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Flocculation properties of several microalgae and a cyanobacterium species during ferric chloride, chitosan and alkaline flocculation

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Abstract

Flocculation holds great potential as a low-cost harvesting method for microalgae biomass production. Three flocculation methods (ferric chloride, chitosan, and alkaline flocculation) were compared in this study for the harvesting of 9 different freshwater and marine microalgae and one cyanobacterium species. Ferric chloride resulted in a separation efficiency greater than 90% with a concentration factor (CF) higher than 10 for all species. Chitosan flocculation worked generally very well for freshwater microalgae, but not for marine species. Alkaline flocculation was most efficient for harvesting of *Nannochloropsis, Chlamydomonas* and *Chlorella sp.* The concentration factor was highly variable between microalgae species. Generally, minimum flocculant dosages were highly variable across species, which shows that flocculation may be a good harvesting method for some species but not for others. This study shows that microalgae and cyanobacteria species should not be selected solely based on their productivity but also on their potential for low-cost separation.

Keywords

Coagulation, microalgae, settling, dewatering, biofuels, dose-response

Highlights

- high variation in optimal dosages between species and flocculation methods
- chitosan was ineffective for harvesting marine species
- species selection for low-cost separation is important

1. Introduction

Microalgae and cyanobacteria attract a lot of interest as new biomass feedstocks for the production of food, feed, fuels, and chemical building blocks (Greenwell et al., 2010; Pienkos and Darzins, 2009; Savage, 2011). However, global production is still very limited (10–20,000 tonnes year^{-1}) and microalgae applications are restricted to niche markets for high-value products (Gerardo et al., 2015; Vanthoor-Koopmans et al., 2013). Upscaling of production is limited by the high cost and energy requirements of different technologies along the entire production chain. Harvesting the microalgal biomass is particularly challenging given the small size of the cells (5–20 µm) and the relatively low biomass concentration in the culture medium (0.5–5 g L^{-1}) (Barros et al., 2015; Wijffels and Barbosa, 2010). Flocculation is widely considered as a promising approach for large-scale and low-cost harvesting of microalgal biomass (Coons et al., 2014; Molina Grima et al., 2003; Vandamme et al., 2013). Using flocculation, small individual microalgal cells are aggregated into large flocs, which can be separated relatively easily from the culture medium using either filtration-based (e.g. membrane filtration) or gravity-based (e.g. sedimentation, centrifugation, flotation) technologies.

Flocculation is generally induced by addition of chemicals that interact with the negatively charged microalgal cell surface (Molina Grima et al., 2003). These chemicals can induce flocculation through different mechanisms: by neutralizing the negative surface charge of the cells (charge neutralization), by connecting individual cells (bridging), or by forming a precipitate that binds and enmeshes the cells (sweeping mechanism) (Vandamme et al., 2013). In the past years, several studies have evaluated the potential of different flocculation methods for harvesting

microalgae. However, these studies generally focused on a single microalgal or cyanobacterial model species such as *Chlorella sp., Scenedesmus*, or *Nannochloropsis sp*. (e.g. 't Lam et al., 2014; Delrue et al., 2015; García-Pérez et al., 2014; Garzon-Sanabria et al., 2012; Vandamme et al., 2012; Xu et al., 2012). Thus, it is currently unknown whether the results can be extrapolated to other economically interesting but less studied species, such as *Pseudanabaena* or *Diacronema*. Microalgae and cyanobacteria are a highly diverse group of aquatic photosynthetic microorganisms, belonging to divergent evolutionary lineages and differing strongly in size, shape, and cell surface properties (Georgianna and Mayfield, 2012; Henderson et al., 2008). Therefore, a flocculation method that is effective for one species may not necessarily be successful for with other species of microalgae or cyanobacteria. Comparison between different studies is complicated because experimental conditions are often different (e.g. biomass concentration and cultivation stage of the culture, parameters of flocculation experiments). A study of the flocculation properties for various species using standard cultivation and evaluation protocols is needed to allow systematic comparison of the flocculation behavior of different microalgae species.

When evaluating the feasibility of a flocculation as a low-cost method for harvesting microalgae, the dosage of flocculant required to induce flocculation is a critical parameter as the quantity of these chemicals will be the main determinant of the harvesting costs. Other parameters are important as well. Flocculation-mediated separation should enable the removal of a large proportion of the cells, i.e. the separation efficiency should be high. The size of the flocs that are formed should also be sufficiently high to obtain flocs that settle easily (Vandamme et al., 2014). Finally, the biomass concentration factor after settling should be maximized to ensure a sufficiently concentrated biomass fraction after settling. Such parameters have never been reported for little-studied but promising species such as *Pseudanabaena*, *Chlamydomonas*, or *Diacronema.* Moreover, the correlation between each of these different parameters has not been analyzed before.

The aim of this study was to systematically compare the flocculation properties of 10 economically interesting microalgal and cyanobacterial species, belonging to different phylogenetic groups and differing in shape, size, and surface charge. For each species, three flocculation methods were tested that differ in the main flocculation mechanism: the metal salt coagulant ferric chloride (charge neutralization), the biopolymer chitosan (bridging), and alkaline flocculation induced by magnesium hydroxide precipitation (sweeping mechanism). The specific objectives of this study were to determine to what extent the flocculant dosage, floc size, and concentration factor differ between species and the impact of these parameters on the cost of harvesting with the respective flocculant.

2. Materials and methods

2.1. Cultivation of microalgae

Nine species of microalgae and one cyanobacterium belonging to different evolutionary groups were selected for this study. They differ strongly in size, shape, and zeta potential (ZP) (Table 1). Cell surface area and volume were calculated using the corresponding formulas for idealized shapes as described by Hillebrand et al. (1999) (Suppl. Table 1). ZP can be used as an indicator of the electrostatic repulsion between the microalgal cells. ZP was estimated from electrophoretic mobility measurements obtained via the phase analysis light scattering (PALS) technique as previously described by Vandamme et al. (2015b).

Four freshwater species (*Chlorella*, *Pseudanabaena*, *Chlamydomonas*, and *Scenedesmus*) were cultivated in Wright's Cryptophyte medium prepared in deionized water. Because alkaline

126 flocculation is caused by precipitation of magnesium hydroxide at high pH and requires a
127 sufficient concentration of magnesium in the medium, the magnesium concentration in this
128 medium was raised to 2 mM (Vandamme et al., 2015a). Six marine species were cultivated in
129 Wright's Cryptophyte medium prepared in artificial seawater (deionized water with 30 g L^{-1}
130 synthetic sea salt; Homarsel, Zoutman, Belgium). Since seawater contains a high concentration of
131 magnesium, no additional magnesium was required to induce alkaline flocculation. The microalgae
132 were cultivated in 30-L bubble column photobioreactors (1 m height, 20 cm diameter). The
133 cultures were mixed by sparging with 0.2-µm-filtered air (5 L min^{-1}) and the pH was maintained at
134 8.5 by addition of 2–3% CO_2 using a pH-stat system. The culture was irradiated on two sides with
135 daylight fluorescent tubes to reach a light intensity of 60 µEinst m^{-2} s^{-1} at the surface of the
136 reactor. Microalgal growth was monitored spectrophotometrically by measuring optical density at
137 750 nm. Absorbance was calibrated against microalgal dry-weight concentration (determined
138 gravimetrically by filtration on Whatman GF-C filters and dried until constant weight at 105°C
139 (Moheimani et al., 2013)). Flocculation experiments were carried out after 12 days when cultures
140 had reached stationary phase. At that stage, the biomass concentration was between 0.35 and 0.45
141 g L^{-1}, except for *Chlamydomonas* and *T-Isochrysis* cultures that had a lower biomass concentration
142 (0.20–0.25 g L^{-1}) (Table 1).
143

144 ***2.2. Flocculation experiments***
145

146 Three flocculation methods, ferric chloride, chitosan, and alkaline flocculation, were tested
147 for each species. These three methods were selected because they are commonly used in studies on
148 microalgae flocculation and they also differ with respect to the flocculation mechanism: the metal
149 salt ferric chloride (Iron (III) chloride, Merck, analytical grade) induces flocculation predominantly
150 through charge neutralization (Wyatt et al., 2012), the cationic polymer chitosan (from crab shells,

Sigma-Aldrich) induces flocculation through a bridging mechanism, and alkaline flocculation causes flocculation predominantly through a sweeping mechanism (Brady et al., 2014; Vandamme et al., 2015a). Alkaline flocculation was induced by addition of sodium hydroxide (Sigma-Aldrich). Since phosphate was depleted in the stationary phase cultures, alkaline flocculation was induced by precipitation of magnesium hydroxide (Brady et al., 2014; Huo et al., 2016; Vandamme et al., 2012). Stock solutions of 0.5 M sodium hydroxide and 10 g L^{-1} ferric chloride were prepared in deionized water. For chitosan, 5 g L^{-1} of stock solution was prepared in 0.01 M HCl. A series of 10–15 jar test experiments were carried out to determine the minimum dosage of flocculant required for induction of flocculation (Suppl. Fig 1). Jar test experiments were carried out in a volume of 100 mL. During addition of the flocculant, the microalgae suspensions were intensively mixed (350 rpm) for 10 min, followed by gentle mixing (250 rpm) for 20 min (Vandamme et al., 2012). The suspensions were subsequently allowed to settle for 30 min. The supernatant was sampled in the middle of the clarified zone and absorbance was measured at 750 nm. The separation efficiency η_a was calculated as:

$$\eta_a = \frac{OD_i - OD_f}{OD_i} \times 100$$

in which OD_i is the absorbance before flocculation and OD_f is the absorbance after flocculation and settling. A four-parameter sigmoidal regression model was empirically fitted on the flocculation dose-response curves (Sigmaplot 11, Systat Software Inc.):

$$Y(x) = y_0 + \frac{a}{1 + exp^{-\left(\frac{x - x_0}{b}\right)}}$$

where x is the unbound variable representing flocculant dosage, x_0 (mg L^{-1}) is the flocculant dosage at the inflection point, $a + y_0 = Y_{max}$ is the maximum separation efficiency (%), and b is the slope of the curve at the inflection point (−) (Suppl. Table 1). Dose-response was compared based on the minimum flocculant dosage for inducing flocculation (estimated as x_0) and the maximum separation efficiency (estimated as Y_{max}). The combination of a relatively large number of jar tests and non-linear modeling allowed estimation of a standard error around the minimum flocculation dosage and to compare the minimum dosages between species. For two species (*Chlorella vulgaris* and *Phaeodactylum tricornutum*) the experiments were repeated for a replicate culture grown with a one-month interval to determine the reproducibility of the minimum flocculant dosage.

2.3. Floc and sludge properties

For effective separation using sedimentation, it is not only important that the flocculant dosage is low, but also that sufficiently large flocs are formed and that flocculation generates a small volume of microalgal sludge (Vandamme et al., 2014). Floc size and sludge volume were determined for the treatments corresponding to the optimum flocculant dosage (corresponding with Y_{max}). Floc size was determined by means of image analysis using ImageJ software (NIH, USA) as previously described (Vandamme et al., 2014). Briefly, a 1 mL subsample of the sludge produced after flocculation was diluted 20 times in fresh culture medium. The flocs were photographed using a stereo zoom microscope (Olympus SZX10) equipped with a digital camera (Lumenera Infinity 2; 5 replicate pictures per treatment, each containing 10–500 separate flocs). The images were transformed to 8 bit, the background was subtracted, and particles were detected based on a threshold of minimum 100 px^2 (Suppl. Fig 2). Floc size was reported as the average Feret's diameter. To determine the volume of sludge produced by flocculation, the flocculated culture was gently poured into a graduated cylinders and the sludge volume was measured after 30 min of sedimentation. The concentration factor (CF) was determined by dividing the total volume (100

mL) by the algae sludge volume. This factor is a measure to report the final biomass solid-liquid ratio (Vandamme et al., 2014).

Correlations between flocculation parameters (Y_{max}, x_0, concentration factor, and floc size) were evaluated using a Spearman rank order test with a level of significance set at $\alpha = 0.05$ (Sigmaplot 11, Systat Software Inc.). Normality of the data was determined using a Shapiro-Wilk normality test.

2.4. Flocculant cost analysis

The flocculant cost was calculated for all species based on the optimum flocculant dose corresponding to Y_{max}, expressed in amount of flocculant per ton of microalgal biomass, and based on bulk price estimations of ferric chloride (500 USD ton^{-1}), chitosan (1500 USD ton^{-1}), and sodium hydroxide (380 USD ton^{-1}) (Farid et al., 2013; Shen et al., 2013; Yang et al., 2016). Additionally, the final flocculant cost per ton of biomass was divided by the concentration factor to give a quantitative overview of its impact on the flocculant cost.

3. Results and discussion

Three flocculation methods were performed in a systematic manner to 10 species of microalgae or cyanobacteria. Flocculation was successful for all species–flocculation method combinations, except for three species of marine microalgae flocculated with chitosan. Only one species, *Tetraselmis*, flocculated spontaneously without addition of a flocculant, but the efficiency of this spontaneous flocculation was low (Supp. Fig 1; only 20%). For each species–flocculation method combination, the response of the separation efficiency to the flocculant dosage was fitted to a sigmoidal model. When flocculation occurred, the fit of the data to this sigmoidal model was

generally good ($R^2 > 0.9$). The model was used to estimate the minimum dose of flocculant needed to induce flocculation (x_0) as well as the maximum separation efficiency (Y_{max}). The standard deviation of the estimated parameters was relatively small (on average 4.2% of mean for x_0 and 3.4% of mean for Y_{max}).

Because microalgae are living organisms, there may be considerable variability between different batch cultures and these differences may affect flocculation conditions. To test whether flocculation was reproducible, the flocculation experiments were repeated on two batch cultures grown with at least a one-month interval for two species (the freshwater *Chlorella* and the marine *Phaeodactylum*) (Fig 1). Differences in x_0 and Y_{max} for these two independent experiments were small, much smaller than the differences that were observed between species (see below). This implies that flocculation is quite predictable for the same species, at least when the species is cultured under the same conditions and harvested during the same cultivation stage. It should be noted, however, that the flocculant dosage may be substantially higher or lower when the species is harvested at a different cultivation stage (e.g. exponential versus stationary phase; Vandamme et al., 2016).

The cost of flocculation is mainly determined by the dosage of flocculant needed to induce flocculation. Therefore, x_0 is a critical parameter. An important outcome of this study is that, for the three flocculation methods tested, x_0 differed by at least an order of magnitude between the different species (Fig 1). For ferric chloride flocculation, the dosage varied between 3 and 69 mg L^{-1}, for chitosan between 5 and 96 mg L^{-1}, and for alkaline flocculation between 18 and 209 mg L^{-1}. The variation in x_0 between species was largest for ferric chloride (coefficient of variation 84%), intermediate for chitosan (coefficient of variation 69%), and lowest for alkaline flocculation (coefficient of variation 48%). These differences can be explained by the flocculation mechanism.

In case of a sweeping mechanism (alkaline flocculation), the flocculant dosage tends to be independent of the particle surface characteristics because particles are enmeshed by a large mass of precipitate. In charge neutralization (ferric chloride), the amount of flocculant required is highly dependent on the number of charges that need to be neutralized, which are in turn a function of the charge density of the cell surface as well as the surface to volume ratio of the cells, parameters that differ strongly between species.

In addition to the flocculant dosage, other parameters are also important when assessing the effectiveness of a flocculation method. The maximum flocculation efficiency (Y_{max}) indicates the proportion of the microalgal population that can be harvested by flocculation. With ferric chloride, a high Y_{max} was achieved for all species of microalgae (average 95%). In case of chitosan, Y_{max} was high for the freshwater species (on average 97%). However, for the marine species, Y_{max} was low (32 to 78%) or no flocculation occurred at all upon addition of chitosan. Polymer flocculants including chitosan often perform poorly in seawater medium (Bilanovic et al., 1988; Lubián, 1989). This can be ascribed to the fact that polymers can undergo coiling at high ionic strengths (Molina Grima et al., 2003). Moreover, with increasing dose of chitosan applied to *Chlamydomonas* and *Dunaliella*, a decrease in flocculation efficiency was observed at the highest dosages. This can be ascribed to dispersion restabilization, a phenomenon that is not caused by charge reversal of the microalgal cell surface (e.g. Morales et al., 1985). In the case of alkaline flocculation, a relatively high Y_{max} was achieved for all species except for *Isochrysis* (Y_{max} = 39%). Nevertheless, Y_{max} was generally lower than in the case of ferric chloride. This might be the result of the fact that an inorganic precipitate is formed during alkaline flocculation. This precipitate can cause a residual turbidity in the medium, especially in marine conditions.

The majority of published studies only report the dosage and separation efficiency when assessing the flocculation behavior of microalgae. The floc size and the concentration factor after flocculation and sedimentation are important parameters for the performance of a flocculation technology as well, as they determine the settling rate of the biomass and the quantity of culture medium that can be removed (Vandamme et al., 2013). In this study, the concentration factor varied strongly between species: from 7 to 50 for ferric chloride, from 5 to 44 for chitosan, and from 5 to 31 for alkaline flocculation (Table 2). A concentration factor < 10 would result in an impractical amount of sludge relative to the volume of culture that is processed. The concentration factor was on average lowest when alkaline flocculation was used. This can be ascribed to the fact that alkaline flocculation is associated with the formation of a large amount of precipitate (Şirin et al., 2012). This precipitate increases the volume of sludge that is formed. Not surprisingly, the mean floc size was highly variable across species–flocculation method combinations, making comparison of floc size between species or flocculation methods more tedious. First, cell size was highly variable amongst the studied species which will directly impact floc size (Table 1). Secondly, the flocculation mechanism will impact floc size as well, as this is different for every method (charge neutralization vs bridging vs sweeping).

Interestingly, the different parameters that highlight different aspects of the flocculation process were all intercorrelated. When the minimum dosage of flocculant was low, maximum separation efficiency tended to be higher (Pearson correlation 0.46, $p = 0.011$), the flocs tended to be larger (Pearson correlation 0.46, $p = 0.012$), and the concentration factor was also higher (Pearson correlation 0.56, $p = 0.001$). This implies that when a low dosage of flocculant is needed for flocculation, other parameters related to the flocculation process will also be acceptable (separation efficiency, floc size, sludge volume).

The present results additionally imply that the cost of harvesting microalgae using flocculation will differ by more than an order of magnitude between species (Table 3). As a result, a flocculant that has been tested and considered cost-effective for harvesting one species of microalgae may not necessarily be cost-effective for another species of microalgae. Ferric chloride was very promising for *Nannochloropsis*, *Tetraselmis*, and *Phaeodactylum* (< 20 USD ton^{-1} biomass) but not for *Pseudanabaena* (150 USD ton^{-1} biomass). On the other hand, the cost of flocculation is not only determined by the dosage but also the cost of the flocculant. Ferric chloride is generally 3 times cheaper than chitosan, while sodium hydroxide is almost 4 times cheaper. For instance, flocculation of *Chlamydomonas* was more cost efficient when using chitosan (65 USD ton^{-1} biomass) than when using ferric chloride (87 USD ton^{-1} biomass). Secondly, the final biomass concentration factor after settling will also determine the cost for secondary dewatering. Flocculation of for example *Chlamydomonas* using chitosan or *Diacronema* using ferric chloride are therefore relatively more effective because of their superior concentration factor. Finally, other implications of the overall process design need consideration as well (Vandamme et al., 2013). For ferric chloride, biomass will be contaminated with iron which could limit biomass applications or value. For alkaline flocculation, sodium hydroxide could be replaced by slaked lime which costs 50% less (Vandamme et al., 2012). Additionally, photosynthesis triggered by a natural pH rise during cultivation should be integrated in the process to minimize the addition of any base. This would reduce the costs by 50–60%. However, a significant difference amongst species would still remain.

Ferric chloride, chitosan, and alkaline flocculation have been proven to be efficient for several model species in previous studies (e.g. ‘t Lam et al., 2014; Delrue et al., 2015; García-Pérez et al., 2014; Garzon-Sanabria et al., 2012; Vandamme et al., 2012; Xu et al., 2012). However, this study reveals that those results cannot be directly extrapolated to many emerging

320 economically interesting species or strains. While for example chitosan was cost-inefficient for
321 most tested species, it can be promising for others such as *Chlamydomonas.* A flocculation method
322 should therefore be assessed based on multiple parameters on the level of each microalgae or
323 cyanobacteria species of interest. This implies that future screening assays should not only select
324 promising strains based on their productivity, but also on their potential for low-cost separation.
325

326 **Conclusions**

327

328 This study demonstrates the importance of species-specific tests to evaluate flocculation
329 and discourages direct extrapolation of the results obtained using known species. The optimal
330 flocculant dosage was highly variable across the different species, with important implications for
331 the cost of flocculation. The results of the present study underline the importance of detailed
332 flocculant screening based on multiple parameters and at the level of microalgae or cyanobacteria
333 species. Microalgae and cyanobacteria should also be selected on their potential for low-cost
334 separation.
335
336

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Figure captions

Figure 1: Maximum separation efficiency (Y_{max}) and minimum flocculant dosage (x_0) for microalgae and a cyanobacterium species using (A) $FeCl_3$, (B) chitosan, and (C) NaOH

Table captions

Table 1: Cell properties of 10 species ($\mu \pm 1\sigma$)

Table 2: Concentration factor and floc size measured at maximum separation efficiency for $FeCl_3$, chitosan, and NaOH flocculation ($\mu \pm 1\sigma$)

Table 3: Flocculant evaluation based on flocculant cost and concentration factor for microalgae and a cyanobacterium species using $FeCl_3$, chitosan, and NaOH

Supplemental material

Suppl. Figure 1: Flocculation dose-response curves from sigmoidal regression analysis for microalgae and a cyanobacterium species using $FeCl_3$, chitosan, and NaOH

Suppl. Figure 2: Original and transformed mask images of flocs used for floc size analysis; flocs formed by $FeCl_3$, chitosan, and NaOH flocculation respectively

Suppl. Table 1: Cell surface area and volume calculations (*V* = volume; *A* = surface area; *d* = diameter; *h* = height; *a* = apical axis (length) ; *b* = transapical axis (width) ; *c* = pervalvar axis (height) (Hillebrand et al., 1999))

Suppl. Table 2: Parameters from sigmoid regression analysis of $FeCl_3$, chitosan, and NaOH dose-response flocculation jar tests ($\mu \pm 1\sigma$)

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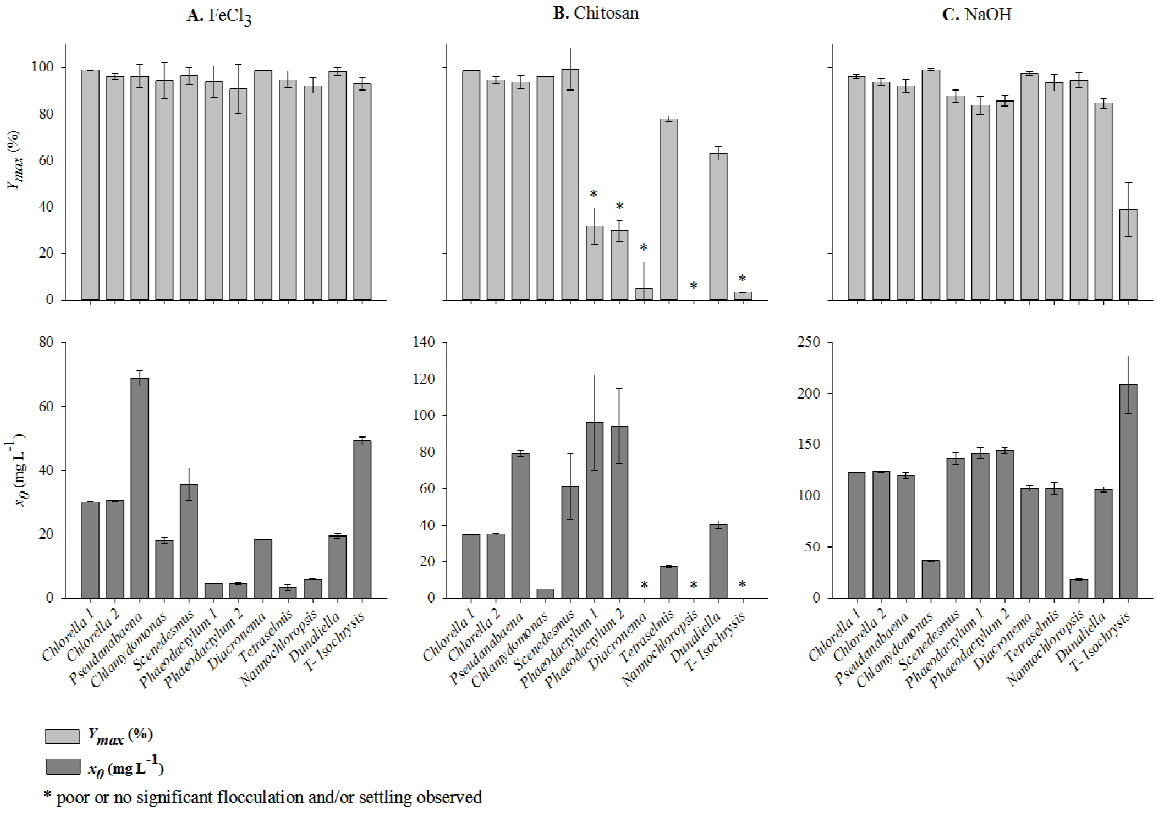
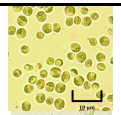
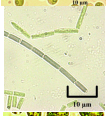
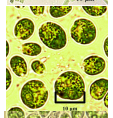
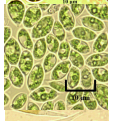
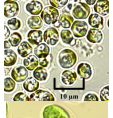
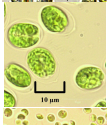
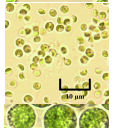
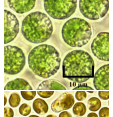
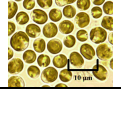


474

Table 1: Cell properties of 10 species (μ ± 1σ)

Species	Image	Class	Shape	DW** (g L^{-1})	Size (μm)		Cell Volume ($μm^3$)	Surface area / Volume ($μm^{-1}$)	Z
					Eq. spherical diameter	Max. linear dimension			
Chlorella vulgaris		Trebouxiophyceae (green algae)	sphere	0.37 ± 0.01	3.0±0.3		15 ± 5	2.0±0.2	-2
Pseudanabaena CY14-1		Cyanophyceae (cyanobacteria)	cylinder	0.45 ± 0.02		79.3± 19.8	163 ± 43	2.5 ± 0.1	
Chlamydomonas reinhardtii		Chlorophyceae (green algae)	sphere, motile	0.25 ± 0.01	7.7±1.7		269 ± 167	0.8 ± 0.2	
Scenedesmus obliquus		Chlorophyceae (green algae)	prolate spheroid	0.43 ± 0.02		8.4±1.3	101 ± 36	1.1 ± 0.1	
Phaeodactylum tricornutum		Bacillariophyceae (diatoms)	half elliptic prism	0.47 ± 0.02		25.7±3.5	55 ± 14	1.9 ± 0.2	
Diacronema lutheri *‡		Pavlovophyceae	sphere, motile	0.39 ± 0.02	5.8±0.9		108 ± 58	1.1 ± 0.2	
Tetraselmis suecica		Chlorodendrophyceae (green algae)	prolate spheroid, motile	0.42 ± 0.01		10.7±0.8	336 ± 103	0.7 ± 0.1	
Nannochloropsis oculata		Chrysophyceae	sphere	0.35 ± 0.03	2.6±0.2		9 ± 3	2.3 ± 0.2	
Dunaliella salina*		Chlorophyceae (green algae)	sphere, motile	0.44 ± 0.02	10.6±1.0		643 ± 183	0.6 ± 0.1	
T- Isochrysis lutea		Coccolithophyceae	sphere, motile	0.21 ± 0.02	4.8±0.6		61 ± 23	1.3 ± 0.2	-

† Measurement on the day of the flocculation experiment (day 12 on culture): late exponential/stationary growth phase
* Experiment on 18th day of culture
** Dry weight concentration
‡ Non-axenic culture

Table 2: Concentration factor and floc size measured at maximum separation efficiency for $FeCl_3$, chitosan and NaOH flocculation ($\mu \pm 1\sigma$)

Species	Concentration Factor (-)			Floc size as Ferret's Diameter (µm)		
	$FeCl_3$	Chitosan	NaOH	$FeCl_3$	Chitosan	NaOH
Chlorella vulgaris	28.6 ± 0.8	24.0 ± 0.9	31 ± 2	270 ± 31	172 ± 80	80.6 ± 1
***Pseudanabaena* CY14-1**	7.0 ± 0.1	4.7 ± 0.1	5.2 ± 0.1	**	**	**
Chlamydomonas reinhardtii	46 ± 4	44 ± 2	24 ± 1	136 ± 18	113 ± 15	205 ± 28
Scenedesmus obliquus	12.2 ± 0.3	11.6 ± 0.1	13.9 ± 0.4	161 ± 42	71 ± 3	187 ± 45
Phaeodactylum tricornutum	12.2 ± 0.2	7.6 ± 0.1	8.3 ± 0.1	126 ± 22	65 ± 5	101 ± 15
Diacronema lutheri	46 ± 4	*	18.2 ± 0.3	141 ± 17	*	122 ± 11
Tetraselmis suecica	39.5 ± 0.9	27.8 ± 0.8	24 ± 1	317 ± 109	45 ±3	92 ± 6
Nannochloropsis oculata	31 ± 2	*	19.2 ± 0.7	297 ± 66	*	141 ± 21
Dunaliella salina	39 ± 2	24.0 ± 0.9	19.4 ± 0.6	186 ± 30	196 ± 18	115 ± 16
T- Isochrysis lutea	50 ± 5	*	9.0 ± 0.1	112 ± 12	*	72 ± 4

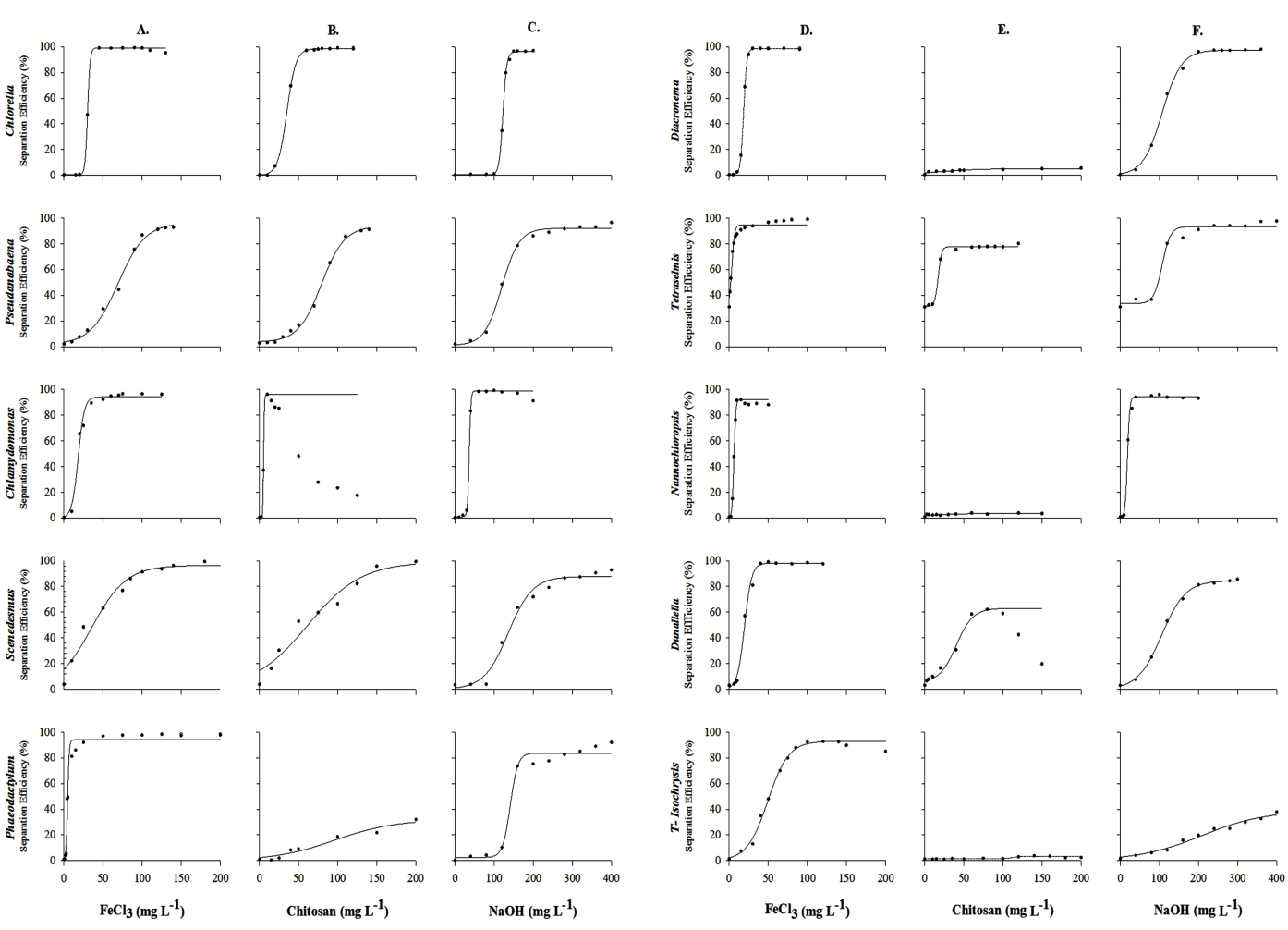
* Poor or no significant flocculation and/or settling observed
** Floc size too large (Filamentous) for image analysis

Table 3: Flocculant evaluation based on flocculant cost and concentration factor for microalgae and cyanobacterium species using $FeCl_3$, chitosan, and NaOH

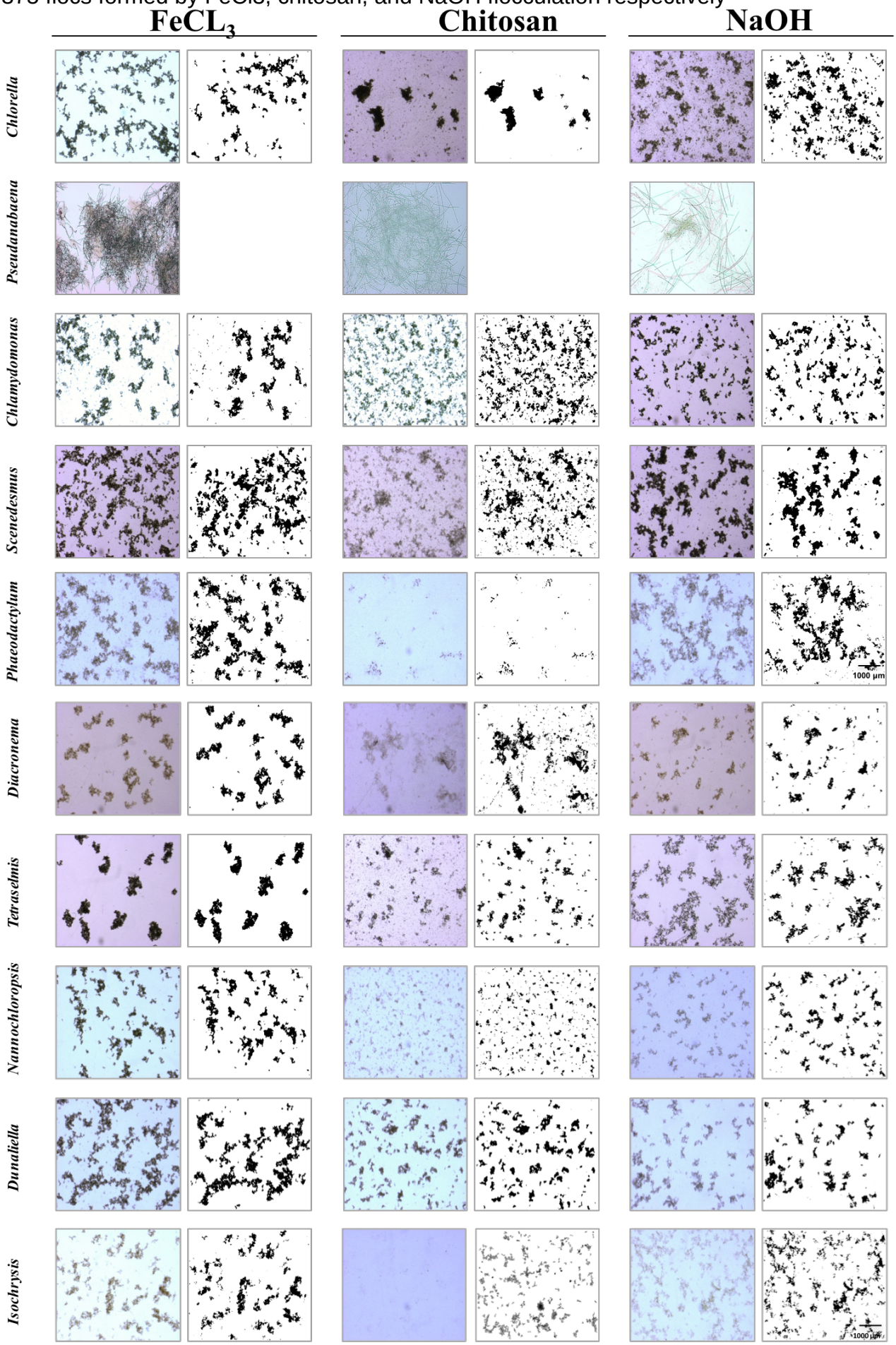
Species	Method	Dose (ton per ton)	Cost[a] ($ per ton)	CF[b]	Cost/CF
Nannochloropsis oculata	$FeCl_3$	0.03	13	31.00	0.4
Tetraselmis suecica	$FeCl_3$	0.04	19	39.50	0.5
Diacronema lutheri	$FeCl_3$	0.09	44	46.00	1.0
Phaeodactylum tricornutum	$FeCl_3$	0.03	13	12.20	1.0
Chlamydomonas reinhardtii	Chitosan	0.04	65	44.00	1.5
Dunaliella salina	$FeCl_3$	0.12	59	39.00	1.5
Chlamydomonas reinhardtii	$FeCl_3$	0.17	87	46.00	1.9
Nannochloropsis oculata	NaOH	0.10	38	19.20	2.0
Chlorella vulgaris	$FeCl_3$	0.12	59	28.60	2.1
Chlamydomonas reinhardtii	NaOH	0.22	83	24.00	3.4
T-Isochrysis lutea	$FeCl_3$	0.43	217	50.00	4.3
Chlorella vulgaris	NaOH	0.38	143	31.00	4.6
Tetraselmis suecica	Chitosan	0.09	131	27.80	4.7
Tetraselmis suecica	NaOH	0.40	152	24.00	6.3
Chlorella vulgaris	Chitosan	0.15	225	24.00	9.4
Dunaliella salina	NaOH	0.50	190	19.40	9.8
Scenedesmus obliquus	$FeCl_3$	0.25	125	12.20	10.2
Diacronema lutheri	NaOH	0.50	190	18.20	10.4
Scenedesmus obliquus	NaOH	0.73	276	13.90	19.8
Pseudanabaena CY14-1	$FeCl_3$	0.30	150	7.00	21.4
Phaeodactylum tricornutum	NaOH	0.49	185	8.30	22.3
Pseudanabaena CY14-1	NaOH	0.50	190	5.20	36.5
Scenedesmus obliquus	Chitosan	0.38	563	11.60	48.5
Pseudanabaena CY14-1	Chitosan	0.33	488	4.70	103.7

[a] based on bulk price estimations: $FeCl_3$ = 500 USD ton^{-1}; Chitosan = 1500 USD ton^{-1}; NaOH = 380 USD ton^{-1}
[b] CF = concentration factor

Suppl. Figure 1: Flocculation dose-response curves from sigmoidal regression analysis for 372 microalgae and a cyanobacterium species using FeCl3, chitosan, and NaOH



Suppl. Figure 2: Original and transformed mask images of flocs used for floc size analysis; 375 flocs formed by FeCl3, chitosan, and NaOH flocculation respectively



Suppl. Table 1: Cell surface area and volume calculations (*V* = volume; *A* = surface area; *d* = diameter; *h* = height; *a* = apical axis (length) ; *b* = transapical axis (width) ; *c* = pervalvar axis (height) (Hillebrand et al., 1999)**)**

Shape	Cell volume	Cell surface area
Sphere	$V = \frac{\pi}{6} . d^3$	$A = \pi . d^2$
Cylinder	$V = \frac{\pi}{4} . d^2 . h$	$A = \pi . d . (\frac{d}{2} + h)$
Prolate spheroid	$V = \frac{\pi}{6} . d^2 . h$	$A = \frac{\pi . d}{2} . \left(d + \frac{h^2}{\sqrt{h^2 - d^2}} \, sin^{-1} \frac{\sqrt{h^2 - d^2}}{h} \right)$
Half elliptic prism	$V = \frac{\pi}{4} . a . b . c$	$A = \frac{\pi}{4} . (a . b + a . c + b . c) + a . c$

Suppl. Table 2: Parameters from sigmoid regression analysis of $FeCl_3$, chitosan, and NaOH dose-response flocculation jar tests ($\mu \pm 1\sigma$)

Species	$FeCl_3$				Chitosan				NaOH			
	Y_{max} (%)[a]	x_0 (mg L^{-1})[b]	b (−)[c]	R^2	Y_{max} (%)[a]	x_0 (mg L^{-1})[b]	b (−)[c]	R^2	Y_{max} (%)[a]	x_0 (mg L^{-1})[b]	b (−)[c]	R^2
Chlorella 1	98.9 ± 0.1	30.17 ± 0.03	1.6 ± 0.3	1.00	98.6 ± 0.2	34.9 ± 0.2	5.8 ± 0.2	1.00	96 ± 1	122.8 ± 0.3	4.8 ± 0.3	0.99
Chlorella 2	96 ± 1	30.5 ± 0.2	3.0 ± 0.5	0.99	95 ± 2	35 ± 1	6.2 ± 0.6	0.99	94 ± 1	123.6 ± 0.4	5.0 ± 0.4	0.99
Pseudanabaena CY14-1	96 ± 5	69 ± 3	17 ± 2	0.99	94 ± 3	79 ± 2	15 ± 1	0.99	92 ± 3	120 ± 2	22 ± 2	0.99
Chlamydomonas reinhardtii	94 ± 7	18 ± 1	4.0 ± 0.9	0.99	96.21 ± 0.01	5.23 ± 0.01	0.47 ± 0.01	1.00	99.0 ± 0.5	36.4 ± 0.1	2.18 ± 0.08	1.00
Scenedesmus obliquus	96 ± 3	36 ± 5	22 ± 5	0.97	99 ± 9	61 ± 18	35 ± 8	0.96	88 ± 3	136 ± 6	31 ± 5	0.99
Phaeodactylum 1	93 ± 7	4.6 ± 0.2	1.0 ± 0.3	0.97	32 ± 8*	96 ± 26	38 ± 15	0.95	84 ± 4	142 ± 5	10 ± 3	0.99
Phaeodactylum 2	91 ± 10	4.6 ± 0.4	1.3 ± 0.5	0.97	30 ± 5*	94 ± 21	39 ± 12	0.95	86 ± 2	144 ± 3	11 ± 2	0.99
Diacronema lutheri	98.6 ± 0.3	18.37 ± 0.04	2.00 ± 0.03	1.00	*	*	*	0.82	97.4 ± 0.8	107 ± 3	25 ± 2	0.99
Tetraselmis suecica	94 ± 4	3 ± 1	1.7 ± 0.6	0.97	78 ± 1	17.1 ± 0.7	2.1 ± 0.5	0.99	93 ± 4	107 ± 6	11 ± 4	0.98
Nannochloropsis oculata	92 ± 3	5.9 ± 0.1	1.18 ± 0.05	0.99	*	*	*	0.59	95 ± 3	18.4 ± 0.6	2.9 ± 0.7	0.99
Dunaliella salina	98 ± 2	19.5 ± 0.8	5 ± 1	0.99	63 ± 3	40 ± 2	10 ± 2	0.99	85 ± 2	106 ± 2	31 ± 2	0.99
T-Isochrysis lutea	93 ± 3	49 ± 1	13 ± 1	0.99	*	*	*	0.69	39 ± 12*	209 ± 29	81 ± 37	0.98

[a] $y_{max} = a + y_0$: the maximum separation efficiency (%)
[b] x_0: flocculant dosage required at the inflection point (mg L^{-1})
[c] b: the slope of the sigmoidal regression curve (−)
* poor or no significant flocculation and/or settling observed