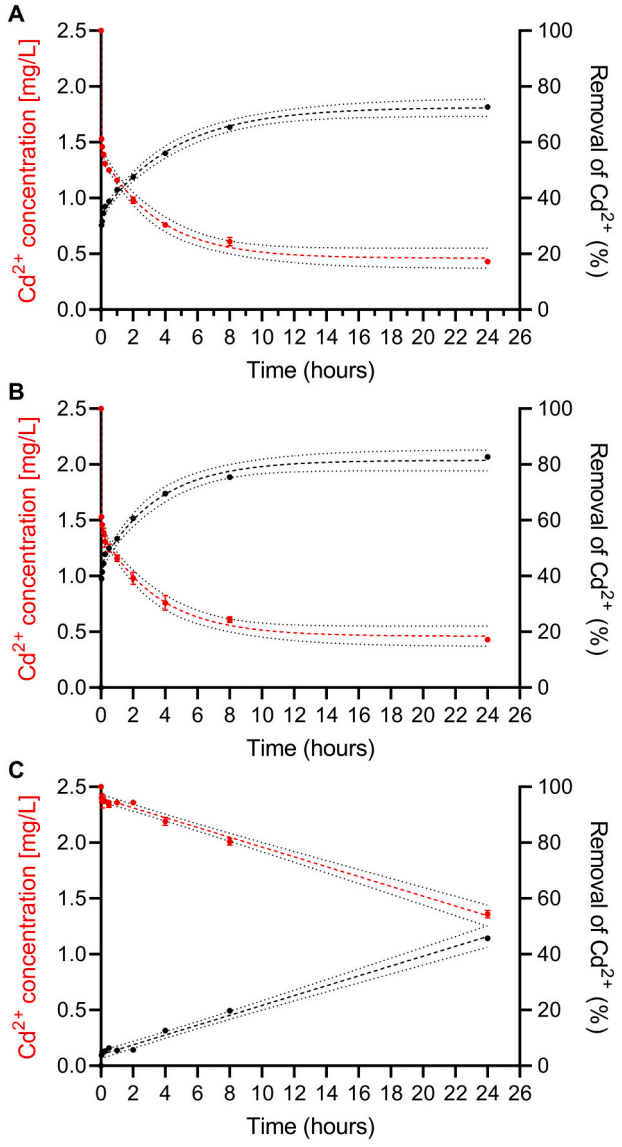

Fig. 2. Removal of Cd^{2+} by the Nordic Microalgae *Chlorella vulgaris* (13-1) (A), *Coelastrella* sp. (3-4) (B) and *Scenedesmus obliquus* (13-8) (C) after exposure to Cd^{2+} (2.5 mg L^{-1}) for 24 h. Data is presented as Mean ± SD of three biological replicates.

slightly smoothed (Savitzky-Golay smoothing, 1st order polynomial, frame = 5). For further analysis of the recorded spectra the 2nd derivative was calculated.

3. Results and discussion

3.1. Determination of microalgae tolerance to Cd(II)

Thirteen native Swedish microalgal strains and one strain originating from the UTEX culture collection, able to grow in waste streams under Nordic climate conditions [16], were screened based on their ability to survive in the presence of cadmium.

Tolerance to cadmium was investigated by growing the microalgae in the absence and presence of Cd(II) at concentrations ranging from 0 to 50 mg L^{-1} (Supplementary Fig. S1A–K). Only three out of the 14 strains showed tolerance to higher concentrations of cadmium (Fig. 1); *Chlorella vulgaris* (13-1) (Fig. 1A), *Coelastrella* sp. (3-4) (Fig. 1B) and *Scenedesmus obliquus* (13-8) (Fig. 1C). The presence of cadmium concentrations ≥5 mg L^{-1} affected growth of *C. vulgaris* and *S. obliquus* already 24 h after

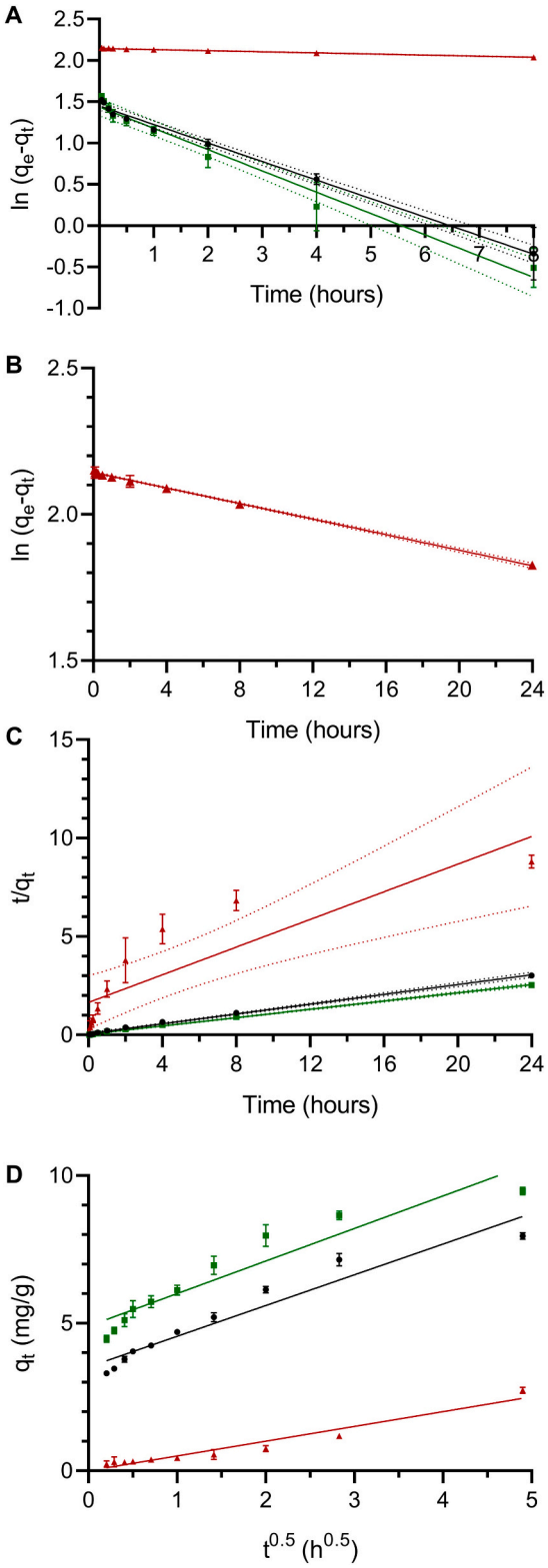

Fig. 3. Kinetic modeling for *Chlorella vulgaris* (13-1) (●, black), *Coelastrella* sp. (3-4) (■, green) and *Scenedesmus obliquus* (13-8) (▲, dark red); data is presented as Mean ± SD of three biological replicates. (A) Pseudo-first-order kinetics of adsorption during the first 8 h of growth in the presence of Cd^{2+}; (B) pseudo-first order kinetics of adsorption for Scenedesmus sp. (13-8) during the total 24 h experiment. (C) Pseudo-second-order kinetics of Cd^{2+} adsorption; (D) Intraparticle Diffusion models describing the Cd^{2+} adsorption. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Kinetic parameters for Cd^{2+} adsorption to Nordic microalgae using the three different models.

Microalgae	Pseudo-first order			Pseudo-second order			Intraparticle diffusion	
	q_e (mg g^{-1})	k_1 (h^{-1})	R^2	q_e (mg g^{-1})	k_2 (g mg^{-1} h^{-1})	R^2	k_i (mg g^{-1} $h^{-0.5}$)	R^2
C. vulgaris (13-1)	4.02	0.186	0.994	8.03	0.237	0.997	[a]	[a]
Coelastrella sp. (3-4)	4.2	0.258	0.978	9.53	0.273	0.998	[a]	[a]
S. obliquus (13-8)	8.54	0.014	0.990	2.85	0.075	0.748	0.5010	0.947

[a] Not applicable, regression line not passing through the origin.

adding the Cd(II). Cultures in the presence of 0 or 2.5 mg L^{-1} Cd(II) continued to grow logarithmic until around day 6 (144 h), with no obvious difference in OD between both cultures (Student's *t*-test, $p = 0.292$).

Coelastrella sp. (3-4) is less tolerant to cadmium compared to *Chlorella vulgaris* (13-1) or *Scenedesmus obliquus* (13-8), since concentrations of around 2.5 mg L^{-1} of Cd(II) already affected the growth (Student's t-test, $p = 0.014$).). Instead, *Coelastrella* sp. (3-4) cultures continued growing, but doubling of the cells was substantially slowed down (Fig. 1, ANOVA [$p < 2e^{-16}$]).

3.2. Removal kinetics of Cd (II) by Nordic microalgae

To study the kinetics of Cd (II) removal, equal amounts of biomass of the three selected microalgae with highest tolerance to the metal were exposed to an initial concentration of Cd(II) of 2.5 mg L^{-1}. The remaining Cd(II) concentration in solution was tracked along an exposure time of 24 h. The results of Cd(II) decay and removal percentage for each microalgae are presented in Fig. 2. Cultures of *Chlorella vulgaris* (13-1) (Fig. 2A) and *Coelastrella* sp. (3-4) (Fig. 2B) removed 31% and 39% Cd (II), respectively, within the first 5 min (Student's *t*-tests: $p = 0.033$ & $p = 0.026$). Over the time course of 24 h *Chlorella vulgaris* (13-1) removed 72% of Cd(II) and *Coelastrella* sp. (3-4) was able to remove up to 82%. Both species reached equilibrium already after 8 h of exposure to Cd(II). The heavy metal seems to be removed in two different phases; initially the largest amount of cadmium seems to be adsorbed onto the algal surfaces followed by a second phase where the metal is taken up by the cell. Such a metabolic uptake is supported by the observed reduced growth after around 24 h in the presence of Cd(II) (Fig. 1A, B). A two-phase process of biosorption of heavy metals has also been described in other macro- and microalgae (*C. vulgaris*, *Vaucheria* sp.) and in fungi (*Aspergillus niger*) [31–33]. In the first rapid phase the heavy metal ions are bound to extracellular components like polysaccharides or proteins present at the outside of the algal cell wall. Functional groups like the carboxyl- or hydroxyl-groups are able to bind heavy metal ions [34]. In the second phase, metal ions are transported through the membrane in an active, but slow and irreversible process [35]. Interestingly, the removal kinetics of cadmium by *Scenedesmus obliquus* (13-8) do not follow a typical two-phase model, they can rather be described by a simple linear regression. Over 24 h around 46% of Cd(II) were removed from the medium, no obvious equilibrium state was observed during the experiment.

Equal amounts of biomass (see Section 2.3) of the three different strains were exposed to Cd(II). Therefore, differences in uptake kinetics and the maximum adsorption capacity must depend on microalgal morphology and physiology rather than the amount of biomass. *Chlorella vulgaris* (13-1) is a small, spherical, mono-cellular microalga with a diameter of around 4 μm, which is protected by a thick cell wall. The shape of *Coelastrella* sp. (3-4) is also spherical, but it is twice as large a *Chlorella*, with a diameter of 8–10 μm [36]. During exponential growth *Coelastrella* reproduces via autosporangia, releasing up to eight mono-cellular daughter cells from a much bigger mother cell [37]. *Scenedesmus obliquus* (13-8), however, is an ellipsoidal microalga with an average size of 4 × 10 μm, forming colonies of two to eight cells, called coenobia [38]. In cultures with the same number of cells *Coelastrella* sp. (3-4) therefore offers the largest surface, followed by *Chlorella vulgaris* (13-1) and then *Scenedesmus obliquus* (13-8), as in coenobia parts of the cell surface is blocked due to cell-cell contact. The very efficient removal of Cd(II) by *Coelastrella* sp. (3-4) therefore can be explained by its large surface offering a large density of functional groups for biosorption. Due to this high biosorption every cell is exposed to a relative higher concentration of heavy metal ions per cell resulting in less tolerance to Cd (II) (Fig. 1). Removal of Cd(II) by *Scenedesmus obliquus* (13-8), however, either is very slow or the metal only adsorbs to its surface without any incorporation into the cells.

3.3. Kinetic modeling of the time-course data

Pseudo-first and pseudo-second-order models presented in 2.4 were employed to describe the time-course profile of cadmium removal. The pseudo-first-order model was only applicable on removal data of *Chlorella vulgaris* (13-1) and *Coelastrella* sp. (3-4) covering the first 8 h of growth in the presence of Cd(II) (Fig. 3A). Pseudo-second-order kinetics resulted in better correlation coefficients (R^2) (Table 2). Instead, all removal data (0 to 24 h) received for *Scenedesmus obliquus* (13-8) were described by the pseudo-first-order equation (Fig. 3B). The reader should keep in mind that only adsorption to the surface affects the kinetics and its parameters of these models [23]. As soon as additional processes like the uptake into the microalgal cell have to be considered, the models are insufficient. The successful description of the removal data of *Scenedesmus obliquus* (13-8) by pseudo-first-order kinetics therefore supported the hypothesis that this strain removes Cd(II) by adsorption to the cell surface, without incorporating the metal into the cells.

The pseudo-second-order equation combines the surface adsorption of the first phase with the incorporation in the second phase, which can also be described as chemisorption [39]. It includes physicochemical interactions and was therefore applicable for the removal of Cd(II) by *Chlorella vulgaris* (13-1) and *Coelastrella* sp. (3-4) (Fig. 3C).

Cadmium removal from the medium can also be described by using the intraparticle diffusion model (see Section 2.4). This model describes the diffusion at the interface of the microalgae and Cd(II) as well as the diffusion within the microalgal surface [40] and assumes that intraparticle diffusion is the rate-limiting step in metal removal [41]. In case intraparticle diffusion is involved in the adsorption plotting q_t versus $t^{0.5}$ will give a straight line that has to pass the origin of the graph. The experimental data in this study resulted in a very good fit for *Scenedesmus obliquus* (13-8), supporting further the hypothesis that Cd(II) adsorbs to the microalgal surface, but is not incorporated into the cell (Fig. 3 D). Data of *Chlorella vulgaris* (13-1) and *Coelastrella* sp. (3-4) could not be described by intraparticle diffusion.

3.4. Modeling the equilibrium of Cd(II) sorption

To model the adsorption isotherms, a large number of different models can be found in the scientific literature. In this study we focused on four commonly used models, namely: Langmuir, Freundlich, Sips and Dubinin-Radushkevich. In order to obtain the parameters to characterize the sorption, the experimental data were fitted to the aforementioned models employing NLLS [27–30]. Fig. 4 shows the resulting plots for *Chlorella vulgaris* (13-1), *Coelastrella* sp. (3-4) and for *Scenedesmus obliquus* (13-8), the amount of adsorbed Cd(II) was plotted against the

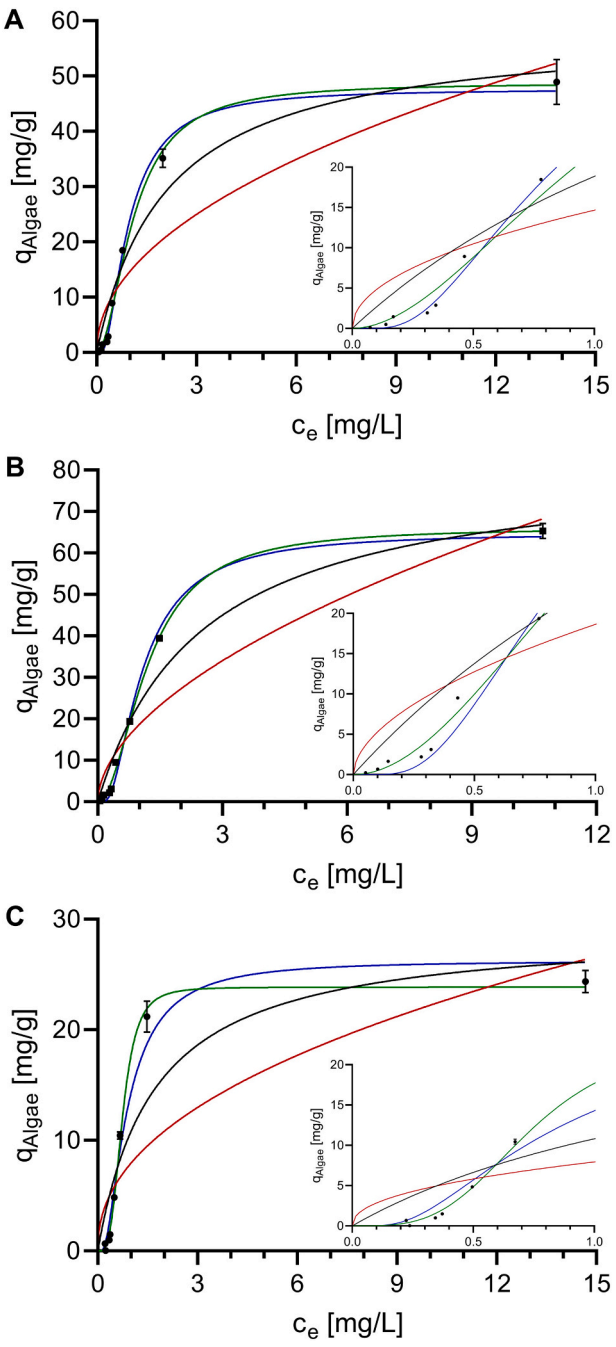

Fig. 4. Langmuir- (black), Freundlich- (red), Sips- (green), and Dubinin-Radushkevich (blue) isotherms on Cd^{2+} removal of (A) *Chlorella vulgaris* (13-1), (B) Coelastrella sp. (3-4) and (C) *Scenedesmus obliquus* (13-8). The concentration of adsorbed Cd^{2+} was plotted against Cd^{2+} remaining in the supernatant; the graphs A–C display all data of the experiment, with the inlet graph focusing on the range with low Cd^{2+} concentrations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Cd(II) concentration remaining in the supernatant. Especially at low Cd (II) concentrations in the supernatant (Fig. 4, inlet graphs) Sips- and the Dubinin-Radushkevich Models revealed the best fitting. The Langmuir model assumes adsorption to occur at a mono-layered homogenous surface until this is saturated [42,43]. The Freundlich model is an empirical model neither assuming a homogenous surface nor a limited number of adsorption sites and can only be applied at low or intermediate ion concentrations [26,44]. While these two models are applicable

Table 3
Summary of the equilibrium parameters calculated by the different models for Cd(II) removal by Nordic microalgae. Results are presented as mean ± SD of three biological replicates.

		Chlorella vulgaris (13-1)	*Coelastrella* sp. (3-4)	*Scenedesmus obliquus* (13-8)
Langmuir	q_{max} (mg g^{-1})	595 ± 5.96	83 ± 4.48	28 ± 2.11
	K_L (L mg^{-1})	0.51 ± 0.06	0.40 ± 0.05	0.749 ± 0.192
	R^2	0.944	0.958	0.848
Freundlich	K_F (mg g^{-1})	15.02 ± 0.37	18.57 ± 0.51	8.15 ± 0.51
	n_F	2.11 ± 0.05	1.82 ± 0.08	2.42 ± 0.21
	R^2	0.840	0.894	0.715
Sips	q_{max} (mg g^{-1})	48.70 ± 6.15	66.80 ± 2.58	23.44 ± 0.13
	K_S (L mg^{-1})	1.02 ± 0.35	0.69 ± 0.15	2.48 ± 0.31
	n_S	2.14 ± 0.25	1.97 ± 0.30	3.21 ± 0.07
	R^2	0.994	0.998	0.995
Dubinin - Radushkevich	q_{max} (mg g^{-1})	48.8 ± 4.48	64.8 ± 2.05	25.3 ± 0.20
	K_{DR} (mol^2 J^{-2})	2.37E−07 ± 2.56E−08	2.79E−07 ± 1.22E−08	1.70E−07 ± 5.16E−08
	R^2	0.995	0.993	0.983

for solid surfaces of inorganic materials, they seem not to be valid modeling removal kinetics of living microalgae containing a combination of adsorption and absorption. The Sips model combines the Langmuir- and the Freundlich model, overcoming the limitations of each single model. According to Foo and Hamed [45] it reduces to the Freundlich isotherm at low concentrations, while predicting a monolayer adsorption at high concentrations. Therefore, especially at very low Cd(II) concentrations, the Freundlich contribution to the description of the sorption profile in the Sips model was sufficient to provide a successful interpretation of the experimental data. The Dubinin-Radushkevich isotherm is used to describe adsorption onto a heterogenous surface with a porous structure. This model showed the best curve-fitting for the adsorption onto the microalgal surface. The cell wall of many green microalgae is composed of a fibrillar skeleton and an amorphous matrix, with mainly polysaccharides and proteins being embedded into it [46]. This biological structure resembles a porous surface explaining the good fitting of the Dubinin-Radushkevich model.

Table 3 summarizes the calculated parameters from the isotherm modeling displaying the good fitting of the experimental data to the Dubinin-Radushkevich as well as Sips-model. Based on these parameters, *Chlorella vulgaris* (13-1) and *Coelastrella* sp. (3-4) are able to adsorb around 48.8 mg Cd(II) g^{-1} and 66.8 mg Cd(II) g^{-1} algal biomass, respectively. Previous studies e.g. on *Chlorella* species showed maximum adsorption capacities between 32.4 mg g^{-1} and 97.3 mg g^{-1}, based on the model used [47,48]. *Scenedesmus obliquus* (13-8) only adsorbed around 25.3 mg Cd(II) g^{-1} algal biomass, but it was reported that other strains of the *Scenedesmus* family can adsorb up to 68.6 mg g^{-1} [49,50]. Other studies have also confirmed that *Chlorellaceae* and *Scenedesmaceae* are indeed capable of removing higher amounts of heavy metals, e.g. Chromium or copper [51–53]. To the best of the knowledge of the authors, there's a lack of information on the potential of *Coelastrella* sp. to remove Cd(II).

3.5. Identification of the functional groups involved in Cd(II) sorption by FTIR

In order to identify the role of the functional groups on the

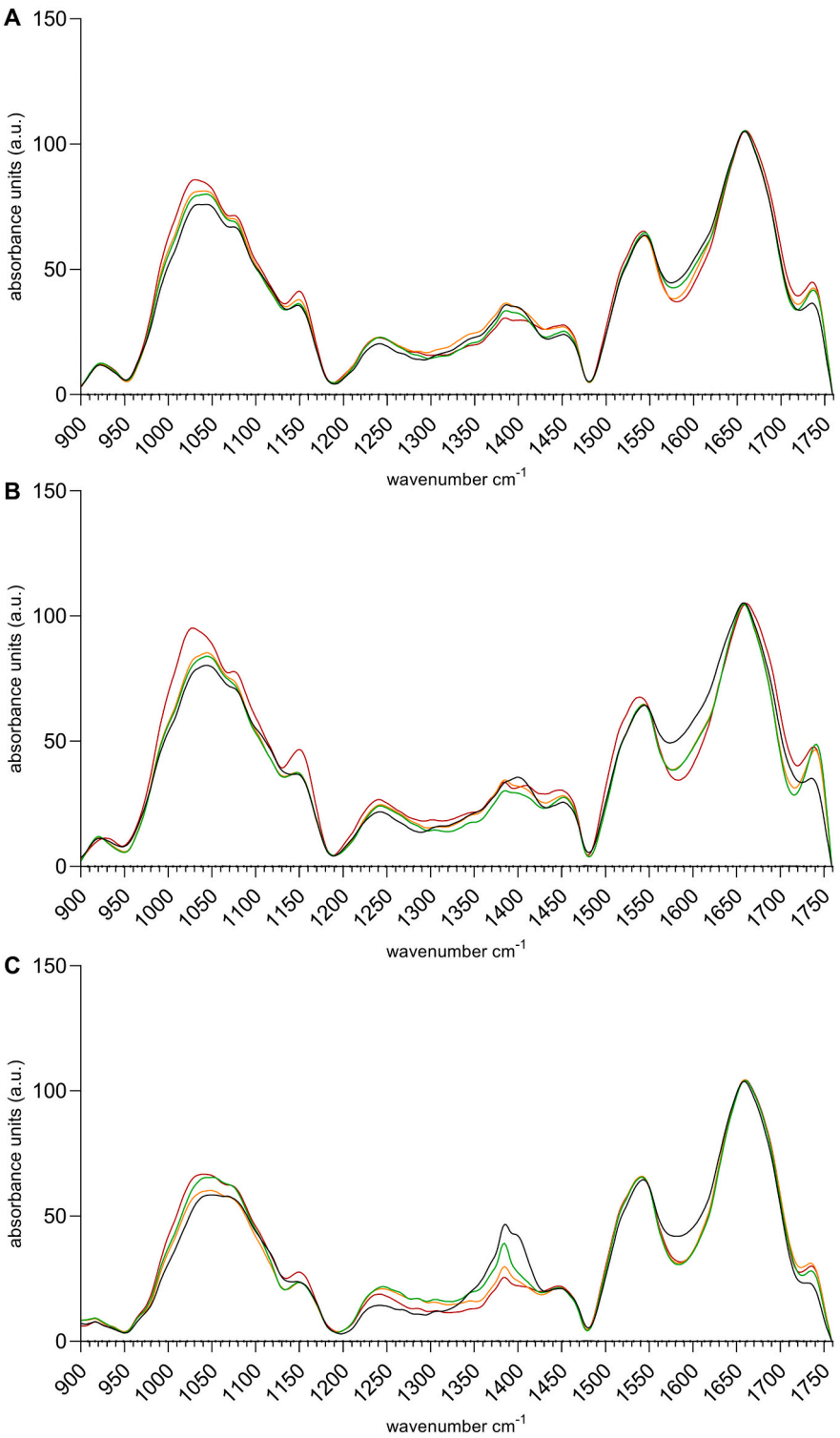

Fig. 5. FTIR analysis of (A) *Coelastrella* sp. (3-4), (B) *Chlorella vulgaris* (13-1) and (C) *Scenedesmus obliquus* (13-8) after growth in the absence or presence of Cd(II) (0 mg L^{-1} (black), 50 mg L^{-1} (green), 100 mg L^{-1} (yellow), 500 mg L^{-1} (red)) for 24 h. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

biosorption of Cd(II) by Nordic microalgae, FTIR measurements were performed on algal biomass received after growth in the absence and in presence of Cd(II) (0, 50, 100 or 500 mg L^{-1}) for 4 (Supplementary Fig. S2) and 24 h (Fig. 5A–C). To highlight changes within the FTIR spectra, 2nd derivatives of the spectra of the biomass grown in absence and in presence of 500 mg L^{-1} Cd(II) were calculated (Fig. 6).

In order to compare the signals, the raw FTIR data was normalized to the amide I band. The most obvious change in the spectra provoked by the presence of Cd(II) was the increase of the broad peak between 1720 and 1740 cm^{-1}. While the intensity of that peak seems to correlate with increasing concentration of Cd(II) for *Coelastrella* sp. (3-4) (Fig. 5A), in *Chlorella vulgaris* (13-1) (Fig. 5B) and *Scenedesmus obliquus* (13-8) (Fig. 5C) it increased even with lower Cd(II) concentrations. Cd(II) might affect either the C═O stretching vibration of carboxylic acids, of

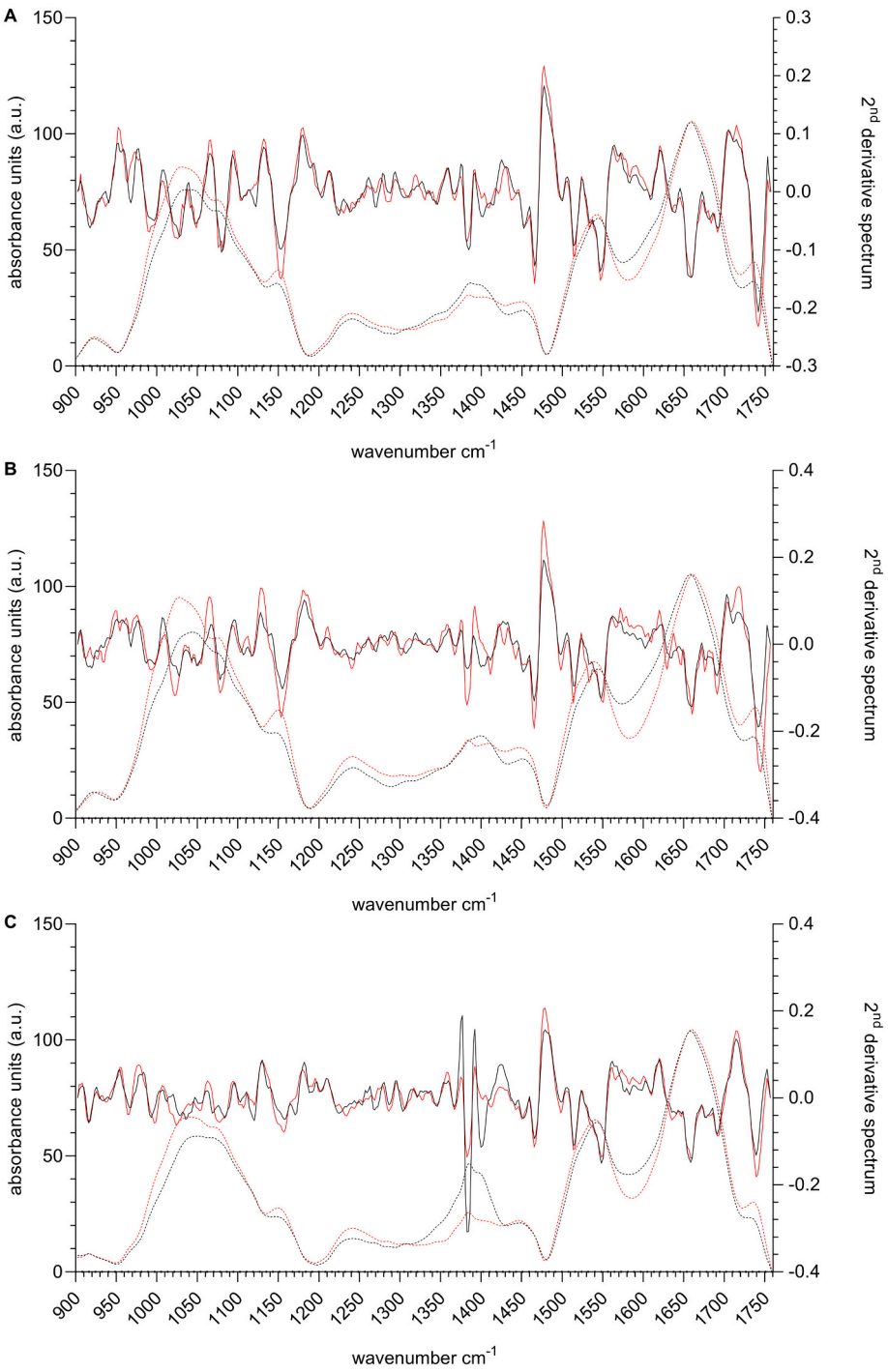

Fig. 6. FTIR spectra (dashed line) and 2nd derivative (solid line) of (A) Coelastrella sp. (3-4), (B) *Chlorella vulgaris* (13-1) and (C) *Scenedesmus obliquus* (13-8) after growth in the absence or presence of Cd(II) (black 0 mg L^{-1}, red 500 mg L^{-1}) for 24 h. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

esters or carbonyl groups [54,55].

An increase of the peak around 1160 cm^{-1} was observed, which could be assigned to C—O stretching vibrations of aliphatic ethers or primary alcohols, or to C-O-C asymmetric stretching vibrations (type I bonding carboxylic acid) [55–57]. The increase correlated to raising Cd (II) concentrations in *Coelastrella* sp. (3-4) and *Scenedesmus obliquus* (13-8), but not in *Chlorella vulgaris* (13-1). The 2nd derivatives calculated for the spectra of *Coelastrella* sp. (3-4) (Fig. 6A) and *Chlorella vulgaris* (13-1) (Fig. 6B) confirmed the relevance of carboxylic acids in sorption of Cd (II). Although there is only a slight change in the intensity around 1740 cm^{-1} the 2nd derivative shows the appearance of a more dominant peak at 1716 cm^{-1}, which can be attributed to the C═O stretching of carboxylic acid. It also confirms the changes around 1160 cm^{-1}, which were assigned to C-O-C asymmetric stretching vibrations. Additionally, the spectra of *Coelastrella* sp. (3-4) and *Chlorella vulgaris* (13-1) showed an increased intensity of a peak around 1070 cm^{-1}, which can be assigned to the C—O stretching within primary alcohols or polysaccharides [55,58].

In the spectra of all three strains the peak around 1040 cm^{-1} was shifted towards 1030 cm^{-1} and increased in intensity in the presence of

the heavy metal. While the shift in the spectra of *Coelastrella* sp. (3-4) and *Chlorella vulgaris* (13-1) was obvious only in the presence of 500 mg L^{-1} Cd(II), in *Scenedesmus obliquus* (13-8) it correlated to increasing Cd (II) concentrations. Change in these wavenumbers can be assigned to effects on v(C-O-C) of glycosidic bonds or v(C-OH) side groups in the carbohydrate structure making them also a possible target for Cd(II) binding [59]. Interestingly, there are only minor changes visible in the 2nd derivative of the spectra from *Coelastrella* sp. (3-4) and *Chlorella vulgaris* (13-1) between 1020 and 1050 cm^{-1}.

The FTIR spectra of *Chlorella vulgaris* (13-1) and *Coelastrella* sp. (3-4) showed interesting changes in the spectral range between 1350–1450 cm^{-1}. While the peak around 1380 cm^{-1}, which could be assigned to the asymmetric stretching of carboxyl groups, disappeared, the peak around 1450 cm^{-1} increased. This one could be assigned either to the bending of methyl/methylene groups within proteins or to C═O stretching of carboxyl or amide-groups (amide I and II band). This again is demonstrating the impact of carboxylic acids on the binding of Cd(II), which was also confirmed by the calculated 2nd derivative.

The spectrum of *Scenedesmus obliquus* (13-8) showed a large decrease of the peak around 1380 cm^{-1}, which again could be related to the asymmetric stretching vibrations of carboxylate [54,60]. An additional peak around 1415 cm^{-1} arouse in the spectrum of *Chlorella vulgaris* (13-1) at 500 mg L^{-1} Cd(II), and could be assigned to the CH_2-bending vibration of carbonyl compounds [61]. Both changes were confirmed by prominent effects on the 2nd derivative around 1380 cm^{-1} as well as around 1415 cm^{-1}.

FTIR analysis greatly pointed towards the carboxylic moieties of the cell walls to be the key player in cadmium removal, as their corresponding peaks at 1150 cm^{-1} and 1730 cm^{-1} increased strongly in the presence of Cd(II). Similar results were shown by Pradhan and coworkers for the removal of chromium by *Scenedesmus* sp. (IMMTCC-13) or by Han et al. who also confirmed the involvement of carboxy groups in *Chlorella miniata* by potentiometric titration [40,46]. The complexation of heavy metals by alcoholic and carboxylate groups in marine algal biomass was confirmed via X-Ray Photoelectron Spectroscopy (XPS) [62]. Nevertheless, heavy metal removal can probably not be assigned to only one functional group, but to a variety of different mechanisms [63].

4. Conclusions

Fourteen Nordic microalgal strains were screened for their tolerance to cadmium with the aim to find the most suitable candidate strains to be further employed in the development of tertiary biotreatment schemes for industrial wastewater polluted with Cd(II). Three of these strains, *Chlorella vulgaris* (13-1), *Coelastrella* sp. (3-4) and *Scenedesmus obliquus* (13-8) showed a remarkably high tolerance towards increasing concentrations of this heavy metal and their sorption performances were tested in kinetics and equilibrium experiments.

The adsorption of Cd(II) occurred fast and reached equilibrium after approximately 8 h of exposure. Data modeling reflected that a pseudo-second order kinetics successfully described the data for the algae *Chlorella vulgaris* (13-1) and *Coelastrella* sp. (3-4), whereas removal kinetics of *Scenedesmus obliquus* (13-8) were best described by intra-particle diffusion. FTIR analyses pointed towards the carboxylic moieties of the cell walls to be the key player in Cd(II) removal. This is the first time Nordic microalgae were investigated for their ability to adsorb/absorb heavy metals, demonstrating their potential to remove Cd(II). This knowledge contributes paving the road for the development of new, cheap and efficient biobased innovative technologies for the bioremediation of industrial wastewaters polluted with heavy metals.

CRediT authorship contribution statement

Christiane Funk and Carlos Escudero-Oñate designed the research project. Martin Plöhn performed the experimental work, data collection, analysis and interpretation under the supervision of Carlos Escudero-Oñate and Christiane Funk. All authors were involved in the preparation of the manuscript and its submission.

CEO and CF: conceptualization, methodology, supervision, reviewing editing; MP: data curation, original draft preparation, investigation. All authors interpreted the data, and approved its submission.

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.

No conflicts, informed consent, human or animal rights applicable.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2021.102471.

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