

**Bio-compatible flotation of *Chlorella vulgaris*: Study of zeta potential and flotation efficiency**

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3 **Bio-compatible flotation of *Chlorella vulgaris*: study of zeta potential and flotation**  
4 **efficiency.**  
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62 **Abstract**  
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65 The energy-intensive dewatering of algae biomass, the first step of most downstream  
66 processes, remains one of the big challenges for economically relevant photoautotrophic  
67 bioprocesses. Due to its scalability and easy construction, foam flotation using the interactions  
68 between cells and bubbles shows considerable potential for this type of cost-efficient initial  
69 dewatering step. Comprehensive knowledge on both the physico-chemical conditions and the  
70 cellular surface properties are an important precondition to harvest cells by flotation. This study  
71 investigates the impact of changing the medium composition, specifically varying the pH and  
72 adding (bio-) collectors on the zeta potential of *Chlorella vulgaris* SAG 211-1B. Decreasing the  
73 pH value from physiological to acidic conditions (pH 1–1.5) resulted in a strongly reduced  
74 cellular zeta potential. As validated by dispersed-air flotation experiments, this yields a  
75 significantly enhanced cell recovery  $R > 95\%$ . The impact of the synthetic collector  
76 cetyltrimethylammonium bromide and the biopolymer chitosan on the cellular zeta potential  
77 and flotation performance was studied, resulting in a 3.3-fold decrease in the surfactant dose  
78 when chitosan was used during dissolved-air flotation. The basic mechanisms of cell-chitosan  
79 interaction were analysed in terms of particle size distribution and surface tension  
80 measurements, revealing interactions between flocculation and adsorption during the  
81 dispersed-air flotation of *Chlorella vulgaris*.  
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## Highlights

- The zeta potential of *C. vulgaris* depends on the medium composition and pH.
- The recovery of cells is > 95% at pHs lower than 1.5 (< -7 mV).
- A lower dose of chitosan (15 mg L<sup>-1</sup>) is needed to adjust the isoelectric point compared to cetyltrimethylammonium bromide (50 mg L<sup>-1</sup>).
- Flotation studies reveal an optimum chitosan concentration range of 12–18 mg L<sup>-1</sup> to yield a recovery > 95 % (concentration factor of 5.3).
- Particle size distribution and surface tension measurements reveal the influence of flocculation and adsorption on flotation efficiency.

## Abbreviations

Abbreviation	Definition	Unit
$\zeta$	Zeta potential	mV
$\sigma$	Surface tension	mN m <sup>-1</sup>
BM	Bristol's Modified Medium	-
$C_{x0}$	Initial cell concentration	Cells mL <sup>-1</sup>
$C_x$	Cell concentration	Cells mL <sup>-1</sup>
CTAB	Cetyltrimethylammonium bromide	-
DiAF	Dispersed-air Flotation	-
IEP	Isoelectric point	mV
PALS	Phase Analysis Light Scattering	-
PBR	Photobioreactor	-
PFD	Photon flux density	$\mu\text{mol m}^{-2} \text{s}^{-1}$

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Q <sub>3</sub>	Cumulative function of the volume weighted size distribution	%
R	Recovery	%
x	Particle (cell) size	μm

**Keywords:**

*Chlorella vulgaris*, flotation, zeta potential, dispersed-air flotation, chitosan, CTAB

## 1. Introduction

Algal biotechnology has drawn increased industrial interest over the last decade, especially the production of biofuels [1–4], high-quality cell metabolites such as dyes [5], polyunsaturated fatty acids [6–8] and antioxidants [9].

The supply of photosynthetic active radiation as the sole source of energy in photobioreactors remains one of the major challenges for photo-biotechnological processes [10–12], resulting in low biomass concentrations of 0.5–5.0 g/L dry weight in large-scale bioprocesses [11]. Taking into account the low cell concentration and high cellular water content [12], the first step of downstream processing, i.e. the energy-intensive separation of the solid and liquid phases (dewatering), becomes the most significant economic aspect of algae-based processes and plant operation [11, 13, 14].

Current techniques for pre-harvesting microalgae cells can be classified into five main technologies: sedimentation [15], flocculation [16, 17], centrifugation [18], filtration [19, 20] and flotation [21]. However, pre-harvesting is often associated with long processing times (sedimentation, flocculation), high energy and investment costs (centrifugation) or membrane clogging and fouling effects (filtration) [22, 23]. To make processes economically efficient and have a broad spectrum of applications, even in the field of low-value products, the development of energy-efficient, low-cost, robust dewatering technologies is indispensable. In this context, the potential of flotation technologies has been unexploited so far.

Flotation utilizes the interaction between gas bubbles and the cell surface, with the aim of attaching the cells to the bubbles and thus discharging them from the liquid phase [24]. Prior to their attachment, the hydrodynamically driven collision of the algal cell and the bubble is necessary [25]. The bubble-algae complexes rise to the surface of the suspension, forming an algae-laden foam which can be skimmed off [26]. Considering the low density of algal cells, which makes them difficult to separate by sedimentation, flotation appears an effective approach to harvest algae biomass [27]. Flotation technologies in biotechnology can be classified into three main branches depending on the bubble generating method [24]:

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298 dissolved-air flotation (DAF) [28], dispersed-air flotation (DiAF) [29] and electro-coagulation  
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300 flotation (EFC) [30]. DiAF has the lowest energy demand compared to the other flotation  
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302 technologies. It generates bubbles by continuously passing air through a porous material or by  
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304 dispersing air via a high-speed mechanical agitator, yielding a heterogeneous bubble size  
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306 distribution between 100  $\mu\text{m}$  and 1500  $\mu\text{m}$  [31]. The recovery efficiency increases if the size  
307  
308 of the bubbles is of the order of the algal cell size [32]. Smaller bubble sizes can be achieved  
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310 in DAF, where part of the medium or water is supersaturated with air under pressure (around  
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312 400 kPa) and re-introduced into the flotation cell [29]. Due to the pressure drop, excess air is  
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314 released in the form of microbubbles of 10  $\mu\text{m}$ –100  $\mu\text{m}$  [23]. Other gases such as  $\text{CO}_2$  [33] or  
315  
316 ozone [36, 37] can also be introduced. In electroflotation; the microbubbles are produced by  
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318 water hydrolysis [8]. This technique is often used in combination with flocculation employing  
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320 coagulants [35] or electro-coagulation [36], where algal flocs are formed by charge  
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322 neutralization. The application of metal electrodes can introduce contaminating metal ions; this  
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324 is prevented by employing non-sacrificial carbon electrodes [37].

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326 Other flotation approaches aim to attach the cells to carrier particles instead of utilizing air  
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328 bubbles. The particle-cell-aggregates which form can be separated either by buoyancy [38] or,  
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330 in the case of magnetic carrier particles, in magnetic field gradients [39–41]. Independently of  
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332 the flotation technique, the zeta potential of the algal surface is an important parameter since  
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334 it influences the interaction both between the algal cells themselves and between the algal  
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336 cells and gas bubbles during attachment. Despite several empirical studies on microalgae  
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338 flotation [42–45], there is only little information on the cellular zeta potential  $\zeta$  in different  
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340 medium conditions. Under physiological growth conditions (pH 4–8), microalgal cells exhibit a  
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342 negative zeta potential  $\zeta$ , predominately caused by dissociated carboxylic groups ( $-\text{COOH}$ ) at  
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344 the cell surface [12]. A highly negative zeta potential indicates that the algal cells are stably  
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346 dispersed in the surrounding medium, counteracting attachment at the bubble surface [46].  
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348 Two strategies can be pursued to modify  $\zeta$ : (1) adjusting the environmental conditions (pH, salt  
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350 concentration) to reach the isoelectric point ( $\zeta = 0$ ) or (2) adding cationic surfactants which  
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352 adsorb at the algal surface and facilitate cell-bubble interactions. Such surfactants (also called  
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357 collectors in the flotation process) compensate for the negative algal surface charge and make  
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359 the algal surface more hydrophobic due to their long hydrocarbon chains [47].  
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362 Classically, polyvalent metal ions and synthetic surfactants such as cetyl trimethylammonium  
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364 bromide (CTAB) are introduced as coagulation and flotation agents [46, 48–50]. Such  
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366 contaminants are undesired in the efficient, economic production of high-value algal  
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368 metabolites, which requires non-toxic, biocompatible substances added in low amounts.  
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370 However, previous studies which added biomolecules such as tea saponin or chitosan to  
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372 promote algae flotation yielded poorer results compared to synthetic surfactants [48], or used  
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374 biological substances in addition to synthetic surfactants [49].  
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377 Hence, biocompatible approaches to adjust the cellular surface properties for the flotation  
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379 process need to be studied in more detail. For that purpose, *Chlorella vulgaris* is used as a  
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381 model algal system in physiological growth conditions. Zeta potential measurements and  
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383 corresponding flotation experiments in a DiAF column are performed with concentration series  
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385 of different additives. First, the feasibility is tested of obtaining floatable cells solely by pH  
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387 variation. Then, chitosan is used (being a biocompatible additive which has already proved  
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389 effective as an algae flocculant) and compared to the performance of CTAB as a reference  
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391 synthetic collector. Finally, particle sizing and surface tension measurements of suspensions  
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393 with chitosan-algae complexes reveal the important effects which the bio-collector has on the  
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395 flotation process.

## 396 **2. Materials and Methods**

### 399 **2.1 Strain and culture conditions**

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401 *Chlorella vulgaris* 211-11b was obtained from the Algae Culture Collection at Göttingen  
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403 University (Göttingen, Germany). The microalgae cultures were initially grown at 26°C  
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405 (Mytron, Heilbad Heiligenstadt, Germany), at light/dark cycles of 16/8 hours, 180 rpm and a  
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407 photon flux density (PFD) of 20  $\mu\text{mol m}^{-2} \text{s}^{-1}$  fluorescent white light in 300 mL shake flasks  
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409 (Schott Duran unbaffled, Wertheim, Germany) containing 100 mL of a BM medium of the  
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414 following composition: 1.5 g L<sup>-1</sup> NaNO<sub>3</sub>, 0.5 g L<sup>-1</sup> K<sub>2</sub>HPO<sub>4</sub>, 1.0 g L<sup>-1</sup> K<sub>2</sub>SO<sub>4</sub>, 1.0 g L<sup>-1</sup> NaCl,  
415  
416 0.2 g L<sup>-1</sup> MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.04 g L<sup>-1</sup> CaCl<sub>2</sub>·2H<sub>2</sub>O and 100 μL L<sup>-1</sup> Hutner's trace elements. All  
417  
418 medium components were obtained from Carl Roth. The cultures were incubated for 100  
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420 hours before studying the zeta potential of microalgal cells under varying conditions (see  
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422 Section 2.3). Lab-scale cultivation was performed in a 1.5 L bubble column which was  
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424 inoculated with a 7-day-old shake flask culture to obtain an initial optical density at 750 nm  
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426 (OD<sub>750</sub>) of 0.1 (BM medium). The process conditions were adjusted to 25 °C ± 2 °C, an initial  
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428 PFD of 200 μmol m<sup>-2</sup> s<sup>-1</sup> fluorescent white light and an aeration rate of 25 L h<sup>-1</sup> (dry air). The  
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430 cultivations were performed as two biological replicates.  
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## 435 **2.2 Correlation of optical density and cell concentration**

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438 A culture of *Chlorella vulgaris* 211-11b was diluted with 0.9% NaCl solution in series to obtain  
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440 OD<sub>750</sub> values between 0.05 and 3.8. The samples were analysed by a Cube8 flow cytometer  
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442 (Sysmex GmbH, Münster, Germany). During hydrodynamic focusing, the cells are excited by  
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444 a 20 mW solid-state laser (488 nm). The red autofluorescence of chlorophyll (FL3 channel,  
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446 675/50 nm band pass) was used as a trigger parameter. The number of *C. vulgaris* 211-11b  
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448 cells in a sample volume of 200 μL was counted on a forward scatter (FSC) chlorophyll  
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450 fluorescence (FL3) dot plot using the 'volumetric counting with electrodes' protocol. The cell  
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452 concentration  $c_X$  [cells mL<sup>-1</sup>] followed a linear correlation on OD<sub>750</sub> in line with Equation 1 ( $R^2$   
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454 = 0.99, three technical replicates):  
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$$457 \quad \text{Cell concentration } c_X [\text{cells mL}^{-1}] = 5 * 10^7 * OD_{750} - 8,4 * 10^5 \quad (1)$$

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## 460 **2.3 Analysis of zeta potential**

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462 In flotation, the zeta potential  $\zeta$  is an important parameter to characterize the surface properties  
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464 of the particles (algae) since it is determined by the surface charge of the particles and by the  
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466 adsorbed matter at the particle surface. The counterions in the diffuse layer around the  
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468 particles balance the charged species at the particle surface. They are not equally distributed  
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475 in the surrounding fluid but are subjected to electrostatic attraction to the particle surface and  
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477 Brownian motion, resulting in a distribution according to the Poisson-Boltzmann equation [51].  
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479 The charge separation in a surface layer and a diffuse layer (electric double layer) leads to an  
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481 electric potential. The zeta potential is defined as the potential in a position near the surface  
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483 where the counterions in the diffuse layer are sheared off by a relative motion of the particle to  
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485 the surrounding fluid. It is frequently used to approximate the surface potential of the particle.  
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487 The zeta potential of the microalgal cells is measured by Phase Analysis Light Scattering  
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489 (NanoBrook 90Plus PALS, Brookhaven Instruments Corp., Holtsville, NY). This technique  
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491 detects the phase shift of the light which is scattered by the particles (algae) moving in an  
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493 applied electric field. From this phase shift, the electrophoretic mobility  $\mu$  is obtained. Hence,  
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495 the zeta potential  $\zeta$  can be determined via the Smoluchowski equation [51]:

$$\mu = \zeta \frac{\varepsilon}{\eta} \quad (2)$$

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498 with the medium viscosity  $\eta$  and permittivity  $\varepsilon$ . The Smoluchowski equation is valid for the limit  
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500 of very thin double layers  $\kappa a \gg 1$  in comparison to the particle radius  $a$ , where the Debye-  
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502 Hückel parameter  $\kappa$  characterizes the inverse double layer thickness. Since the algal samples  
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504 introduce a considerable ionic strength (i.e.  $1/\kappa$  is small) and the cells are typically in the  $\mu\text{m}$   
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506 range (i.e.  $a$  is large), Equation 2 is applicable. Due to the high sensitivity of Phase Analysis  
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508 Light Scattering (PALS), reliable measurements are possible even for low zeta potential values  
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510 and high salt concentrations in the surrounding medium.

### 511 **2.3.1 Zeta potential at varying pH values**

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513 Before conducting the PALS measurement, the cell concentration was adjusted to an  $\text{OD}_{750}$  of  
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515 0.05 (=  $1.66 \cdot 10^6$  cells  $\text{mL}^{-1}$ ) using BM medium. By adding 1 M HCl, the pH of the samples  
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517 was adjusted to the desired value in the range of pH 1.0 to 7.0 with three technical replicates.  
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519 Zeta potential measurements were performed in each condition as seven measurement  
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521 replicates to achieve a reliable data basis for  $\zeta$  values.  
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### 2.3.2 Preparation of surfactant solutions

The cationic surfactant cetyltrimethylammonium bromide (CTAB, Carl Roth, Karlsruhe, Germany) stock solution was prepared by dissolving 0.1 g CTAB in 20 ml deionized water. The chitosan solution was prepared by dissolving 0.1 g of chitosan (ACROS Organics, New Jersey, USA) in 10 ml of 1 vol% acetic acid [44]. Zeta potential measurements were performed as in Section 2.3.1.

### 2.4 Flotation experiments

A laboratory-scale dispersed-air flotation column was made from polymethylmethacrylate (PMMA) with a working volume of 28 mL and a sintered air-stone at the bottom to release macroscopic gas bubbles (see Graphical Abstract). A foam collector was attached at the top of the flotation column. Before flotation, the microalgal suspension was diluted with BM medium to an OD<sub>750</sub> of 0.8 (= 3.9 \* 10<sup>7</sup> cells mL<sup>-1</sup>) to obtain comparable initial conditions between different runs. Subsequently, either HCl (pH variations) or surfactant solution (CTAB or chitosan) was added to the cell suspension. The flotation was performed for 180 seconds at 20 L h<sup>-1</sup> aeration (dry air, DASGIP 4/4, Eppendorf, Hamburg). The depleted suspension in the DiAF flotation column was examined using photometric measurements at 750 nm (helios β, Thermo Fisher Scientific GmbH, Dreieich, Germany) to calculate the recovery R [52] expressed by Equation 3:

$$R = \frac{c_{X0} - c_X}{c_{X0}} * 100 [\%] \quad (3)$$

Here,  $c_{X0}$  and  $c_X$  represent the cell concentration in the pulp before and after flotation, respectively. All flotation experiments were performed in triplicate technical runs.

### 2.5 Laser diffraction measurements for size distribution

To characterize the flocculating effect of chitosan, changes in the particle size distribution of the algal cells were monitored with laser diffraction measurements (Helos, Sympatec GmbH, Clausthal-Zellerfeld, Germany). First, the particle size distribution of the native algal cells was determined. Then, a chitosan concentration of 18 mg L<sup>-1</sup> was adjusted in the algal suspension

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593 by adding the appropriate amount of chitosan stock solution under continuous stirring. The  
594 samples were diluted for the laser diffraction measurements using a BM medium with the same  
595 chitosan concentration. Each laser diffraction measurement was repeated in two technical  
596 replicates.  
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## 601 **2.6 Adsorption at air-water interface**

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603 The adsorption of surface-active matter at the air-water interface was determined by profile  
604 analysis tensiometry (PAT1M, SINTERFACE Technologies e.K., Berlin, Germany). This  
605 measurement involves a pendant drop of the respective liquid sample being created at the tip  
606 of a capillary. The droplet shape is recorded over time via a camera. Under quiescent  
607 conditions, the droplet shape corresponds to a Young-Laplace profile [53]. This profile is  
608 determined by the interaction between the surface tension tending to make the drop  
609 spherical and deformation by gravity. With the density and the volume of the liquid, the  
610 surface tension at every time step can be calculated by fitting the droplet shape to the  
611 Young-Laplace profile, yielding the dynamic surface tension curve. Before each  
612 measurement, the surface tension of deionized water was checked as a reference to verify  
613 the absence of impurities in the capillary and dosing system of the PAT1M device.  
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## 626 **3. Results and discussion**

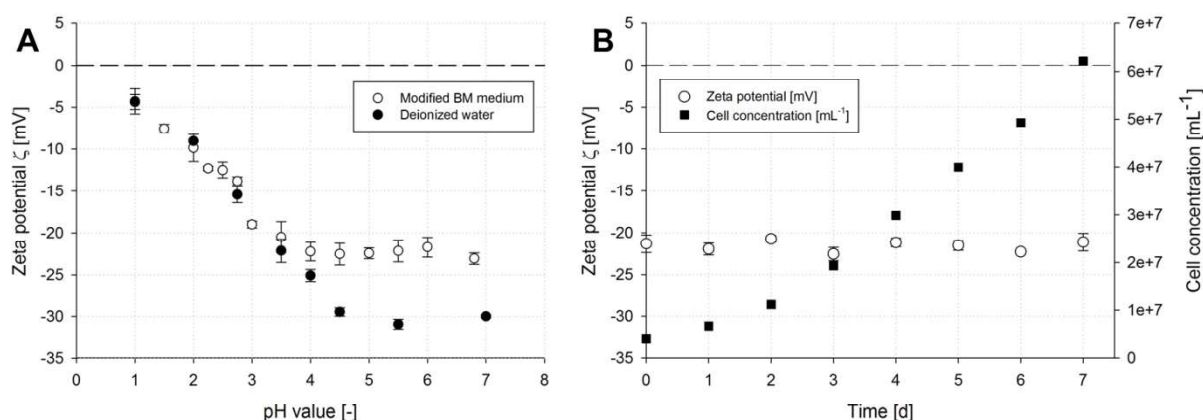
### 627 **3.1 Zeta potential**

#### 628 **3.1.1 Zeta potential depending on medium conditions**

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631 Figure 1A presents the effect of varying pHs on the zeta potential of *C. vulgaris* SAG 211-11b  
632 either suspended in BM medium or deionized water. Under physiological pH conditions (pH 4–  
633 7), the zeta potential of *C. vulgaris* SAG 211-11b reached values of  $-22.4 \pm 0.45$  mV and  
634  $-30.1 \pm 0.6$  mV in modified BM or deionized water, respectively. This is in accordance with  
635 studies by Kurniawati et al. [54] and Ozkan et al. [42], who measured  $\zeta = -30.3$  mV (*C. vulgaris*  
636 from a Taiwan shrimp pond, probably within deionized water, pH 6.89, Malvern Zetasizer 2000)  
637 and  $\zeta = -23.3$  mV (*C. vulgaris* UTEX 2714, BG-11 medium, pH 7.42, ZetaCompact).  
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At pH 4–7,  $|\zeta|$  was lower under BM conditions compared to deionized water, as is generally the case at higher ionic strengths of an indifferent electrolyte due to the increasing adsorption of counter-ions caused by electrostatic attraction and compression of the diffuse layer. When the pH was lowered from 3.5 to 1, both curves coincided while approaching the isoelectric point (IEP). This indicates that protonation of the carboxylate groups exceeded the impact of medium components and became the predominant factor influencing the zeta potential of suspended *C. vulgaris* SAG 211-11b cells.

At pH 1.0, the cellular zeta potential amounted to  $-4.29$  mV in both media, which is close to the IEP. Nevertheless, IEP ( $\zeta \approx 0$ ) was not fully reached by adjusting the pH in the range of 1.0–7.0 for both deionized water and BM.



**Figure 1:** (A) Zeta potential measurements of *C. vulgaris* SAG 211-11b subjected to pH variation ranging from 1.0 to 7.0 in deionized water and BM medium. Error bars represent standard variations in seven independent measurements; (B) Zeta potential measurements of *C. vulgaris* SAG 211-11b during batch-mode growth in a 1.5 L bubble column. Error bars represent standard deviations of two biological replicas (cell concentration) and seven independent technical measurements thereof (zeta potential).

To the best of our knowledge, this is the first comprehensive study presenting data on the microalga zeta potential of *C. vulgaris* in growth medium conditions in a wide physiological pH range. In the literature, either model media were employed or only selective zeta potential measurements for a fixed pH value were performed in growth media (see Table 1). Hadjoudja et al. [55] analysed the zeta potential  $\zeta$  of *C. vulgaris* CCAP1110/4 at pH 2.0–8.0. Prior to the measurement, the cells were washed and resolved in a 0.1 M sodium nitrate solution by the authors. Under these model conditions, Hadjoudja et al. detected an isoelectric point ( $\zeta \approx 0$ ) at

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711 pH 2.9. A Czech group tested the zeta potential of *C. vulgaris* Beijerinck strain P12 in model  
712 environments (10 mM KCl, pH 2–12) and found that *C. vulgaris* maintains a negative zeta  
713 potential subjected to pH values ranging from 4.0–12.0; yielding an isoelectric point at pH 2.0.  
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715 Indeed, defined parameter variations in model media provide a fundamental understanding for  
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717 the mechanisms influencing the algal surface properties. The technological flotation process  
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719 however aims to harvest the algal biomass directly by adjusting conditions in the growth  
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721 medium. Whereas, the qualitative trend (increasing  $\zeta$  with decreasing pH) agrees between  
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723 different studies, concrete values significantly differ depending on additional medium  
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725 components. Since the recovery is sensitive to such moderate variations (see later in Sec.  
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727 3.3), measurements under technological conditions are indispensable.  
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### 731 **3.1.2 Zeta potential dependent on cultivation time during bubble column** 732 **cultivation** 733 734

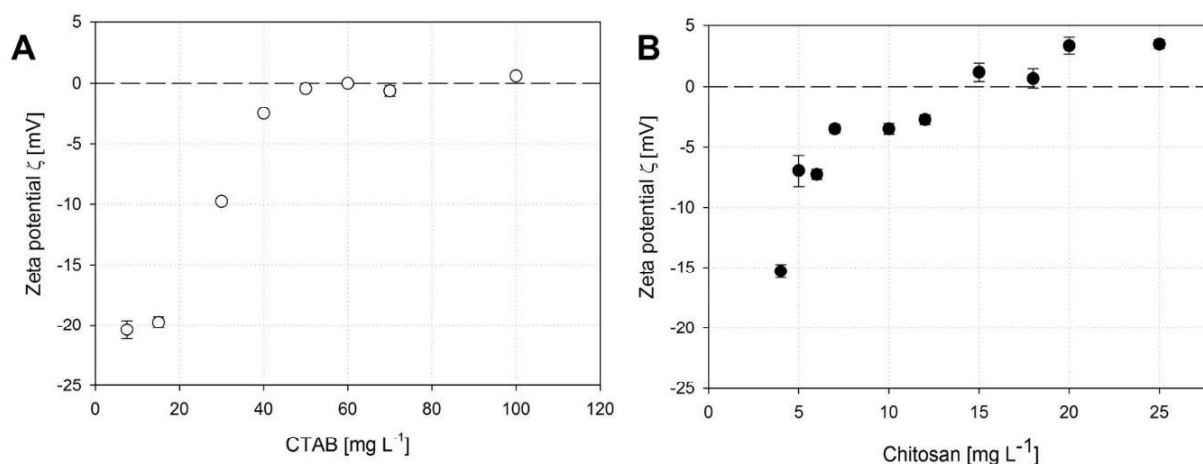
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736 Various parameters such as the cell wall composition or the secretion of extracellular polymeric  
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738 substances change during the different growth phases of algal cells, which affects the  
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740 operational efficiency in flocculation and flotation. The zeta potential measurements in this  
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742 section aim to provide defined conditions regarding the physiological state of the cells (i.e. the  
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744 age of the culture) rather than to identify the underlying biological mechanisms which affect  
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746 the algal surface properties during the cultivation time. Therefore, *C. vulgaris* 211-11b were  
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748 grown in photoautotrophic batch mode (1.5 L bubble column) in a BM medium and analysed  
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750 at specific cultivation times. As shown in Figure 1B, an increasing cell concentration during  
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752 photoautotrophic cultivation (0–7 days) did not show any significant effect on the zeta potential  
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754 yielding stable  $\zeta$  values of  $-21.3 \pm 1.2$  mV. Based on these results, all DiAF studies were  
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756 performed with 5-day-old cultures at a cell concentration of approx.  $3.9 \cdot 10^7$  cells mL<sup>-1</sup>. As  
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758 reported in other studies, the physiological state of the cells can actually have a significant  
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760 impact on the surface properties [50, 56], in turn influencing flotation or flocculation efficiency.  
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762 The dependence on the growth state may differ with the type of microorganism [50, 57],  
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764 therefore two specific examples are given in the following. Matter et al. [45] analysed the  
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770 influence of the pH (6–10), cell concentration and chitosan dose on the bioflocculation  
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772 efficiency of *Scenedesmus obliquus* at different growth states. They concluded that stationary  
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774 growing cells (> 15-day-old cultures) under moderate pH conditions showed facilitated  
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776 flocculation behaviour compared to late-exponentially growing cells (< 10-day-old cultures).  
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778 The cultivation time not only influences the physiological state of the cells, but also the cell  
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780 concentration. In the study by Matter et al., higher cell concentrations could be correlated to  
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782 higher flocculation efficiency. Similar observations were presented by Maji et al. [57] who  
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784 tested the flocculation behaviour of *C. vulgaris*, *S. obliquus* and *Chlorococcum* sp. at varying  
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786 pHs (pH 3.5–12) and biomass concentrations. This is in line with the fact that increasing cell  
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788 concentration leads to lower mean distance between the cells and higher collision probability  
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790 which are factors promoting flocculation. As indicated by the constant cellular zeta potential in  
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792 Figure 1B *C. vulgaris* 211-11b, the cells used in this study (5-day-old cultures) are still under  
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794 optimum physiological conditions and not suffering from any medium component limitations.  
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796 This allows neglecting the effect of different growth phases in the following results.  
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### 798 **3.1.3 Zeta potential dependent on CTAB and chitosan dose**

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801 This section compares the usage of the cationic surfactant CTAB as a classical synthetic  
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803 collector and the cationic biopolymer chitosan to change the zeta potential of *C. vulgaris* SAG  
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805 211-11b. Figure 2A shows the zeta potential in presence of varying CTAB concentrations  
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807 ranging from 7.5 mg L<sup>-1</sup> to 100 mg L<sup>-1</sup>. A noticeable impact of CTAB on  $\zeta$  was detected at  
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809 30 mg L<sup>-1</sup> yielding  $\zeta = -9.86$  mV. By further increasing the CTAB concentration up to 100 mg  
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811 L<sup>-1</sup>, a neutralization of the zeta potential was obtained with an optimum lower dosage of 50 mg  
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813 L<sup>-1</sup>. These results are comparable with flotation studies by Kurniawati et al. [54], Wen et al.  
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815 [46] and Alkarawi et al. [58], who reported recoveries of >90 % using CTAB concentrations of  
816  
817 60 mg L<sup>-1</sup>, 50 mg L<sup>-1</sup> and 35 mg L<sup>-1</sup>, respectively.  
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**Figure 2:** Zeta potential of *C. vulgaris* SAG 211-1b at the presence of the collectors (A) CTAB at 7.5–100 mg L<sup>-1</sup> and (B) chitosan at 4–25 mg L<sup>-1</sup>. Error bars represent standard deviation of seven measurement replicates.

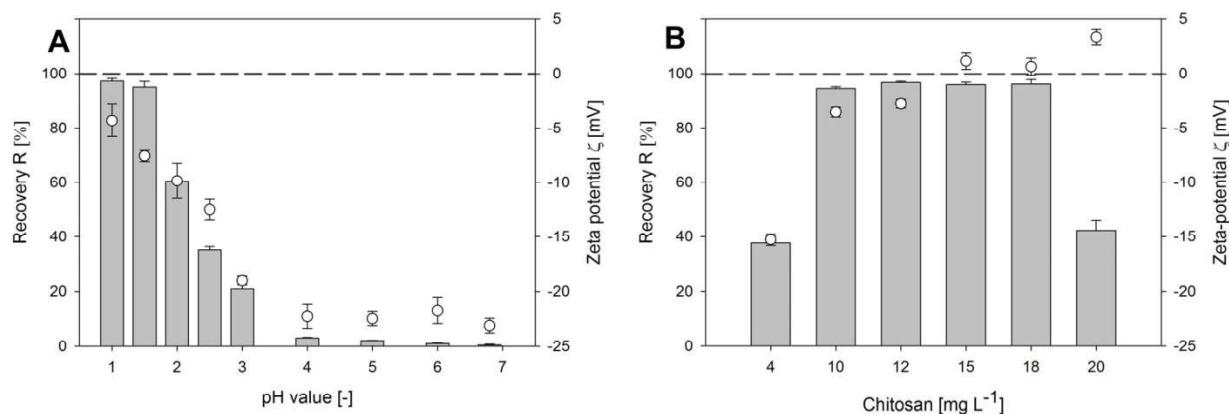
Figure 2B shows the zeta potential which was obtained at 4–25 mg L<sup>-1</sup> chitosan. Compared to the surfactant CTAB (50 mg L<sup>-1</sup>), using the biopolymer chitosan (15 mg L<sup>-1</sup>) reduced the dose needed to approach the isoelectric point of microalgal cells by a factor of 3.3. The polycation chitosan is able to interact very efficiently with the negatively charged functional groups (amide and carboxylic groups) on the cell surface [59, 60]. Rashid et al. [15] examined different acidic solutions of chitosan as flocculants and found the maximum separation efficiency using HCl-dissolved chitosan to occur at 30 mg L<sup>-1</sup>. The authors reported an IEP of *C. vulgaris* UTEX 0000265 at comparably high chitosan concentrations of > 100 mg L<sup>-1</sup>. This effect might be due to the experimental conditions (dead cells in the stationary phase at pH 8.7) which differed significantly from this work. In a further study by Low & Lau [61], an optimum dose of 30–40 mg chitosan per g biomass was applied at pH 7.6–7.8 to flocculate 8–10-week-old *C. vulgaris* cultures. Even if the exact chitosan dose is not directly comparable to the results of the present work due to the difference in experimental conditions, these studies underline the potential of chitosan to adjust the algal surface properties. A further important finding of the measurements in Figure 2B concerns the evolution at the highest chitosan concentrations. Above 20 mg L<sup>-1</sup>, the zeta potential becomes positive and increases in magnitude again after crossing IEP. The next section shows that this strongly affects the performance of the flotation process.

### 3.2 Flotation experiments



### 3.2.1 Flotation experiments at varying pH

In order to validate the results of varying pHs on the zeta potential of *C. vulgaris* 211-11b, flotation experiments were performed under corresponding medium conditions using a model DiAF bubble column (Fig. 3). After 5 days of photoautotrophic growth, the cell suspension was transferred to the DiAF column (see Graphical Abstract). Except for the adjustment of the pH to the desired value, no further collector was added to the algal suspension. Due to the strongly negative zeta potential of  $\zeta < -20$  mV at pH 6.8–4.0, less than 1 % of cells were recovered from the original cell suspension. The recovery R of cells increased with stronger acidity at pH 3, pH 2.5 and pH 2.0 to  $21.0 \pm 1.4$  %,  $35.0 \pm 1.3$  % and  $60.4 \pm 0.9$  %, respectively. This agrees with the stepwise reduction of the cellular surface charge resulting in an increase in bubble-cell-interactions. Although IEP was not fully reached at pH 1.0 and pH 1.5, yielding zeta potentials  $|\zeta|$  of lower than 7 mV, a recovery R of > 95% was obtained in this study. Similar results on this critical lower boundary of  $|\zeta|$ , which has to be passed to achieve efficient harvesting, were obtained by Henderson et al. [43], who reported an optimum flotation performance for *C. vulgaris* 211/11B at  $\zeta$  values between  $-8$  mV and  $+2$  mV. Indeed, the efficiency of flotation is affected by multiple factors, e.g. the particle and bubble size, hydrodynamic conditions and surface properties of particles and bubbles. Changing a process parameter such as the pH can influence several of those factors. For example, in addition to the algal surface properties, the surface properties of the bubbles are also expected to vary. This section indicates that in principle, it is possible to obtain a floatable algal suspension purely by pH variation without any additional collector. However, the adjustment of extreme pH values lower than 1.5 may not be suitable as a first dewatering step in industrial processes. Thus, we further focus on the more practicable case of flotation with chitosan as a bio-compatible collector and complement the flotation experiments with further measurements to understand the mechanisms responsible for the recovery trends for this system.



**Figure 3:** Recovery of microalgal cells (grey bars) in a 28 mL dispersed-air flotation column and corresponding zeta potential values (non-filled circles) from Figure 1A and Figure 2B, respectively, varying the pH conditions for (A) and varying the chitosan dose for (B). Error bars represent standard deviation of three independent flotation experiments.

### 3.2.2 Flotation experiments at varying chitosan concentrations

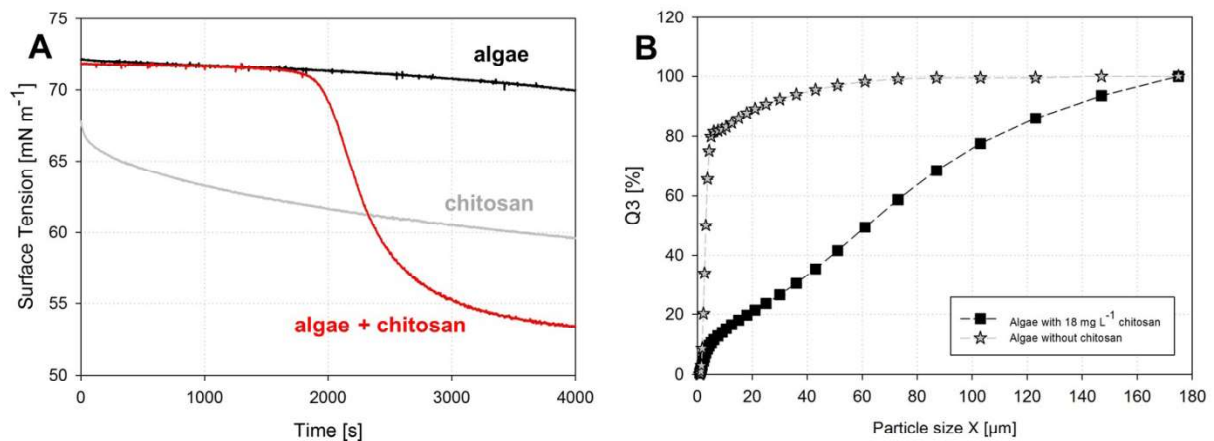
Flotation experiments were performed at selected chitosan concentrations (4–20 mg L<sup>-1</sup>), resulting in negative  $\zeta$  (4 mg L<sup>-1</sup>), neutral  $\zeta$  (10–18 mg L<sup>-1</sup>) and positive  $\zeta$  (20 mg L<sup>-1</sup>). Figure 3B shows the zeta potential and corresponding recovery rates of cells from the suspension. A constant, high recovery of  $R > 94\%$  was obtained at  $-3.5\text{ mV} > \zeta < 0.66\text{ mV}$ . If the cellular zeta potential was above or below this range, the recovery rate dropped significantly. Kurniawati and co-workers [54] analysed the combined use of the bio-collectors chitosan and saponin (to initiate flocculation and flotation) and achieved separation efficiencies of  $> 93\%$  at 20 mg L<sup>-1</sup> saponin and 5 mg L<sup>-1</sup> chitosan, respectively. Another study [49] employs 10 mg L<sup>-1</sup> chitosan and 20 mg L<sup>-1</sup> SDS, yielding  $R > 90\%$ . The experiments in the present study show that the sole addition of chitosan is sufficient to obtain comparable recovery rates in algae flotation. Since the recovery remains high in the range from 10–18 mg L<sup>-1</sup>, the process can be expected to run stably for moderate variations in the collector concentration. The same aspect applies to variations in the cell concentration, which determines the consumption of collector molecules.

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1006 **3.3 Underlying mechanisms for dispersed-air flotation of *C. vulgaris* using**  
1007 **chitosan as bio-collector**  
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1011 The upper limit of the chitosan concentration range with a high recovery  $R$  ( $18 \text{ mg L}^{-1}$ ) was  
1012 chosen to provide the most robust conditions for the surface tension and particle size  
1013 measurement. Prior to this, the native algal suspension with no added chitosan was  
1014 characterized. According to the dynamic surface tension measurements, shown in Figure 4A,  
1015 the algal suspension shows negligible surface activity. If  $18 \text{ mg L}^{-1}$  chitosan is added to the  
1016 medium without algae, the surface tension decreases with time as more and more chitosan  
1017 molecules are adsorbed at the interface. After around 4000 s, the surface tension reaches a  
1018 value of  $58 \text{ mN m}^{-1}$ . The chitosan-algae complexes first show no noticeable deviation from the  
1019 native algal suspension. After this induction period of approx. 1500 s, the surface tension  
1020 decreases to  $53 \text{ mN m}^{-1}$  at around 4000 s, which is even lower than in the case of a medium  
1021 supplemented with chitosan. This indicates that the chitosan-algae complexes have a high  
1022 tendency to adsorb at the gas-liquid interface in comparison to native algae or the pure  
1023 chitosan solution, since their neutralized charge makes them more hydrophobic. The existence  
1024 of an induction period is a known phenomenon for the adsorption of large-size surface-active  
1025 matter [62]. In addition to the extremely slow diffusion, multi-step adsorption processes and  
1026 the existence of adsorption barriers, e.g. due to electrostatic interaction, can lead to such  
1027 adsorption dynamics. However, profile analysis tensiometry employs a quiescent system,  
1028 whereas convective mixing leads to considerably faster adsorption [63]. Through the flow field  
1029 around the rising bubbles in our flotation column, adsorption occurs within the residence time  
1030 of the bubbles.  
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1051 The results of the particle size measurements are plotted in Figure 4B as the cumulative  
1052 function of the volume weighted size distribution  $Q_3(x)$ . This quantity describes the particle  
1053 mass fraction, which is smaller than the respective particle size  $x$ . Similarly,  $1-Q_3(x)$   
1054 corresponds to the particle mass fraction which is larger than  $x$ . Without chitosan, the algal  
1055 cells are typically a few micrometres in size, which corresponds to microscopic observations.  
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Between 6 and 10  $\mu\text{m}$ ,  $Q_3(x)$  reaches a small plateau, i.e. the fraction of algal cells in this size range is low. Roughly 20% of the algal mass is larger than 10  $\mu\text{m}$ , which can probably be ascribed to a minor degree of self-agglomeration. With 18  $\text{mg L}^{-1}$  chitosan, 85 % of the algal mass is larger than 10  $\mu\text{m}$  and 50 % is still larger than 60  $\mu\text{m}$ . This flocculating effect of chitosan shifts the particle size towards the floatable particle size range between 20 and 150  $\mu\text{m}$  [64]. Finer particles follow the streamlines around the rising bubbles without bubble-particle collision, because of their low inertia [65]. For the algal flocs, the collision probability increases, while their detachment rate in a shear flow is still low, leading to an improved recovery.



**Figure 4:** (A) Dynamic surface tension of microalgal cell suspension in BM medium, in BM medium with 18  $\text{mg L}^{-1}$  chitosan, and chitosan solution in BM medium without algae, (B) Cumulative function of the volume weighted size distribution for microalgal cells in BM medium and in BM medium with 18  $\text{mg L}^{-1}$  chitosan. Data are shown as single measurements, representing a representative course.

#### 4. Conclusion

Due to their flexibility, easy construction [31], and scalability for high-throughput applications, harvesting approaches using flotation are of particular interest to dewater microalgal biomass in an energy- and cost-efficient first step [54]. To gain reliable data on the zeta potential of microalgal cells at changing process conditions, both physico-chemical parameters of the cell environment (pH and ionic strength of the medium) and biological parameters (cell state of the culture) must be considered. Our results confirmed that a characterization of algal surface properties is advisable for new process routes due to the dependence on the medium

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1124 composition and pH, which may change for different cultivation procedures. In strongly acidic  
1125 conditions, floatable algal cells were obtained even without using additional collector  
1126 substances. For physiological pH conditions, which are more practicable in technological  
1127 processes, the biopolymer chitosan proved to be an effective collector, which adsorbs at the  
1128 oppositely charged algal surface. Only a 3.3-fold lower application dose ( $15 \text{ mg L}^{-1}$ ) was  
1129 necessary for chitosan, compared to the classical synthetic collector CTAB ( $50 \text{ mg L}^{-1}$ ). This  
1130 study showed a striking correlation between the recovery R and the isoelectric point IEP ( $\zeta \approx$   
1131  $0 \text{ mV}$ ) of the algal suspension. Under this condition, cell-bubble interactions during flotation  
1132 are enhanced. Our tensiometry and particle size measurements reveal the main operating  
1133 principles of chitosan during this process: increasing surface activity along with flocculation of  
1134 algal cells. The collision probability rises with larger particle sizes, according to the flocculation  
1135 tendency at the isoelectric point. Furthermore, the attachment of the algal cells to the air  
1136 bubbles is facilitated due to the increasing hydrophobicity with a reduced surface charge.  
1137 Chitosan's properties, i.e. biodegradability, non-toxicity, and its high cationic charge, make it  
1138 highly suitable for adjusting the cellular zeta potential of microalgae even for medical,  
1139 nutritional, and functional food applications. Moreover, it can be produced sustainably by  
1140 isolating it from fungi or by processing chitin. For the employed experimental conditions, a  
1141 recovery  $R = 95\%$  corresponds to a concentration factor of 5.3. Increasing this target value  
1142 requires the foam properties (stability, wetness) and flotation cell design to be optimised:  
1143 important steps for future work to make the process efficient at a technological scale. Our  
1144 results already carry an immediate practical implication for the operation of such flotation cells.  
1145 To a certain extent, the process is robust against variations in the chitosan dose. However, the  
1146 principle "a lot helps a lot" should be treated with caution. Exceeding the optimum  
1147 concentration range is not only connected to higher operational costs due to the chitosan  
1148 consumption, but also leads to a less effective flotation process.

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1186 diffraction measurements.  
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## 1557 **7. Contributions declaration**

1558  
1559 CM was responsible for performing the experiments on microalgae cultivation, zeta potential  
1560 determination and flotation experiments. The profile analysis tensiometry experiments were  
1561 carried out by BK and CM. FK, KS and CM wrote the manuscript. All studies were planned  
1562 by KE, KS, JS, FK, CM and TW. All co-authors carefully reviewed the manuscript with focus  
1563 on microalgae technology (TW, FK, JS, CM), zeta potential, tensiometry and particle size  
1564 analysis and flotation studies (KE, KS).  
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## 1571 **8. Declaration of interest**

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1573 The authors declare that there is no conflict of interest.  
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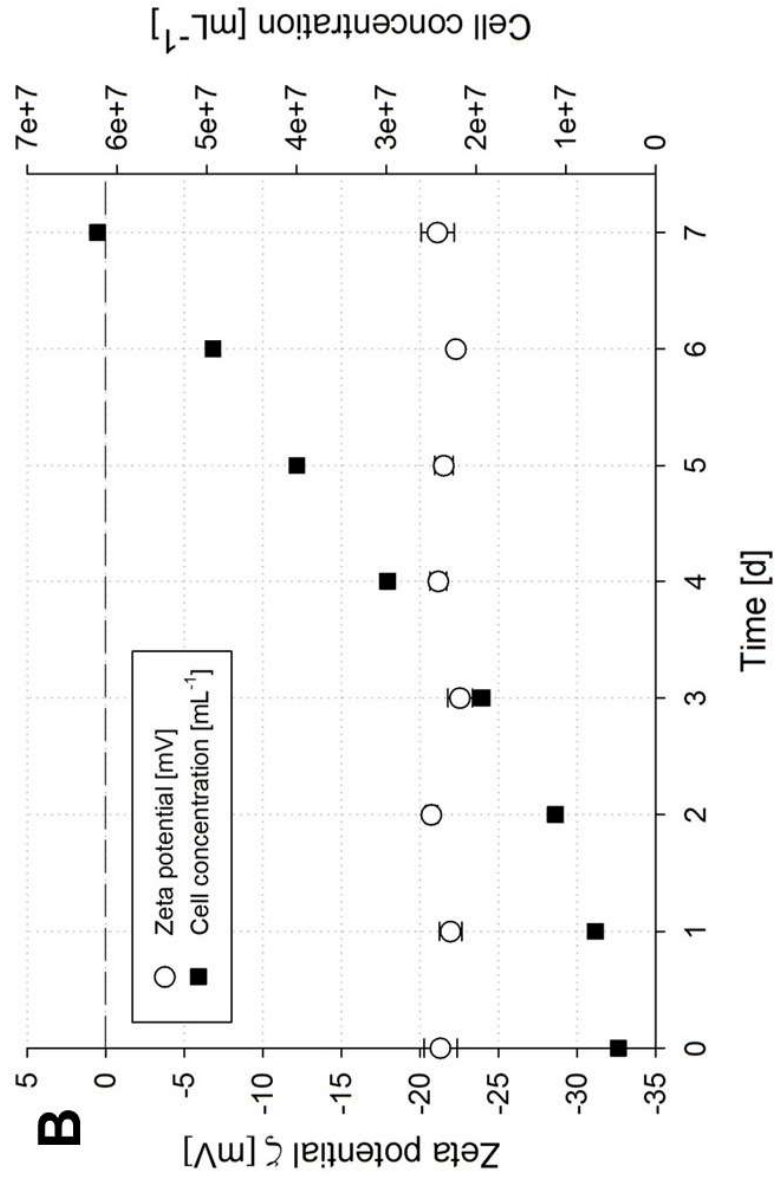
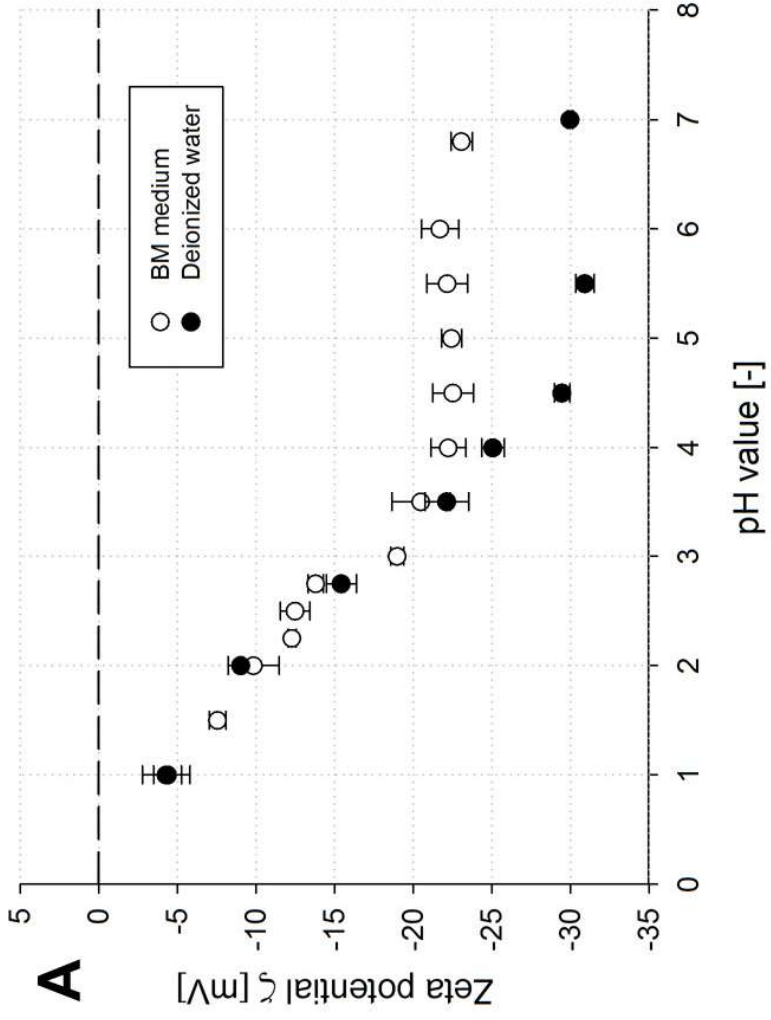
**Table 1:** Comparison of zeta potential of *Chlorella* spp. at different process conditions (data of this study are presented as mean value and standard deviation of seven technical replicates).

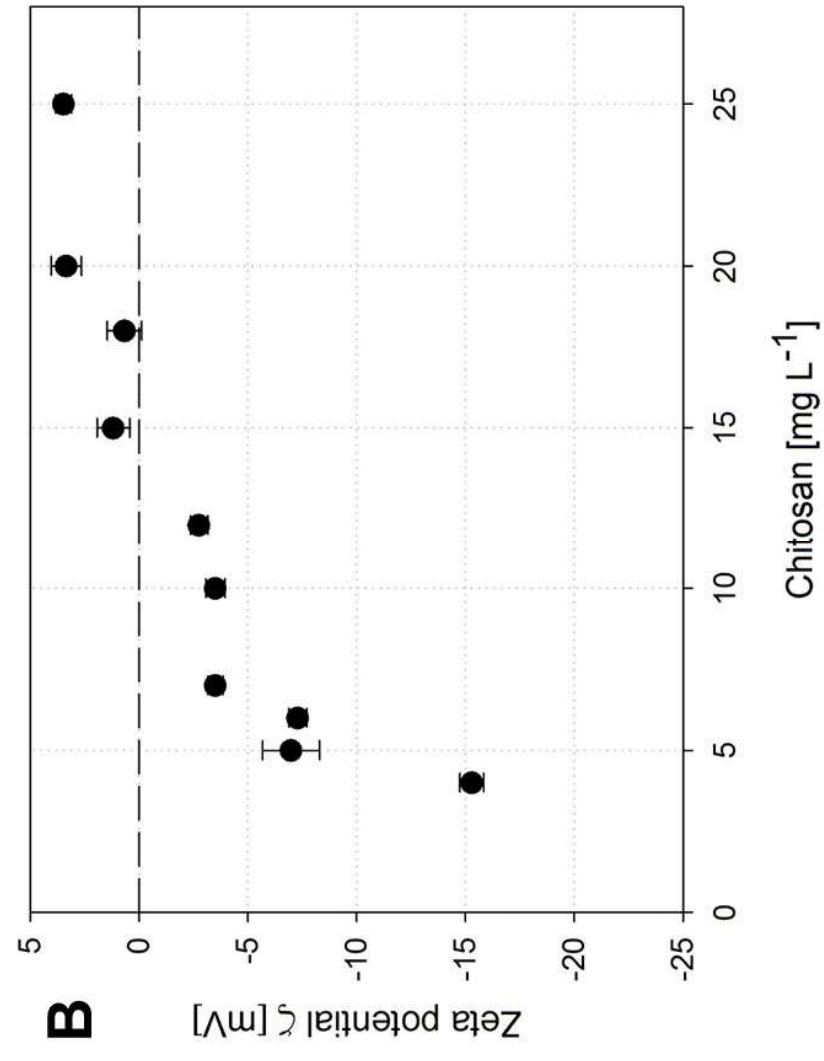
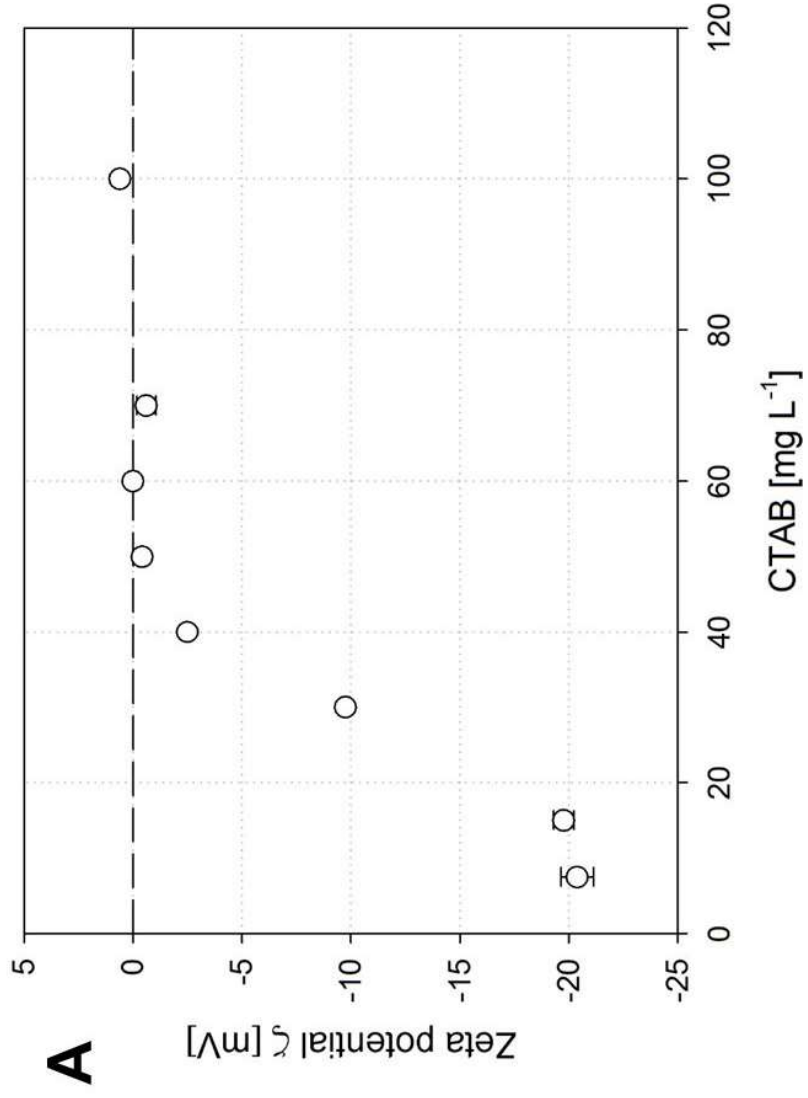
Microalgae stain	Medium conditions	pH	Measuring device	Additives	Zeta Potential	Source
<b><i>Chlorella vulgaris</i> SAG 211-11b</b>	Modified BM	6.8	NanoBrook 90Plus	-	-23.1 ± 0.7 mV	This Study
<b><i>Chlorella vulgaris</i> SAG 211-11b</b>	Deionized water	7	NanoBrook 90Plus	-	-29.98 ± 0.2 mV	This Study
<b><i>Chlorella vulgaris</i> SAG 211-11b</b>	Modified BM	6.8	NanoBrook 90Plus	+ 18 mg L <sup>-1</sup> Chitosan	0.66 ± 0.8 mV	This Study
<b><i>Chlorella vulgaris</i> SAG 211-11b</b>	Modified BM	6.8	NanoBrook 90Plus	+ 50 mg L <sup>-1</sup> CTAB	-0.42 ± 0.05 mV	This Study
<b><i>Chlorella vulgaris</i></b>	Deionized water	6.89	Malver Mastersizer 2000	-	-30.3 ± 2.4 mV	[54]
<b><i>Chlorella vulgaris</i></b>	Culture medium	6.8	Zeta-Compact,CAD, France	-	-23.3 mV	[42]
<b><i>Chlorella vulgaris</i> CCAP1110/4</b>	n.d.	2-8	Sephy zetaphoremeter Z3000	0.1 M NaNO <sub>3</sub> solution	-15 mV <sup>1</sup>	[55]
<b><i>Chlorella vulgaris</i> 211/11B</b>	n.d.	n.d.	Zetasizer 2000HSA, Malvern Co., UK	0.5 mM NaHCO <sub>3</sub> and 1.8 mM NaCl	-32.6 ± 0.6 mV	[66]
<b><i>Chlorella vulgaris</i></b>	n.d.	6.5	Nano-ZS, Malvern Co., UK	10 mM KCl	-21 mV	[39]
<b><i>Chlorella vulgaris</i></b>	n.d.	n.d.	Zeta potential analyzer (Photal, ELS-Z)	0,1 g L <sup>-1</sup> Chitosan	-6.5 mV	[15]
<b><i>Chlorella</i> sp. ESP-6</b>	n.d.	7.0	Nano-ZS, Malvern Co., UK	pH adjustment with NaOH + 200 mg L <sup>-1</sup> Chitosan	-3 mV, -4 mV and -8 mV	[19]
<b><i>Chlorococcum</i> sp.</b>	n.d.	n.d.	Malvern Zetasizer 90 (Malvern Instruments Ltd., USA)	120 mM AlSO <sub>4</sub>	-3 mV	[67]

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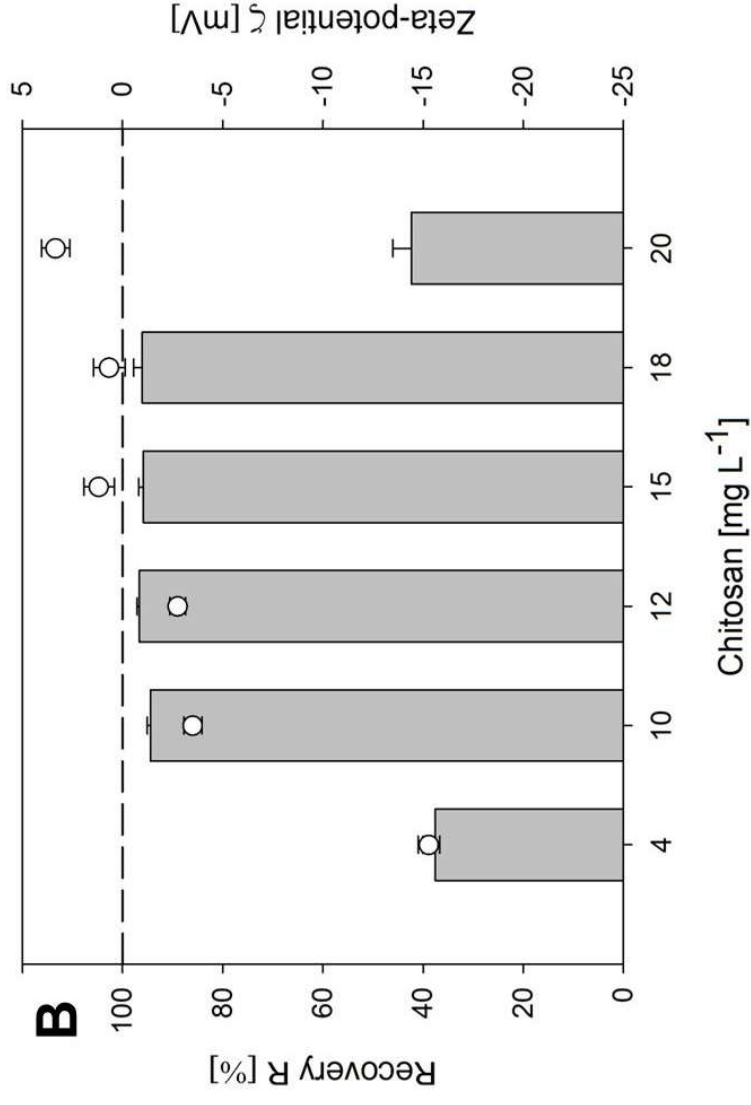
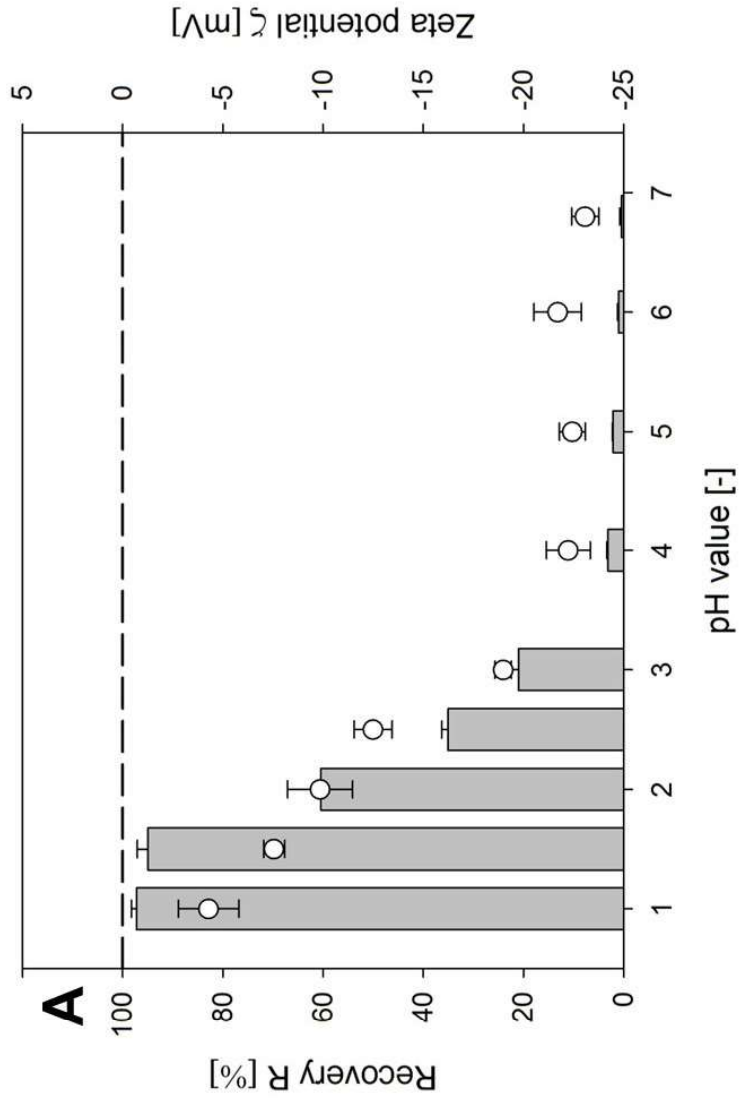
<sup>1</sup> value was estimated from figure.

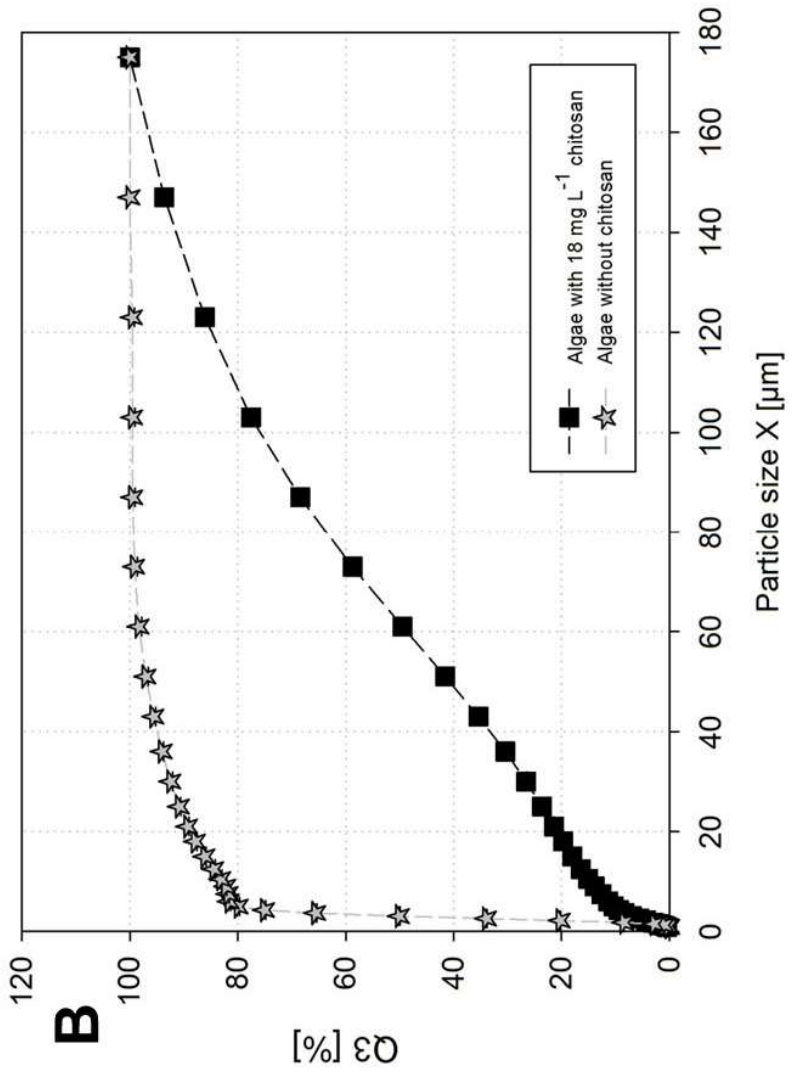
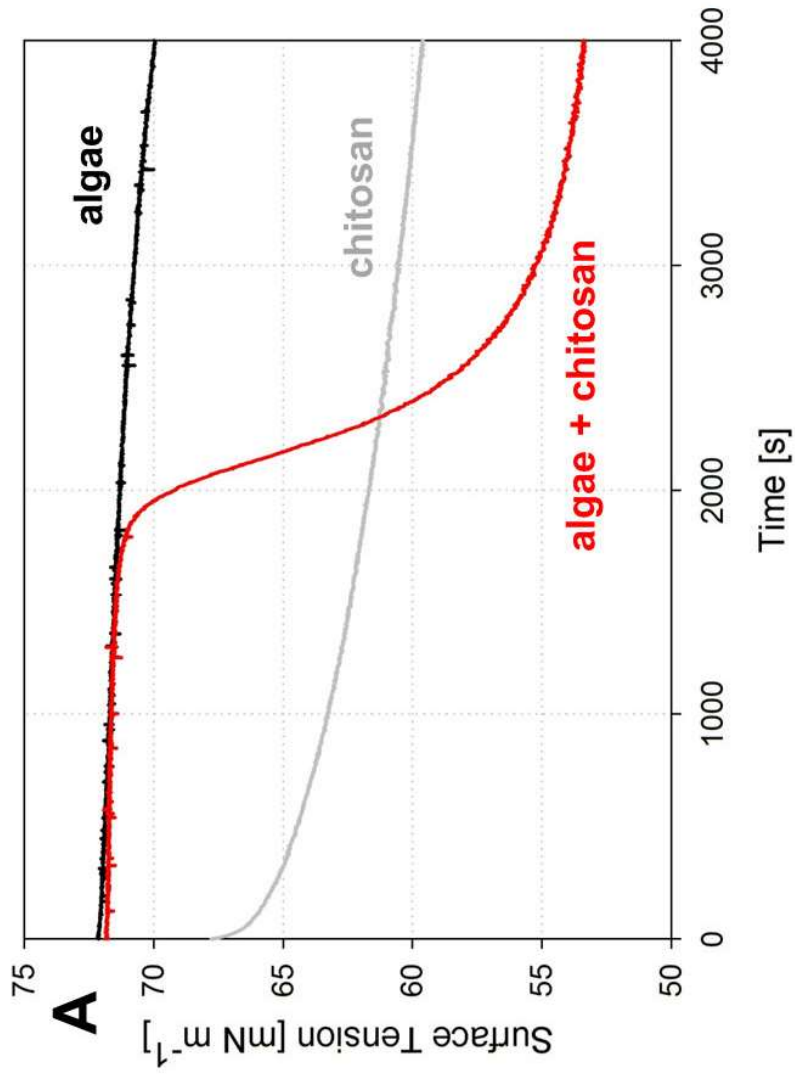
***Chlorococcum sp.***      n.d.      n.d.      Malvern Zetasizer 90 (Malvern Instruments Ltd., USA)      70 mM FeCl<sub>3</sub>      - 4 mV      [67]











**Declaration of interest**

The authors declare that there is no conflict of interest.