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

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Abstract

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

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^{-1}) is needed to adjust the isoelectric point compared to cetyltrimethylammonium bromide (50 mg L^{-1}).
- Flotation studies reveal an optimum chitosan concentration range of 12–18 mg L^{-1} 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
ζ	Zeta potential	mV
σ	Surface tension	mN m^{-1}
BM	Bristol's Modified Medium	-
c_{X0}	Initial cell concentration	Cells mL^{-1}
c_X	Cell concentration	Cells mL^{-1}
CTAB	Cetyltrimethylammonium bromide	-
DiAF	Dispersed-air Flotation	-
IEP	Isoelectric point	mV
PALS	Phase Analysis Light Scattering	-
PBR	Photobioreactor	-
PFD	Photon flux density	µmol m^{-2} s^{-1}

Q_3	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]:

dissolved-air flotation (DAF) [28], dispersed-air flotation (DiAF) [29] and electro-coagulation flotation (EFC) [30]. DiAF has the lowest energy demand compared to the other flotation technologies. It generates bubbles by continuously passing air through a porous material or by dispersing air via a high-speed mechanical agitator, yielding a heterogeneous bubble size distribution between 100 µm and 1500 µm [31]. The recovery efficiency increases if the size of the bubbles is of the order of the algal cell size [32]. Smaller bubble sizes can be achieved in DAF, where part of the medium or water is supersaturated with air under pressure (around 400 kPa) and re-introduced into the flotation cell [29]. Due to the pressure drop, excess air is released in the form of microbubbles of 10 µm–100 µm [23]. Other gases such as CO_2 [33] or ozone [36, 37] can also be introduced. In electroflotation; the microbubbles are produced by water hydrolysis [8]. This technique is often used in combination with flocculation employing coagulants [35] or electro-coagulation [36], where algal flocs are formed by charge neutralization. The application of metal electrodes can introduce contaminating metal ions; this is prevented by employing non-sacrificial carbon electrodes [37].

Other flotation approaches aim to attach the cells to carrier particles instead of utilizing air bubbles. The particle-cell-aggregates which form can be separated either by buoyancy [38] or, in the case of magnetic carrier particles, in magnetic field gradients [39–41]. Independently of the flotation technique, the zeta potential of the algal surface is an important parameter since it influences the interaction both between the algal cells themselves and between the algal cells and gas bubbles during attachment. Despite several empirical studies on microalgae flotation [42–45], there is only little information on the cellular zeta potential ζ in different medium conditions. Under physiological growth conditions (pH 4–8), microalgal cells exhibit a negative zeta potential ζ, predominately caused by dissociated carboxylic groups (−COOH) at the cell surface [12]. A highly negative zeta potential indicates that the algal cells are stably dispersed in the surrounding medium, counteracting attachment at the bubble surface [46]. Two strategies can be pursued to modify ζ: (1) adjusting the environmental conditions (pH, salt concentration) to reach the isoelectric point ($\zeta = 0$) or (2) adding cationic surfactants which adsorb at the algal surface and facilitate cell-bubble interactions. Such surfactants (also called

collectors in the flotation process) compensate for the negative algal surface charge and make the algal surface more hydrophobic due to their long hydrocarbon chains [47].

Classically, polyvalent metal ions and synthetic surfactants such as cetyl trimethylammonium bromide (CTAB) are introduced as coagulation and flotation agents [46, 48–50]. Such contaminants are undesired in the efficient, economic production of high-value algal metabolites, which requires non-toxic, biocompatible substances added in low amounts. However, previous studies which added biomolecules such as tea saponin or chitosan to promote algae flotation yielded poorer results compared to synthetic surfactants [48], or used biological substances in addition to synthetic surfactants [49].

Hence, biocompatible approaches to adjust the cellular surface properties for the flotation process need to be studied in more detail. For that purpose, *Chlorella vulgaris* is used as a model algal system in physiological growth conditions. Zeta potential measurements and corresponding flotation experiments in a DiAF column are performed with concentration series of different additives. First, the feasibility is tested of obtaining floatable cells solely by pH variation. Then, chitosan is used (being a biocompatible additive which has already proved effective as an algae flocculant) and compared to the performance of CTAB as a reference synthetic collector. Finally, particle sizing and surface tension measurements of suspensions with chitosan-algae complexes reveal the important effects which the bio-collector has on the flotation process.

2. Materials and Methods

2.1 Strain and culture conditions

Chlorella vulgaris 211-11b was obtained from the Algae Culture Collection at Göttingen University (Göttingen, Germany). The microalgae cultures were initially grown at 26°C (Mytron, Heilbad Heiligenstadt, Germany), at light/dark cycles of 16/8 hours, 180 rpm and a photon flux density (PFD) of 20 µmol m^{-2} s^{-1} fluorescent white light in 300 mL shake flasks (Schott Duran unbaffled, Wertheim, Germany) containing 100 mL of a BM medium of the

following composition: 1.5 g L^{-1} $NaNO_3$, 0.5 g L^{-1} K_2HPO_4, 1.0 g L^{-1} K_2SO_4, 1.0 g L^{-1} NaCl, 0.2 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.04 g L^{-1} $CaCl_2 \cdot 2H_2O$ and 100 µL L^{-1} Hutner's trace elements. All medium components were obtained from Carl Roth. The cultures were incubated for 100 hours before studying the zeta potential of microalgal cells under varying conditions (see Section 2.3). Lab-scale cultivation was performed in a 1.5 L bubble column which was inoculated with a 7-day-old shake flask culture to obtain an initial optical density at 750 nm (OD_{750}) of 0.1 (BM medium). The process conditions were adjusted to 25 °C ± 2 °C, an initial PFD of 200 µmol m^{-2} s^{-1} fluorescent white light and an aeration rate of 25 L h^{-1} (dry air). The cultivations were performed as two biological replicates.

2.2 Correlation of optical density and cell concentration

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

$$Cell\ concentration\ c_X\ [cells\ mL^{-1}] = 5 * 10^7 * OD_{750} - 8{,}4 * 10^5 \quad (1)$$

2.3 Analysis of zeta potential

In flotation, the zeta potential ζ is an important parameter to characterize the surface properties of the particles (algae) since it is determined by the surface charge of the particles and by the adsorbed matter at the particle surface. The counterions in the diffuse layer around the particles balance the charged species at the particle surface. They are not equally distributed

in the surrounding fluid but are subjected to electrostatic attraction to the particle surface and Brownian motion, resulting in a distribution according to the Poisson-Boltzmann equation [51]. The charge separation in a surface layer and a diffuse layer (electric double layer) leads to an electric potential. The zeta potential is defined as the potential in a position near the surface where the counterions in the diffuse layer are sheared off by a relative motion of the particle to the surrounding fluid. It is frequently used to approximate the surface potential of the particle. The zeta potential of the microalgal cells is measured by Phase Analysis Light Scattering (NanoBrook 90Plus PALS, Brookhaven Instruments Corp., Holtsville, NY). This technique detects the phase shift of the light which is scattered by the particles (algae) moving in an applied electric field. From this phase shift, the electrophoretic mobility μ is obtained. Hence, the zeta potential ζ can be determined via the Smoluchowski equation [51]:

$$\mu = \zeta \frac{\varepsilon}{\eta} \tag{2}$$

with the medium viscosity η and permittivity ε. The Smoluchowski equation is valid for the limit of very thin double layers $\kappa a >> 1$ in comparison to the particle radius a, where the Debye-Hückel parameter κ characterizes the inverse double layer thickness. Since the algal samples introduce a considerable ionic strength (i.e. $1/\kappa$ is small) and the cells are typically in the µm range (i.e. a is large), Equation 2 is applicable. Due to the high sensitivity of Phase Analysis Light Scattering (PALS), reliable measurements are possible even for low zeta potential values and high salt concentrations in the surrounding medium.

2.3.1 Zeta potential at varying pH values

Before conducting the PALS measurement, the cell concentration was adjusted to an OD_{750} of 0.05 (= 1.66 * 106 cells mL^{-1}) using BM medium. By adding 1 M HCl, the pH of the samples was adjusted to the desired value in the range of pH 1.0 to 7.0 with three technical replicates. Zeta potential measurements were performed in each condition as seven measurement replicates to achieve a reliable data basis for ζ values.

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_{750} of 0.8 (= 3.9 * 10^7 cells mL^{-1}) 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^{-1} 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^{-1} was adjusted in the algal suspension

by adding the appropriate amount of chitosan stock solution under continuous stirring. The samples were diluted for the laser diffraction measurements using a BM medium with the same chitosan concentration. Each laser diffraction measurement was repeated in two technical replicates.

2.6 Adsorption at air-water interface

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

3. Results and discussion

3.1 Zeta potential

3.1.1 Zeta potential depending on medium conditions

Figure 1A presents the effect of varying pHs on the zeta potential of *C. vulgaris* SAG 211-11b either suspended in BM medium or deionized water. Under physiological pH conditions (pH 4–7), the zeta potential of *C. vulgaris* SAG 211-11b reached values of −22.4 +/−0.45 mV and −30.1 +/−0.6 mV in modified BM or deionized water, respectively. This is in accordance with studies by Kurniawati et al. [54] and Ozkan et al. [42], who measured ζ = −30.3 mV (*C. vulgaris* from a Taiwan shrimp pond, probably within deionized water, pH 6.89, Malvern Zetasizer 2000) and ζ = −23.3 mV (*C. vulgaris* UTEX 2714, BG-11 medium, pH 7.42, ZetaCompact).

At pH 4–7, |ζ| 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 (ζ ≈ 0) was not fully reached by adjusting the pH in the range of 1.0–7.0 for both deionized water and BM.

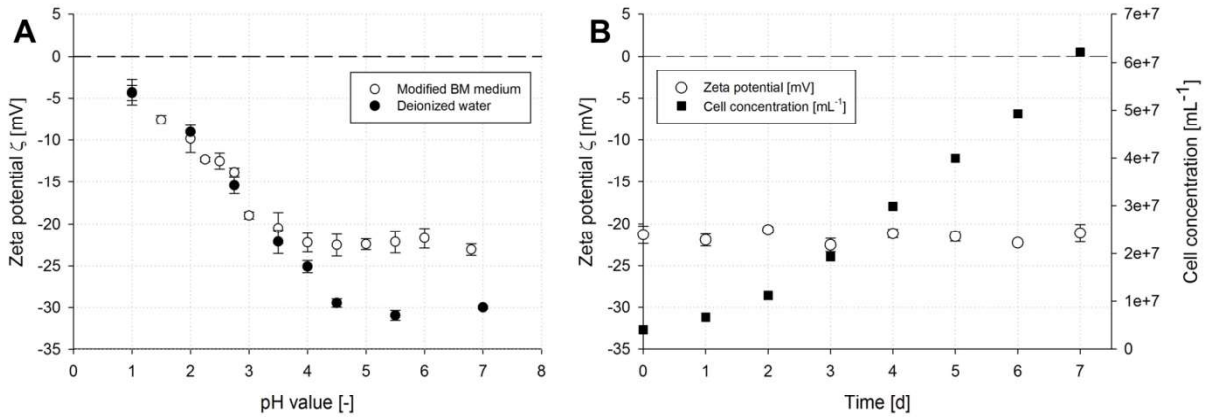

Figure 1: (A) Zeta potential measurements of *C. vulgaris* SAG 211-11b subjected to pH variation ranging from 1.0 to 7.0 in deionized water and BM medium. Error bars represent standard variations in seven independent measurements; (B) Zeta potential measurements of *C. vulgaris* SAG 211-11b during batch-mode 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 ζ 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 (ζ ≈ 0) at

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

3.1.2 Zeta potential dependent on cultivation time during bubble column cultivation

Various parameters such as the cell wall composition or the secretion of extracellular polymeric substances change during the different growth phases of algal cells, which affects the operational efficiency in flocculation and flotation. The zeta potential measurements in this section aim to provide defined conditions regarding the physiological state of the cells (i.e. the age of the culture) rather than to identify the underlying biological mechanisms which affect the algal surface properties during the cultivation time. Therefore, *C. vulgaris* 211-11b were grown in photoautotrophic batch mode (1.5 L bubble column) in a BM medium and analysed at specific cultivation times. As shown in Figure 1B, an increasing cell concentration during photoautotrophic cultivation (0–7 days) did not show any significant effect on the zeta potential yielding stable ζ values of −21.3 ± 1.2 mV. Based on these results, all DiAF studies were performed with 5-day-old cultures at a cell concentration of approx. $3.9 * 10^7$ cells mL^{-1}. As reported in other studies, the physiological state of the cells can actually have a significant impact on the surface properties [50, 56], in turn influencing flotation or flocculation efficiency. The dependence on the growth state may differ with the type of microorganism [50, 57], therefore two specific examples are given in the following. Matter et al. [45] analysed the

influence of the pH (6–10), cell concentration and chitosan dose on the bioflocculation efficiency of *Scenedesmus obliquus* at different growth states. They concluded that stationary growing cells (> 15-day-old cultures) under moderate pH conditions showed facilitated flocculation behaviour compared to late-exponentially growing cells (< 10-day-old cultures). The cultivation time not only influences the physiological state of the cells, but also the cell concentration. In the study by Matter et al., higher cell concentrations could be correlated to higher flocculation efficiency. Similar observations were presented by Maji et al. [57] who tested the flocculation behaviour of *C. vulgaris*, *S. obliquus* and *Chlorococcum* sp. at varying pHs (pH 3.5–12) and biomass concentrations. This is in line with the fact that increasing cell concentration leads to lower mean distance between the cells and higher collision probability which are factors promoting flocculation. As indicated by the constant cellular zeta potential in Figure 1B *C. vulgaris* 211-11b, the cells used in this study (5-day-old cultures) are still under optimum physiological conditions and not suffering from any medium component limitations. This allows neglecting the effect of different growth phases in the following results.

3.1.3 Zeta potential dependent on CTAB and chitosan dose

This section compares the usage of the cationic surfactant CTAB as a classical synthetic collector and the cationic biopolymer chitosan to change the zeta potential of *C. vulgaris* SAG 211-11b. Figure 2A shows the zeta potential in presence of varying CTAB concentrations ranging from 7.5 mg L^{-1} to 100 mg L^{-1}. A noticeable impact of CTAB on ζ was detected at 30 mg L^{-1} yielding ζ = −9.86 mV. By further increasing the CTAB concentration up to 100 mg L^{-1}, a neutralization of the zeta potential was obtained with an optimum lower dosage of 50 mg L^{-1}. These results are comparable with flotation studies by Kurniawati et al. [54], Wen et al. [46] and Alkarawi et al. [58], who reported recoveries of >90 % using CTAB concentrations of 60 mg L^{-1}, 50 mg L^{-1} and 35 mg L^{-1}, respectively.

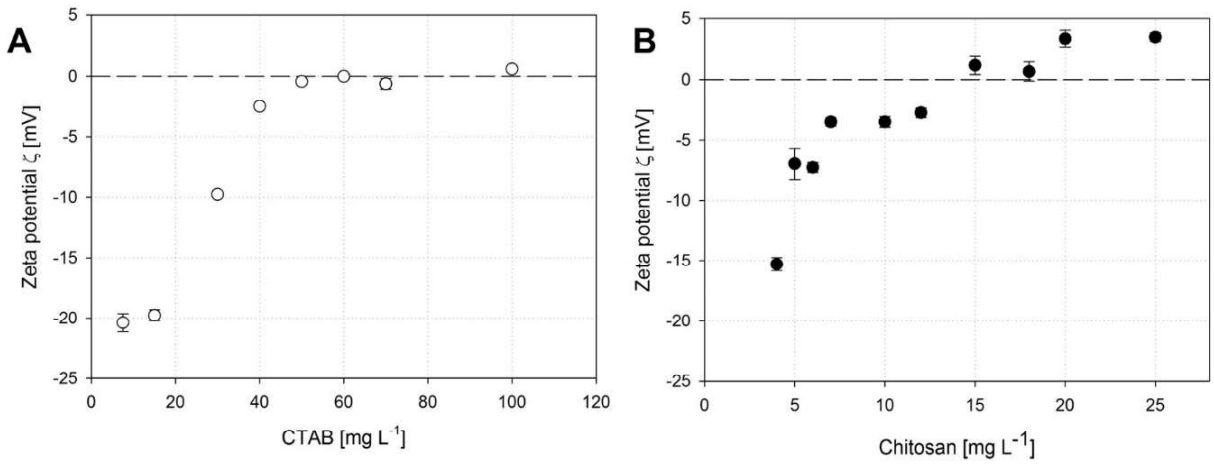

Figure 2: Zeta potential of *C. vulgaris* SAG 211-1b at the presence of the collectors (A) CTAB at 7.5–100 mg L^{-1} and (B) chitosan at 4–25 mg L^{-1}. Error bars represent standard deviation of seven measurement replicates.

Figure 2B shows the zeta potential which was obtained at 4–25 mg L^{-1} chitosan. Compared to the surfactant CTAB (50 mg L^{-1}), using the biopolymer chitosan (15 mg L^{-1}) 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^{-1}. The authors reported an IEP of *C. vulgaris* UTEX 0000265 at comparably high chitosan concentrations of > 100 mg L^{-1}. 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^{-1}, 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 ζ <−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 ± 1.4 %, 35.0 ± 1.3 % and 60.4 ± 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 ζ 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.

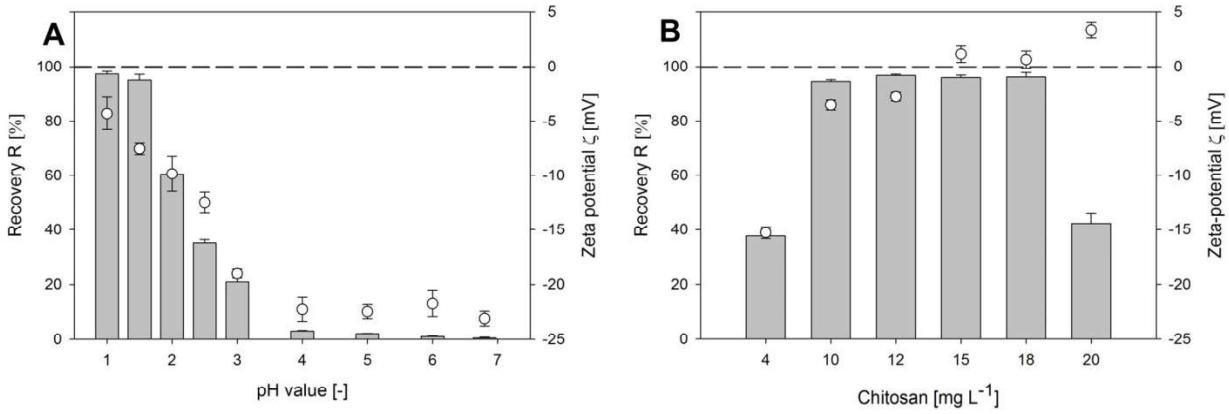

Figure 3: Recovery of microalgal cells (grey bars) in a 28 mL dispersed-air flotation column and corresponding zeta potential values (non-filled circles) from Figure 1A and Figure 2B, respectively, varying the pH conditions for (A) and varying the chitosan dose for (B). Error bars represent standard 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^{-1}), resulting in negative ζ (4 mg L^{-1}), neutral ζ (10–18 mg L^{-1}) and positive ζ (20 mg L^{-1}). 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 mV > ζ < 0.66 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^{-1} saponin and 5 mg L^{-1} chitosan, respectively. Another study [49] employs 10 mg L^{-1} chitosan and 20 mg L^{-1} 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^{-1}, 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.

3.3 Underlying mechanisms for dispersed-air flotation of *C. vulgaris* using chitosan as bio-collector

The upper limit of the chitosan concentration range with a high recovery R (18 mg L^{-1}) was chosen to provide the most robust conditions for the surface tension and particle size measurement. Prior to this, the native algal suspension with no added chitosan was characterized. According to the dynamic surface tension measurements, shown in Figure 4A, the algal suspension shows negligible surface activity. If 18 mg L^{-1} chitosan is added to the medium without algae, the surface tension decreases with time as more and more chitosan molecules are adsorbed at the interface. After around 4000 s, the surface tension reaches a value of 58 mN m^{-1}. The chitosan-algae complexes first show no noticeable deviation from the native algal suspension. After this induction period of approx. 1500 s, the surface tension decreases to 53 mN m^{-1} at around 4000 s, which is even lower than in the case of a medium supplemented with chitosan. This indicates that the chitosan-algae complexes have a high tendency to adsorb at the gas-liquid interface in comparison to native algae or the pure chitosan solution, since their neutralized charge makes them more hydrophobic. The existence of an induction period is a known phenomenon for the adsorption of large-size surface-active matter [62]. In addition to the extremely slow diffusion, multi-step adsorption processes and the existence of adsorption barriers, e.g. due to electrostatic interaction, can lead to such adsorption dynamics. However, profile analysis tensiometry employs a quiescent system, whereas convective mixing leads to considerably faster adsorption [63]. Through the flow field around the rising bubbles in our flotation column, adsorption occurs within the residence time of the bubbles.

The results of the particle size measurements are plotted in Figure 4B as the cumulative function of the volume weighted size distribution $Q_3(x)$. This quantity describes the particle mass fraction, which is smaller than the respective particle size x. Similarly, $1-Q_3(x)$ corresponds to the particle mass fraction which is larger than x. Without chitosan, the algal cells are typically a few micrometres in size, which corresponds to microscopic observations.

Between 6 and 10 µm, $Q_3(x)$ 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 µm, which can probably be ascribed to a minor degree of self-agglomeration. With 18 mg L^{-1} chitosan, 85 % of the algal mass is larger than 10 µm and 50 % is still larger than 60 µm. This flocculating effect of chitosan shifts the particle size towards the floatable particle size range between 20 and 150 µm [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.

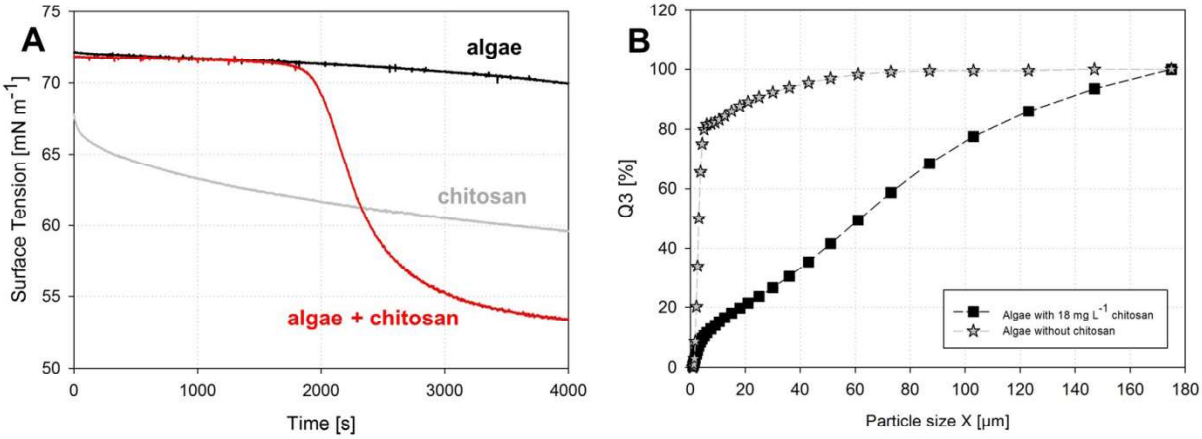

Figure 4: (A) Dynamic surface tension of microalgal cell suspension in BM medium, in BM medium with 18 mg L^{-1} chitosan, and chitosan solution in BM medium without algae, (B) Cumulative function of the volume weighted size distribution for microalgal cells in BM medium and in BM medium with 18 mg L^{-1} 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

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

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No conflicts, informed consent, or human or animal rights are applicable to this study.

6. References

[1] Y. Chisti, Raceways-based production of algal crude oil. *C Posten C Walter Eds Microalgal Biotechnol. Potential Prod. Gruyter Berl.* 2012, 113–146.

[2] T. Ndikubwimana, J. Xiao, W. Shao, Flotation: A promising microalgae harvesting and dewatering technology for biofuels production. *Biotechnol. J. 11* 2016, 315–326.

[3] C.-Y. Chen , K.-L. Yeh, R. Aisyah, D.-J. Lee, J.-S. Chang, Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. *Bioresour. Technol. 102* 2011, 71–81.

[4] A. Converti, A. A. Casazza, E. Y. Ortiz, P. Perego, M. Del Borghi, Effect of temperature and nitrogen concentration on the growth and lipid content of *Nanoochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chem. Eng. Process. Process Intensif. 48* 2009, 1146–1151.

[5] K. Yamaguchi, Recent advances in microalgal bioscience in Japan, with special reference to utilization of biomass and metabolites. *J. Appl. Phycol. 8* 1996, 487–502.

[6] L. Borges, J. A. Morón-Villarreyes, M. G. Montes D'Oca, P. C. Abreu, Effects of flocculants on lipid extraction and fatty acid composition of the microalgae *Nannochloropsis Oculata* and *Thalassiosira Weissflogii* Biomass. *Bioenergy 35* 2011, 4449–4454.

[7] K. H. M. Cardozo, T. Guaratini, M. P. Barros, V. R. Falcão, A. P. Tonon, Metabolites from algae with economical impact. *Comp. Biochem. Physiol.* 2007, *Part C 146*, 60–78.

[8] I. Valencia, D. Ansorena, I. Astiasarán, Development of dry fermented sausages rich in docosahexaenoic acid with oil from the microalgae *Schizochytrium* sp.: influence on

nutritional properties, sensorial quality and oxidation stability. *Food Chem.* 2007, *Volume 104, Issue 3*, 1087–1096.

[9] L. Gouveia, A. Raymundo, A. Paula Batista, I. Sousa, J. Empis, *Chlorella vulgaris* and *Haematococcus pluvialis* biomass as colouring and antioxidant in food emulsions. *Eur. Food Res. Technol.* 2006, *222*, 362–367.

[10] A. Janoska, V. Andriopoulos, R. H. Wijffels, M. Janssen, Potential of a liquid foam-bed photobioreactor for microalgae cultivation. *Algal Res. Vol. 36* 2018, 193–208.

[11] N. Uduman, Y. Qi, M.K. Danquah, G.M. Forde, A. Hoadley, Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. *J. Renew. Sustain. Energy 2* 2010, 1–15.

[12] C. A. Laamanen, G. M. Ross, J. A. Scott, Flotation harvesting of microalgae. *Renew. Sustain. Energy Rev. 58* 2016, 75–86.

[13] E. Molina Grima , E.-H. Belarbi , F. G. Acién Fernández , A. Robles Medina, Y. Chisti, Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnol. Adv. 20* 2003, 491–515.

[14] M. K. Danquah , B. Gladman, N. Moheimani, G. M. Forde, Microalgal growth characteristics and subsequent influence on dewatering efficiency. *Chem. Eng. J. 151* 2009, 73–78.

[15] N. Rashid, M. S. U. Rehman, J.-I. Han, Use of chitosan acid solutions to improve separation efficiency for harvesting of the microalga *Chlorella vulgaris*. *Chem. Eng. J. 226* 2013, 238–242.

[16] D. Vandamme, I. Foubert, K. Muylaert, Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. *Trends Biotechnol. 31* 2013, 233–239.

[17] P. Das, M. I. Thaher, M. A. Q. M. A. Hakim,, H. M. S. .J. Al-Jabri, G. S. H. S. Alghasal, Microalgae harvesting by pH adjusted coagulation-flocculation, recycling of the coagulant and the growth media. *Bioresour. Technol. 216* 2016, 824–829.

[18] M. Heasman, J. Diemar, W. O' Connor, T. Sushames, L. Foulkes, J.-A. Nell, Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs—a summary. 2000, 637–659.

[19] Y.-R. Chang , D.-J. Lee, Coagulation–membrane filtration of *Chlorella vulgaris* at different growth phases. *Dry Technol 30* 2012, 1317–1322.

[20] X. Sun, C. Wang, Y. Tong, W. Wang, J. Wei, A comparative study of microfiltration and ultrafiltration for algae harvesting. *Algal Res. 2* 2013, 437–444.

[21] Y.M. Chen, J.C. Liu, Y.H. Ju, Flotation removal of algae from water. *Colloids Surf B Biointerf 12* 1998, 49–55.

[22] G. Shelef, A. Sukenik, M. Green, Microalgae Harvesting and Processing: A Literature Review. *Tech. Res. Dev. Found. Ltd* 1984.

[23] L. Christenson, R. Sims, Production and harvesting of microalgae for waste water treatment, biofuels and bioproducts. *Biotechnol. Adv. 29* 2011, 686–702.

[24] A. I. Barros, A. L. Gonçalves, M.Simões, J. C. M. Pires, Harvesting techniques applied to microalgae: A review. *Renew. Sustain. Energy Rev. 41* 2015, 1489–1500.

[25] H. Zhang , X. Zhang, Microalgal harvesting using foam flotation: A critical review. *Biomass Bioenergy 120* 2019, 176–188.

[26] K. Muylaert, L. Bastiaens, D. Vandamme, L. Gouveia, Harvesting of microalgae: Overview of process options and their strengths and drawbacks. *Microalgae-Based Biofuels Bioprod.* 2017, 113–132.

[27] G. Singh, S. K. Patidar, Microalgae harvesting techniques: A review. *J. Environ. Manag. 217* 2018, 499–508.

[28] J. K. Edzwald, Dissolved air flotation and me. *Water Res. 44* 2010, 2077–2106.

[29] K.-Y. Show, D.-J. Lee, Chapter 5 - Algal Biomass Harvesting. *Biofuels Algae* 2014, 85–110.

[30] L. Xu, F. Wang, H.-Z. Li, Z.-M. Hu, C. Guo, C.-Z. Liu, Development of an efficient electroflocculation technology integrated with dispersed-air flotation for harvesting microalgae. *Chem. Technol. Biotechnol. 85* 2010, 1504–1507.

[31] J. Rubio, M. L. Souza , R. W. Smith, Overview of flotation as a waste water treatment technique. *Miner. Eng. 15* 2002, 139–155.

[32] J. Hanotu, E. Karunakaran, H. Bandulasena, C. Biggs, W. B. Zimmerman, Harvesting and dewatering yeast by microflotation. *Biochem. Eng. J. 82* 2014, 174–182.

[33] M. S. Kim, D. H. Kwak, Effect of zeta potential on collision-attachment coefficient and removal efficiency for dissolved carbon diox-ide flotation. *Environ. Eng. Sci. 34* 2017, 272–280.

[34] Y. L. Cheng, Y. C. Juang, G. Y. Liao, P. W. Tsai, S. H. Ho, K. L. Yeh, D. J. Lee, Harvesting of *Scenedesmus obliquus* FSP-3 using dispersed ozone flotation. *Bioresour. Technol. 1021* 2011, 82–87.

[35] W. Zhou, L. Gao, W. Cheng, L. Chen, J. Wang, H. Wang, T. Liu, Electro-flotation of *Chlorella* sp. assisted with flocculation by chitosan. *Algal Res. 18* 2016, 7–14.

[36] E. Poelman, N. De Pauw, B. Jeurissen, Potential of electrolytic flocculation for recovery of micro-algae. *Resour. Conserv. Recycl. 191* 1997, 1–10.

[37] A. Guldhe, R. Misra, P. Singh, I. Rawat, F. Bux, An innovative electrochemical process to alleviate the challenges for harvesting of small size microalgae by using non-sacrificial carbon electrodes. *Algal Res. 19* 2016, 292–298.

[38] H. Wen, X. Zou, K. Xu, Z. Shen, X. Ren, Y. Li, Buoy-bead flotation application for the harvesting of microalgae and mechanistic analysis of significant fac-tors. *Bioprocess Biosyst. Eng.* 2018, 1–10.

[39] G. Prochazkova, I. Safarik, T. Branyik, Harvesting microalgae with microwave synthesized magnetic microparticles. *Bioresour. Technol. 130* 2013, 472–477.

[40] R. Stange, F. Lenk, K. Eckert, S. Lenk, T. Bley, E. Boschke, A new method for mixing of suspended superparamagnetic beads using variable electromagnetic fields. *Eng. Life Sci. 157* 2015, 727–732.

[41] P. Fraga-García, P. Kubbutat, M. Brammen, S. Schwaminger, S. Berensmeier, Bare Iron Oxide Nanoparticles for Magnetic Harvesting of Microalgae: From Interaction Behavior to Process Realization. *Nanomater. 85* 2018, 292.

[42] A. Ozkan, H. Berberoglu, Physico-chemical surfaceproperties of microalgae. *Colloids Surf. B Biointerfaces 112* 2013, 287–293.

[43] R. K. Henderson , S. A. Parsons, B. Jefferson, Successful Removal of Algae through the Control of Zeta Potential. *Sep. Sci. Technol. 437* 2008, 1653–1666.

[44] A. L. Ahmad, N. H. Mat, Yasin C. J., C. Derek, J. K. Lim, Optimization of microalgae coagulation process using chitosan. *Chem. Eng. J. Vol. 173* 2011, 879–882.

[45] I. A. Matter, O. M. Darwesh, M. F. Eida, Harvesting of *Scenedesmus obliquus* by Bioflocculation: Appropriate Chitosan Concentrations with Various pH Values at Different Growth Stages. *Jordan J. Biol. Sci.* 2018.

[46] W. Hao, L. Yanpeng, S. Zhou, R. Xiangying, Z. Wenjun, L. Jun, Surface characteristics of microalgae and their effects on harvesting performance by air flotation. *Int. J. Agric. Biol. Eng.* 2017, 125–133.

[47] M. Alhattab, M. S. L. Brooks, Dispersed air flotation and foam fractionation for the recovery of microalgae in the production of biodiesel. *Sep. Sci. Technol. 5212* 2017, 2002–2016.

[48] Z. Shen, Y. Li, H. Wen, X. Ren, J. Liu, L. Yang, Investigation on the role of surfactants in bubble-algae interaction in flotation harvesting of *Chlorella vulgaris*. *Sci. Rep. 81* 2018, 3303.

[49] J. C. Liu, Y. M. Chen, Yi-Hsu Ju, Separation of Algal Cells from Water by Column flotation. *Seperation Sci. Technol. Vol. 34* 1998.

[50] L. Xia, R. Huang, Y. Li, S. Song, The effect of growth phase on the surface properties of three oleaginous microalgae (*Botryococcus* sp. FACGB-762, *Chlorella* sp. XJ-445 and *Desmodesmus bijugatus* XJ-231). *PloS One 1210* 2017.

[51] Hunter, R. J., *Found. Colloid Sci. Vol 1* 1989, *Oxford University Press*.

[52] J. Hanotu, C. H. Bandulasena, W. B. Zimmerman, Micro flotation performance for algal separation. *Biotechnol. Bioeng. 109* 2012, 1663–1673.

[53] Liggieri, L., & Miller, R., Bubble and drop interfaces, CRC Press, 2011.

[54] H. A. Kurniawati, S. Ismadji, J. C. Liu, Microalgae harvesting by flotation using natural saponin and chitosan. *Bioresour. Technol. 166* 2014, 429–434.

[55] S. Hadjoudja, V. Deluchat, M. Baudu, Cell surface characterisation of *Microcystis aeruginosa* and *Chlorella vulgaris*. *J. Colloid Interface Sci. 342* 2010, 293–299.

[56] X. Zhang, P. Amendola, J. C. Hewson, M. Sommerfeld, Q. Hu, Influence of growth phase on harvesting of *Chlorella zofingiensis* by dissolved air flotation. *Bioresour. Technol. 116* 2012, 477–484.

[57] G. K. Maji, S. Choudhury, S. Hamid, R. Prashanth , S. Arjunan, G. Sibi, Microalgae Harvesting via Flocculation: Impact of PH, Algae Species and Biomass Concentration. *Methods Microbiol. Mol. Biol.* 2018.

[58] M. A. S. Alkarawi, G. S. Caldwell, J. G. M. Lee, Continuous harvesting of microalgae biomass using foam flotation. *Algal Res. Vol. 36* 2018, 125–138.

[59] R. Pranowo, D. J. Lee, J. C. Liu, J. S. Chang, Effect of O3 and O3/H2O2 on algae harvesting using chitosan. *Water Sci. Technol. Vol. 67* 2013.

[60] A. Pugazhendhi, S. Shobana, P. Bakonyi, N. Nemestothy, A. Xia, R Banu, G. Kumar, A review on chemical mechanism of microalgae flocculation via polymers. *Biotechnol. Rep.* 2018, *Volume 21*.

[61] Y. J. Low, S. W. Lau, Effective flocculation of *Chlorella vulgaris* using chitosan with zeta potential measurement. *IOP Conf. Ser. Mater. Sci. Eng. 206* 2017.

[62] B. Noskov, A. Mikhailovskaya, Adsorption kinetics of globular proteins and protein/surfactant complexes at the liquid–gas interface. *Soft Matter 9* 2013, *39*, 9392–9402.

[63] M. Lotfi, D. Bastani, V. Ulaganathan, R. Miller, A. Javadi, Bubble in flow field: A new experimental protocol for investigating dynamic adsorption layers by using capillary pressure tensiometry. *Colloids Surf. Physicochem. Eng. Asp. 460* 2014, 369–376.

[64] G. J. Jameson, Advances in fine and coarse particle flotation. *Can. Metall. Q. 49* 2010, *4*, 325–330.

[65] A. E. Sommer, M. Nikpay, S. Heitkam, M. Rudolph, K. Eckert, A novel method for measuring flotation recovery by means of 4D particle tracking velocimetry. *Miner. Eng. 124* 2018, 116–122.

[66] R. K. Henderson , S. A. Parsons, B. Jefferson, The impact of differing cell and algogenic organic matter (AOM) characteristics on the coagulation and flotation of algae. *Water Res. 44* 2010, 3617–3624.

[67] S. B. Ummalyma, A. K. Mathew, A. Pandey, R. K. Sukum, Harvesting of microalgal biomass: Efficient method for flocculation through pH modulation. *Bioresour. Technol. 213* 2016, 216–221.

7. Contributions declaration

CM was responsible for performing the experiments on microalgae cultivation, zeta potential determination and flotation experiments. The profile analysis tensiometry experiments were carried out by BK and CM. FK, KS and CM wrote the manuscript. All studies were planned by KE, KS, JS, FK, CM and TW. All co-authors carefully reviewed the manuscript with focus on microalgae technology (TW, FK, JS, CM), zeta potential, tensiometry and particle size analysis and flotation studies (KE, KS).

8. Declaration of interest

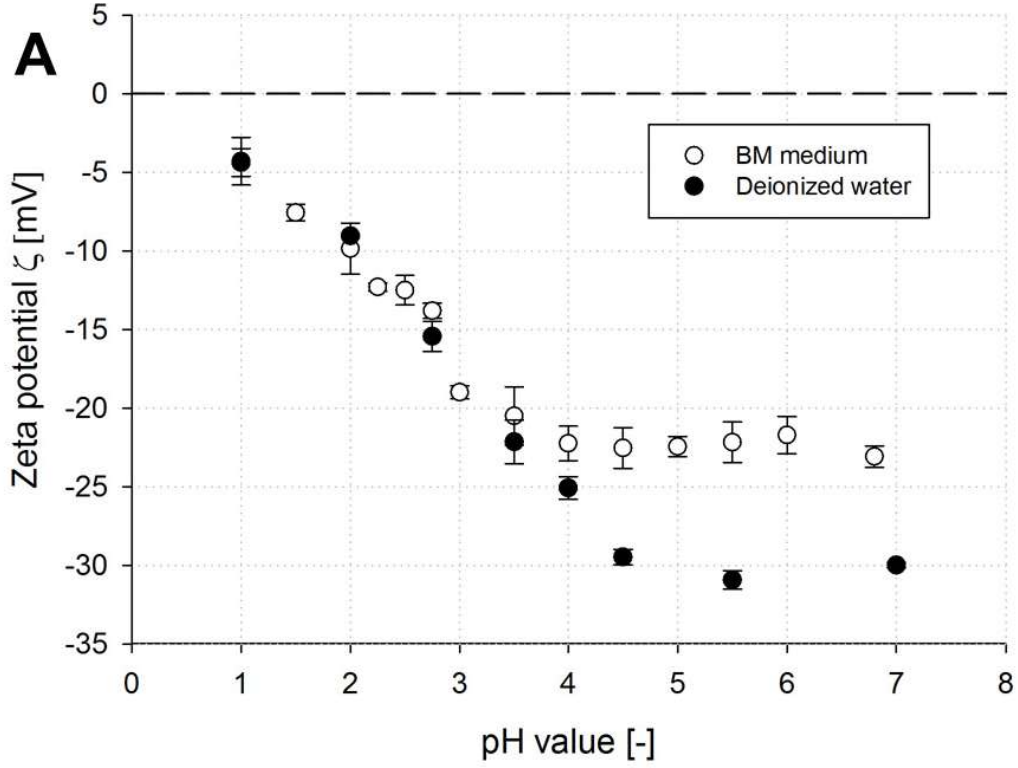
The authors declare that there is no conflict of interest.

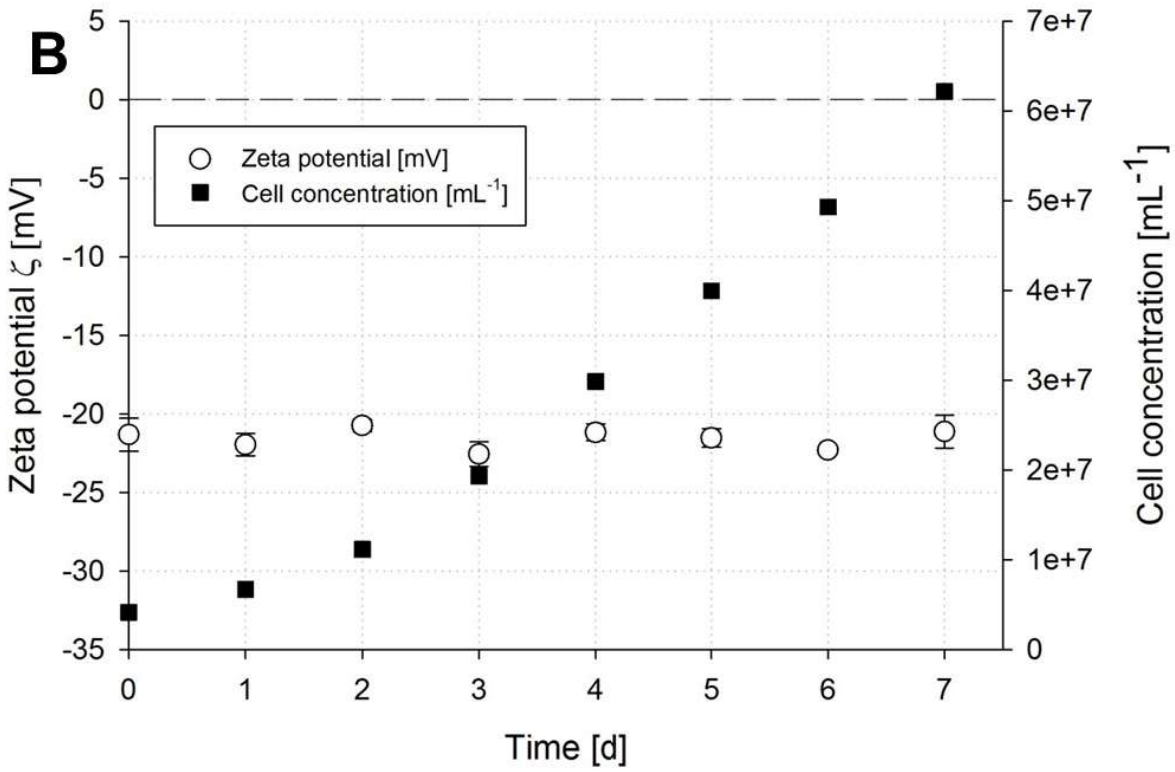
Table 1: Comparison of zeta potential of *Chlorella* ssp. at different process conditions (data of this study are presented as mean value and standard deviation of seven technical replicates).

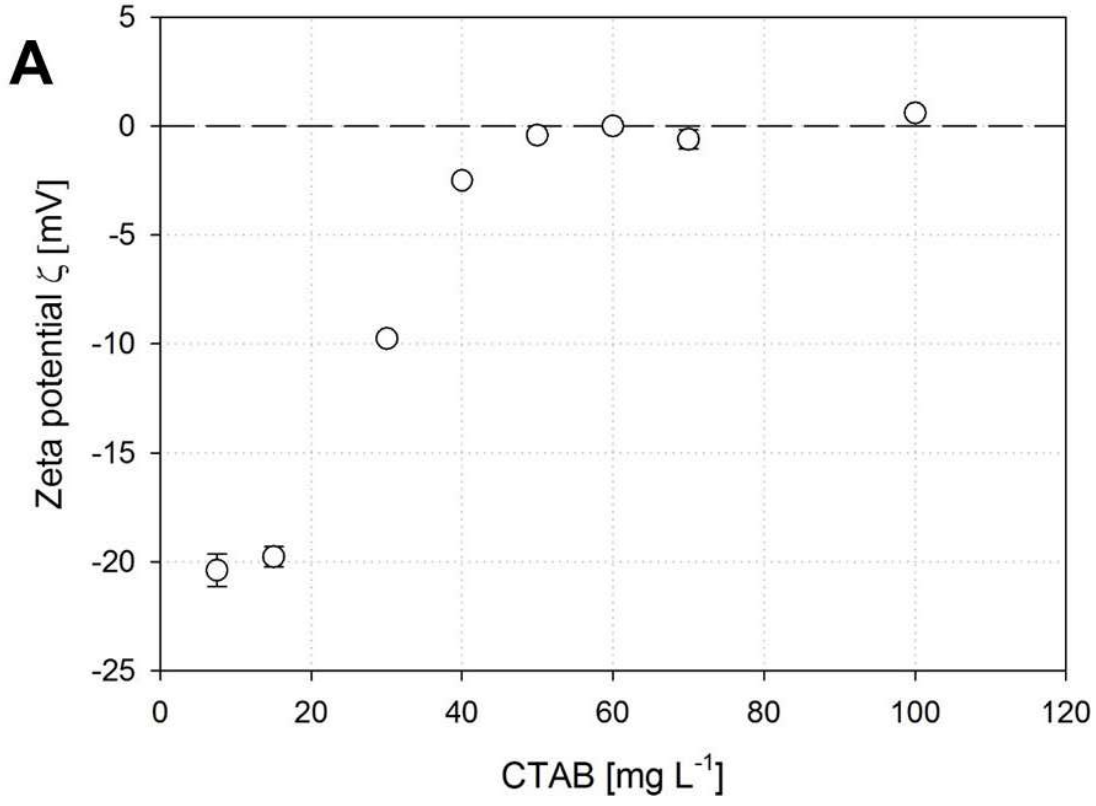
Microalgae stain	Medium conditions	pH	Measuring device	Additives	Zeta Potential	Source
***Chlorella vulgaris* SAG 211-11b**	Modified BM	6.8	NanoBrook 90Plus	-	−23.1 ± 0.7 mV	This Study
***Chlorella vulgaris* SAG 211-11b**	Deionized water	7	NanoBrook 90Plus	-	−29.98 ± 0.2 mV	This Study
***Chlorella vulgaris* SAG 211-11b**	Modified BM	6.8	NanoBrook 90Plus	+ 18 mg L^{-1} Chitosan	0.66 ± 0.8 mV	This Study
***Chlorella vulgaris* SAG 211-11b**	Modified BM	6.8	NanoBrook 90Plus	+ 50 mg L^{-1} CTAB	−0.42 ± 0.05 mV	This Study
Chlorella vulgaris	Deionized water	6.89	Malver Mastersizer 2000	-	−30.3 ± 2.4 mV	[54]
Chlorella vulgaris	Culture medium	6.8	Zeta-Compact,CAD, France	-	−23.3 mV	[42]
***Chlorella vulgaris* CCAP1110/4**	n.d.	2–8	Sephy zetaphoremeter Z3000	0.1 M $NaNO_3$ solution	−15 mV[1]	[55]
***Chlorella vulgaris* 211/11B**	n.d.	n.d.	Zetasizer 2000HSA, Malvern Co., UK	0.5 mM $NaHCO_3$ and 1.8 mM NaCl	−32.6 ± 0.6 mV	[66]
Chlorella vulgaris	n.d.	6.5	Nano-ZS, Malvern Co., UK	10 mM KCl	−21 mV	[39]
Chlorella vulgaris	n.d.	n.d.	Zeta potential analyzer (Photal, ELS-Z)	0,1 g L^{-1} Chitosan	−6.5 mV	[15]
***Chlorella sp.* ESP-6**	n.d.	7.0	Nano-ZS, Malvern Co., UK	pH adjustment with NaOH +200 mg L^{-1} Chitosan	−3 mV, −4 mV and −8 mV	[19]
Chlorococcum sp.	n.d.	n.d.	Malvern Zetasizer 90 (Malvern Instruments Ltd., USA)	120 mM $AlSO_4$	−3 mV	[67]

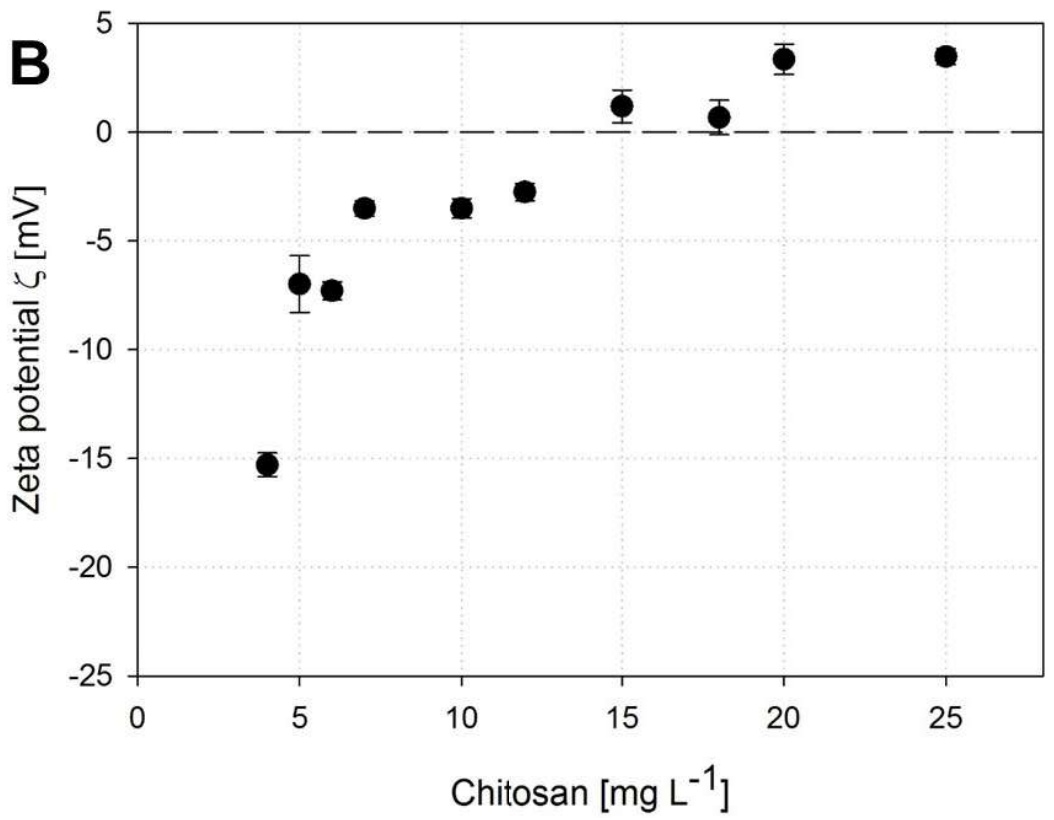
Chlorococcum sp.	n.d.	n.d.	Malvern Zetasizer 90 (Malvern Instruments Ltd., USA)	70 mM FeCl	− 4 mV	[67]

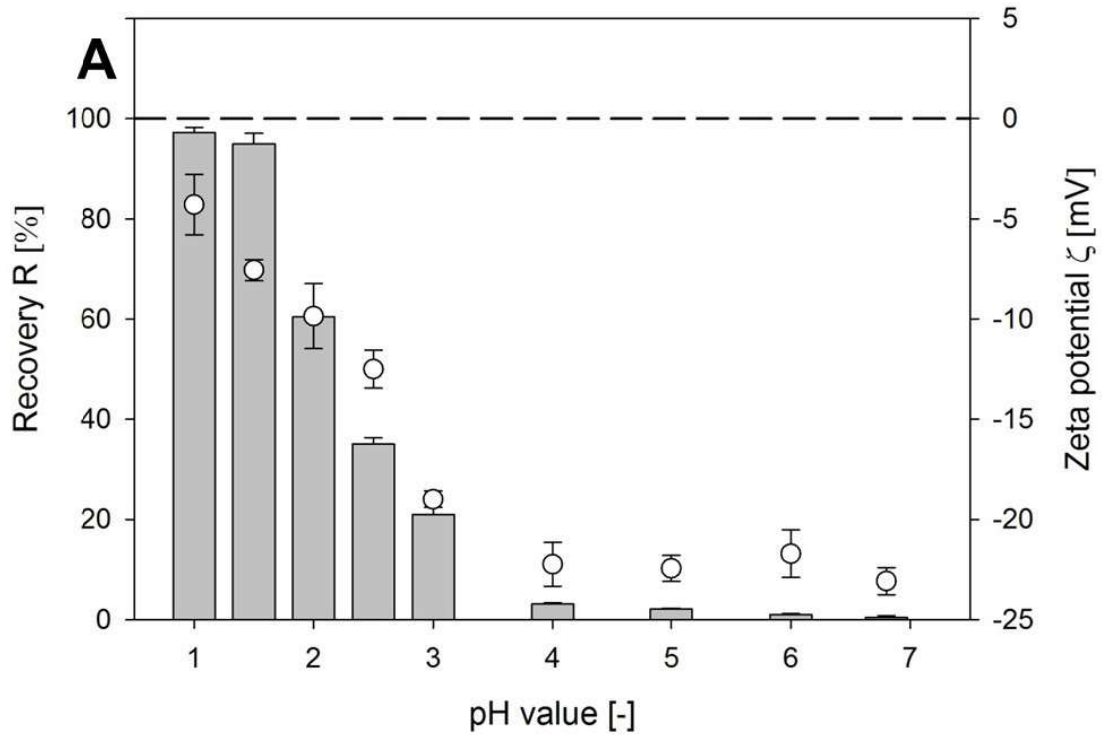
[1] value was estimated from figure.

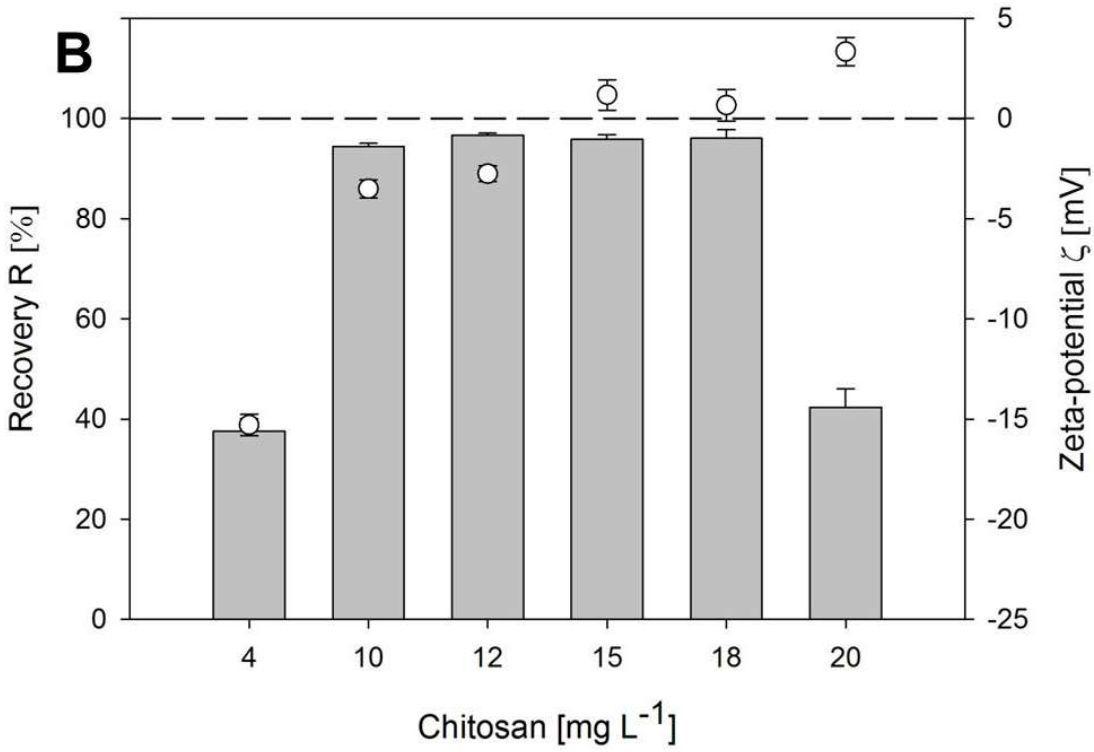

A
Zeta potential ζ [mV]
pH value [-]
BM medium
Deionized water

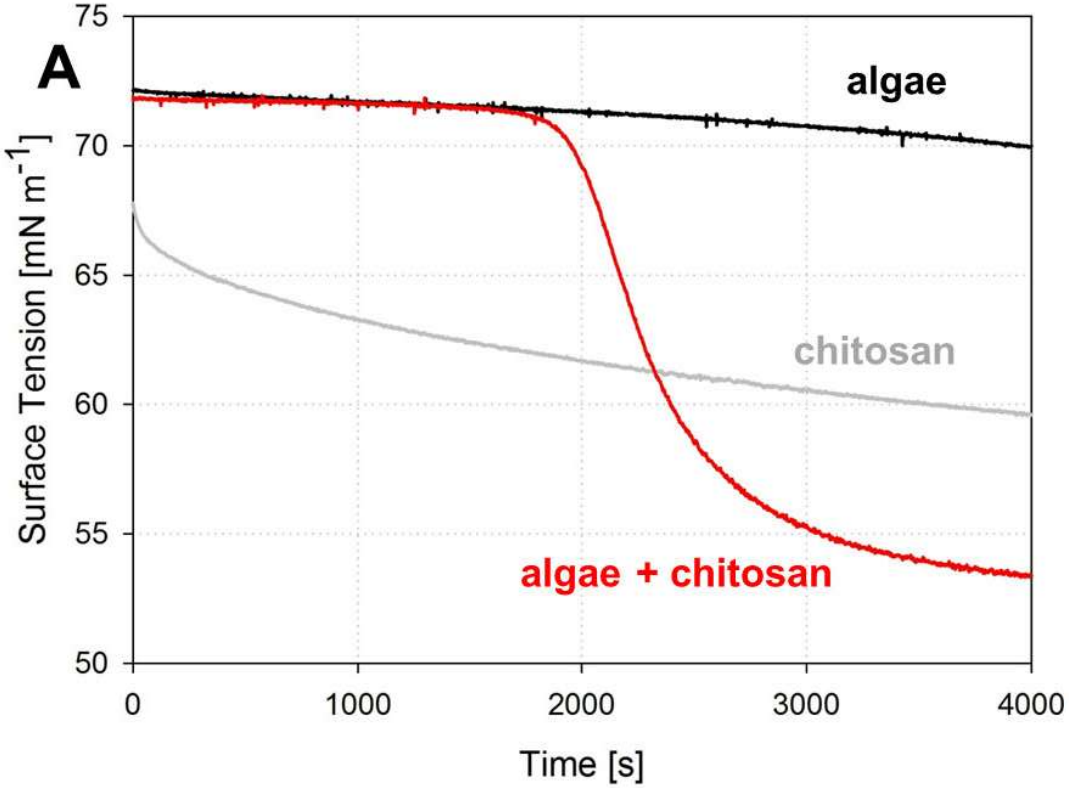

B
Zeta potential ζ [mV]
Cell concentration [mL-1]
Time [d]
Zeta potential [mV]
Cell concentration [mL-1]

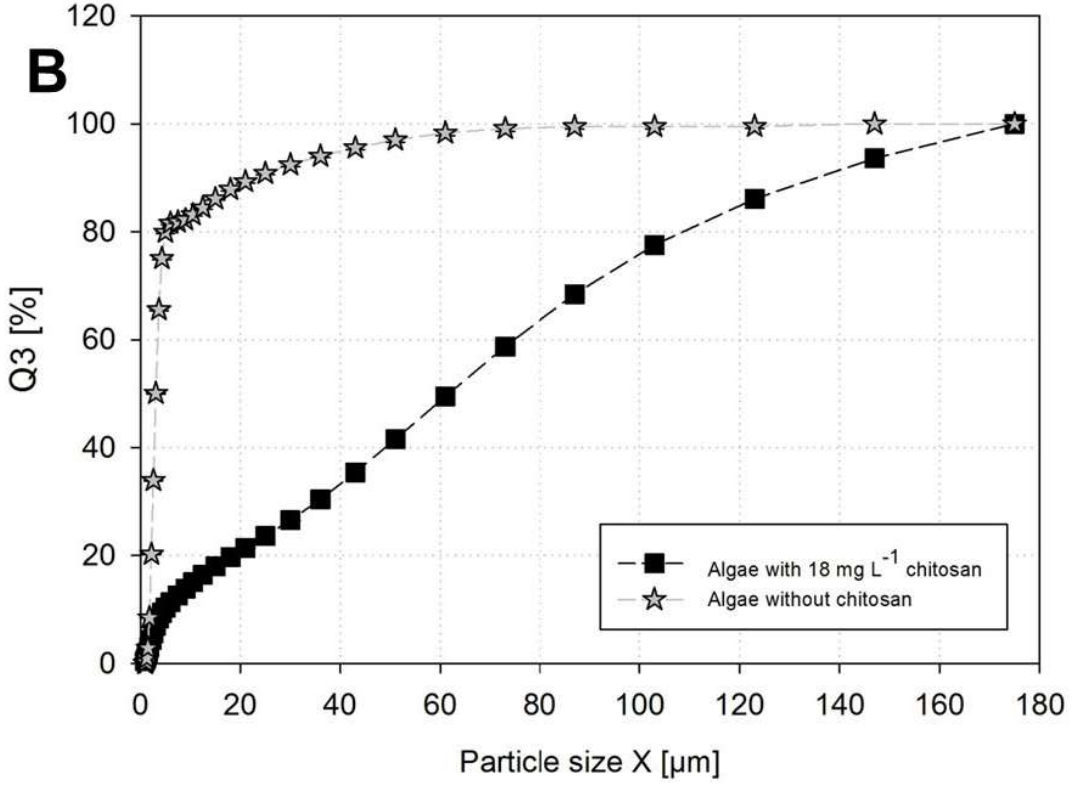

A
5
0
-5
-10
-15
-20
-25
0
20
40
60
80
100
120
Zeta potential ζ [mV]
CTAB [mg L-1]


B
5
0
-5
-10
-15
-20
-25
0
5
10
15
20
25
Zeta potential ζ [mV]
Chitosan [mg L-1]


A
Recovery R [%]
Zeta potential ζ [mV]
pH value [-]
0
20
40
60
80
100
5
0
-5
-10
-15
-20
-25
1
2
3
4
5
6
7


B
Recovery R [%]
Zeta-potential ζ [mV]
Chitosan [mg L-1]
0
20
40
60
80
100
5
0
-5
-10
-15
-20
-25
4
10
12
15
18
20


A
algae
chitosan
algae + chitosan
Surface Tension [mN m-1]
Time [s]
75
70
65
60
55
50
0
1000
2000
3000
4000


B
Q3 [%]
Particle size X [µm]
Algae with 18 mg L-1 chitosan
Algae without chitosan
120
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
180

Declaration of interest

The authors declare that there is no conflict of interest.