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Screening of commercial natural and synthetic cationic polymers for flocculation of freshwater and marine microalgae and effects of molecular weight and charge density

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Abstract

Twenty-five natural and synthetic cationic polymers of different molecular weights and charge densities were evaluated for microalgae flocculation. Tanfloc is a natural low molecular weight tannin polymer whereas Zetag and Flopam are both synthetic high molecular weight polyacrylamide polymers. Five exponential concentrations (0.55, 1.66, 5, 15 and 45 mg L^{-1}) were tested for freshwater *Chlorella vulgaris* and marine *Nannochloropsis oculata*. All polymers were efficient (>90% at $\geq$ 1.66 mg L^{-1}) for *C.*

vulgaris. However, for *N. oculata,* only Tanfloc was effective. Charge density positively influenced flocculation decreasing the required polymer dosage. Restabilisation was observed only for synthetic polymers when overdosed. Natural polymers performed similarly for both species. In overall, Tanfloc SL and Flopam FO 4990 SH were the most efficient polymers for microalgae flocculation though Tanfloc is a more economic option (US\$ 37 ton^{-1} of biomass) and environmentally friendly than Flopam (US\$ 171 ton^{-1} of biomass).

Keywords: Microalgae; Coagulation; Biopolymer; Harvesting; Dewatering

1. Introduction

Microalgae are attracting a lot of interest as a new source of biomass for production of food, feed, bulk chemicals, or biofuels [1]. Harvesting is currently one of the major bottlenecks to large-scale production of microalgae [2]. Because of their small size (3 to 30 μm) and low biomass concentration (< 5 g L^{-1}), harvesting using centrifuges is too energy-intensive and costly, being only justified for high value bioproducts such as carotenoids or poly-unsaturated fatty acids [3-5]. For bulk production of biomass for commodities, a low-cost harvesting method is needed that can process large volumes of microalgae culture at a minimal cost.

Spontaneous flocculation of microalgae in suspension is prevented by electrostatic repulsion caused by the negative surface charge of the cells [6]. This negative charge is related to the presence of carboxyl, sulfate or phosphate groups on the microalgae cell surface. Hence, positively charged chemicals that interact with those negative surface charges can induce flocculation. In flocculation, small particles are combined into larger aggregates. These large aggregates can be much more easily separated from the liquid medium than the individual cells [2]. Thus, flocculation has a lot of potential to be used as a low-cost and high-throughput method for harvesting microalgae.

An important class of chemicals used in flocculation is metal salts, such as ferric chloride or aluminum sulfate [7]. When dissolved in water, these metal salts form positively charged hydroxides that cause flocculation by neutralizing the negative charge of the microalgae cells or by causing a positively charged precipitate that enmeshes the microalgae cells and removes them from suspension (‘sweep flocculation’). Metal salts have been successfully applied for flocculating microalgae [8-10]. However, these elements have the disadvantage that they require a relatively high dosage and that the biomass is contaminated with high concentrations of metals, limiting the application of the biomass due to metal toxicity [11].

Another class of chemicals that are widely used for microalgae flocculation is organic polymers. They can induce flocculation by neutralizing the negative surface charge, similar as for metal salts, and by forming bridges between the microalgae cells. The effectiveness of such polymers depends on their size, secondary structure in solution as well as on their charge density [7]. Organic polymers are generally preferred over metal salts because they require a much lower dosage. The majority of organic polymers that are commercially available are synthetic based on polyacrylamide [7]. Some studies have successfully applied synthetic polyacrylamide polymers for flocculating microalgae (e.g. [12-16]). Nevertheless, these studies have made clear that there are often large disparities in the effectiveness of different polymers when applied to microalgae (e.g. [12, 16]). It is not clear, however, which properties of polymers (e.g. charge density, polymer size, secondary structure) determine this variation in effectiveness.

Although synthetic polyacrylamide polymers as such are non-toxic, they may contain acrylamide residues that are presumable carcinogenic or display a high toxicity towards aquatic organisms [17]. Therefore, it is preferable to use natural based polymers, particularly when fractions of the microalgae biomass are to be used for animal feed, which may be economically attractive in a biorefinery context [1]. A well-known natural cationic polymer is chitosan, a derivative of chitin obtained from shrimp shells. Several studies have shown that chitosan is quite effective for flocculating microalgae (e.g. [18, 19]). Other natural based polymers include derivatives of cassia gum [20] or starch [21]. Tanfloc is a relatively recently developed commercial biopolymer that is based on tannin [22]. It differs from other natural polymers in that it is not based on a polysaccharide but on a phenolic polymer. Tannins are branched polymers and thus have a different secondary structure than linear polymers such as chitosan or polyacrylamide. While Tanfloc has been used for removal of chemical contaminants [23] and turbidity in wastewater treatment [24], its potential for flocculating microalgae has not been thoroughly evaluated, although Roselet et al., [25] have recently analyzed the effect of pH, salinity, polymers dose and biomass concentration on Tanfloc efficiency in concentrating the marine microalga *N. oculata*, with good results.

A disadvantage of both synthetic and natural polymers is that they often undergo coiling when used in high ionic strength medium such as seawater (e.g. [8, 26]). Coiling changes the secondary structure of the polymer and this generally results in a decrease in the flocculation efficiency [27]. Many species of microalgae, including those that have a lot of potential for biodiesel production, are marine species. Therefore, it is important to evaluate whether synthetic and natural polymers have potential for harvesting of marine microalgae species.

The main objective of this study was to evaluate the potential of 25 different commercially available cationic polymers for flocculating microalgae. These polymers included different charge density variants of a low molecular weight natural tannin polymer (Tanfloc) and two high molecular weight synthetic polyacrylamide polymers (Flopam and Zetag). To evaluate the potential of these polymers for harvesting marine as well as freshwater microalgae, screening was performed on two model species, the freshwater *Chlorella vulgaris* and the marine *Nannochloropsis oculata*. The effects of molecular weight and charge density on the microalgae flocculation were evaluated and cost analysis was conducted for all tested polymers and compared with hydrolyzing metal salts and chitosan.

2. Materials and methods

2.1. Microalgae cultivation

The two microalgae model species used in this study were freshwater *Chlorella vulgaris* (SAG 211-11b) and marine *Nannochloropsis oculata* (SAG 38.85), obtained from the Culture Collection of Algae at Göttingen University (SAG, Germany). The microalgae were cultured in Wright's Cryptophyte medium prepared from pure salts and deionized water. For *N. oculata*, synthetic sea salt (Homarsel, Zoutman, Belgium) was added at a final concentration of 30 g L^{-1}. Both species were cultured for 6 days in 30 liters plexiglass bubble column photobioreactors mixed by sparging with 0.2 µm filtered air (5 L min^{-1}) in a temperature-controlled room (20ºC) [9]. The pH was maintained at 8 by addition of CO_2 (2-3%) using a pH-controller system. Each photobioreactor was continuously irradiated with daylight fluorescent tubes (100 µmol photons m^{-2} s^{-1}).

Microalgae biomass concentration was monitored daily by measuring the absorbance at 750 nm. Optical density measurements were calibrated against dry weight measured gravimetrically on pre-weighed GF/F glass fiber filters (R^2 = 0.998). The marine microalga was washed with 0.5 M ammonium formate, prior to filtration to remove salts absorbed on the cell surface. The final biomass concentrations after 6 days were 260 mg L^{-1} and 290 mg L^{-1} for *C. vulgaris* and *N. oculata*, respectively. The final concentrations were later confirmed by dry weight measurements.

2.2. Flocculation experiments

After day 6, the microalgae cultures were collected from the photobioreactors to be used in the flocculation experiments. All 25 polymers were simultaneously screened and flocculation experiments lasted approximately 4 hours. Microalgae may excrete large amounts of dissolved organic matter (DOM) into the culture medium and this may interfere with flocculation [9]. To avoid DOM interference in the flocculation experiments, the microalgae was centrifuged from the medium and resuspended in the same volume of fresh medium. This treatment reduced carbohydrate concentrations in the medium from 10 and 58 mg L^{-1} to 2 and 10 mg L^{-1} of glucose equivalent for *C. vulgaris* and *N. oculata*, respectively. Previous experiments had demonstrated that centrifugation and subsequent resuspension in fresh medium had no significant effect on flocculation [9].

Twenty-five cationic polymers were compared. Table 1 lists the properties of the polymers used. Tanfloc is a natural low molecular weight quaternary ammonium polymer based on tannins extracted from the black wattle tree (*Acacia mearnsii*) and manufactured by TANAC (Brazil). Flopam and Zetag are both synthetic copolymers of acrylamide and quaternized cationic monomer polymers manufactured by SNF Floerger (France) and BASF (Germany), respectively. For Flopam, a series of polymers with similar molecular weight (4.1 – 8.6 x 10^6 Da) but increasing charge densities (2.5 – 100 mole %) was used. For Zetag, we compared polymers with high (8125, 8160, 8180) and very high (7652, 8165, 8185) molecular weight and variable charge densities. For each polymer a 1 g L^{-1} stock solution was prepared by adding 50 mg of polymers to 50 mL of deionized water and mixed for 1 hour. Zetag was initially moistened with 3% acetone as indicated by the manufacturer. For each polymer, five exponential concentrations (0.55, 1.66, 5, 15 and 45 mg L^{-1}) were selected to determine the order of magnitude of the dosage required to induce flocculation. All polymers used in this study were kindly provided by the manufacturers.

Jar test experiments were used to quantify the efficiency of *C. vulgaris* and *N. oculata* flocculation. During addition of polymers, the microalgae suspensions were intensively mixed (350 rpm) for 10 minutes, to allow uniform polymer dispersal, followed by gentler mixing (250 rpm) for 20 minutes to allow floc formation. Subsequently, the microalgae suspensions were allowed to settle for 30 minutes and then samples were collected in the middle of the clarified zone. Optical density at 750 nm was measured prior to polymer addition (OD_i) and after settling (OD_f) and the flocculation efficiency (η_a) was calculated as:

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

Only flocculation efficiencies higher than 90% were considered effective.

2.3. Statistical analysis

Polymers doses and flocculation efficiencies were log transformed and a nonlinear regression analysis with least square iteration was performed to describe the polymers effectiveness. Each dose-response curve was compared by extra sum-of-squares F test ($P < 0.05$) and D'Agostino-Pearson omnibus test was performed to verify dataset normality.

2.4. Cost analysis

Analysis was conducted to quantify the cost of flocculating *C. vulgaris* and *N. oculata* using hydrolyzing metal salts ($Al_2(SO_4)_3$ and $AlCl_3$), synthetic (Flopam and Zetag) and natural (chitosan and Tanfloc) flocculants. Initial biomass concentration, flocculant dose and efficiency for hydrolyzing metal salts and chitosan were obtained from previous studies for both species [9, 10] and are presented in Table 3. Costs of Tanfloc, Flopam and Zetag were provided by the manufacturers whereas costs of hydrolyzing metal salts and chitosan were obtained from bulk vendors of industrial chemicals (Alibaba). All flocculant costs were calculated in US$ per metric ton of dried microalgae. Costs related to harvesting apparatus or energy consumption were not considered.

3. Results and discussion

3.1. Screening results

The polyacrylamide polymers Flopam and Zetag were very effective at flocculating the freshwater *C. vulgaris* and no differences were observed within each polymer series as the dose-response curves did not differ ($P > 0.05$). However, Flopam and Zetag were not capable of flocculating the marine *N. oculata* and performance within polymer series varied significantly ($P < 0.05$) due to differences in charge density. The tannin polymers, on the other hand, were effective at flocculating both *C. vulgaris* and *N. oculata* and no differences ($P > 0.05$) were observed within Tanfloc variants (Table 1). The poor performance of Flopam and Zetag polymers in marine medium is not surprising, as it is well known that polymers often undergo coiling because of the high ionic strength of saltwater. Bilanovic et al. [26] employed Zetag to harvest the marine *Chlorella stigmatophora* and reported that reducing the medium salinity significantly improved flocculation. König et al. [28] employed Flopam to harvest the marine microalga *Conticribra weissflogii*, reporting that salinity negatively impacted flocculation. A poor performance in marine medium has also been observed for polymers based on natural polysaccharides such as chitosan [8] and cationic starch [21].

Flopam and Zetag generally had high flocculation efficiency at a dosage of 1.66 mg L^{-1} while a dosage of 5 mg L^{-1} was required for effective flocculation with Tanfloc. At the highest dosages, the flocculation efficiency of the polyacrylamide polymers declined.

This is an indication of restabilisation, caused by charge reversal of the microalgae cell surface. Restabilisation has also been observed for other natural polymers, such as chitosan [29] or cationic starch [21]. However, no such restabilisation was observed when using Tanfloc.

To date, hydrolyzing metal salts, synthetic and natural polymers were reported for flocculating freshwater and marine microalgae (Table 2). For example, Vandamme et al. [9] employed $Al_2(SO_4)_3$ to harvest *C. vulgaris* whereas Garzon-Sanabria et al. [10] used $AlCl_3$ for *N. salina*. However, the required dosage for such flocculants is higher than the dosage needed for synthetic or natural polymers (20-50 mg L^{-1}). In this study, several Flopam polymers were evaluated. For *N. oculata*, efficiency ranged from 8 to 90% at 0.55 mg L^{-1} polymer concentration (Table 1). Garzon-Sanabria et al. [10], working with *N. salina*, also employed four Flopam polymers (4550, 4650, 4800 and 4990), reporting efficiencies ranging from 73% to 94% at 3 mg L^{-1} dose, similar with the present study (Table 2). The higher biomass concentration (700 mg L^{-1}) employed in the Garzon-Sanabria et al. [10] experiment may explain the increased optimal dosage used by the authors. In the present study, *C. vulgaris* was readily harvested (100%) with 5 mg L^{-1} of Zetag 8185, a very high molecular weight and high charge density polymer. For *N. oculata* the same polymer resulted in 75% removal at 0.55 mg L^{-1}. Udom et al. [30] employed Zetag polymers of high and very high molecular weight (8846, 8848, 8814, 8816 and 8819), ranging from medium to very high charge densities, to concentrate *Chlorella sp.* grown on wastewater. Zetag 8819, according to the authors, presented the highest efficiency (98%) at the lowest optimal dosage (34 mg L^{-1}). However, Eldridge et al. [31] reported Zetag 7570 (of high molecular weight and charge density) as being ineffective for *N. salina* at doses up to 20 mg L^{-1}. Both studies reported higher dosages, which may be explained by the higher biomass concentration employed and by the presence of DOM in the medium, which may have inhibited flocculation [9] (Table 2).

The present work tested Tanfloc, a tannin polymer, for harvesting *C. vulgaris* and *N. oculata*. Flocculation was achieved at 5 mg L^{-1} for both species, resulting in more than 97% removal. These results are in accordance with Roselet et al. [25], who achieved 95-98% removal for *N. oculata* employing Tanfloc doses between 1 and 10 mg L^{-1}. Wang et al. [32] recently tested a quaternized-modified tannin to harvest *Microcystis aeruginosa*. Applying a dose of 10 mg L^{-1} also resulted in 97% removal efficiency, though in a medium containing DOM. Comparing with chitosan, Vandamme et al. [9] and Garzon-Sanabria et al. [10] required 8 mg L^{-1} and 3 mg L^{-1} to flocculate *C. vulgaris* and *N. salina* achieving 85% and 98% efficiency, respectively (Table 2). This study confirms that Tanfloc works well in marine medium and therefore has potential to be used for harvesting other marine microalgae species. The fact that the flocculation efficiency of Tanfloc does not differ between freshwater and marine medium may be due to different secondary structure of tannin in comparison to polyacrylamide or polysaccharides, being Tanfloc a branched rather than a linear polymer. As a result, it may be less affected by coiling than polyacrylamide polymers.

3.2. Effect of molecular weight and charge density

The Tanfloc series is only composed of low molecular weight polymers with low-medium charge densities. Considering the aggregation mechanism, low molecular weight polymers act mostly by charge neutralization [12] and require higher dosages than high molecular weight polymers [33]. However Tanfloc dosages were much lower than other low molecular weight flocculants like $AlCl_3$ and $Al_2(SO_4)_3$ and similar to high molecular weight Flopam and Zetag (Tables 1 and 2). Regarding charge neutralization, molecular weight has little importance, thus increasing charge density should prove most effective [33]. Therefore, the different flocculation efficiencies observed for Tanfloc may be related to differences in charge density though no significant ($P > 0.05$) differences were observed within variants (Table 1).

On the other hand, the Flopam series is composed of high molecular weight polymers ($4.1 – 8.6 \times 10^6$ Da) with charge densities ranging from very low (2.5 mole %) to very high (100 mole %). It is acknowledged that high molecular weight polymers act better as bridging agents [3]. Interestingly, results demonstrate that increasing the molecular weight negatively affected the flocculation efficiency (Figure 1). From the Flopam series, we notice that those polymers with the highest molecular weight presented lower charge densities. This can be explained as, for high molecular weight polymers, size depends on the interaction between polymer segments. Thus, increasing the charge density, the polymer adopts a more expanded configuration [7]. Figure 1 exemplifies that effect for *C. vulgaris* and *N. oculata*. For 0.55 mg L^{-1}, increasing the charge density improved the flocculation efficiency from 1% to 80% and from 8% to 90% for *C. vulgaris* and *N. oculata*, respectively. Despite having high molecular weights, those with lower charge densities were unable to expand the polymer segments or to neutralize the cell surface charge.

For *N. oculata*, however, we can distinguish four statistically different ($P < 0.05$) regions relating Flopam efficiency and charge density (Table 1). For very low charge density polymers (≤ 10 mole %), efficiency improves as charge increases, with an optimal dosage exceeding 45 mg L^{-1}. Therefore, very low charge density polymers require larger dosages than polymers with higher charges. Low (≤ 25 mole %) and medium charge densities polymers (≤ 45 mole %) attained maximal efficiency between 1.66 and 5 mg L^{-1} whereas restabilisation was evident to occur at higher doses. However, low and medium charge density polymers composed two different groups ($P < 0.05$). At last, for high (≤ 70 mole %) and very high charge densities polymers (≥ 80 mole %), the optimal dosage seems to be under 0.55 mg L^{-1} and increasing dosages induced restabilisation.

Similarly, the Zetag series is constituted of high molecular weight (8125, 8160, 8180) and very high molecular weight (7652, 8165, 8185) polymers, with charge densities ranging from low to high. The effects of charge density are comparable to those described for Flopam. In general, three statistically different ($P < 0.05$) regions were observed, mostly related to charge density than to molecular weight (Table 1). Region 1, with lower efficiencies, was composed of Zetag 8125 and 7652. Region 2, with medium efficiency, was composed of polymers 8160 and 8165. At last, Region 3 was composed of high charge densities Zetag 8180 and 8185 polymers.

Garzon-Sanabria et al. [10] evaluated the effect of polymer molecular weight and charge density on harvesting of *N. salina* comparing a low molecular weight polyamine polymer (Floquat FL 2949) with four high molecular weight polyacrylamide polymers from the Flopam series (4550, 4650, 4800 and 4990). The authors concluded that Floquat did not resulted in a substantial flocculation even at concentrations up to 100 mg L^{-1} whereas Flopam achieved >90% at concentrations between 20-30 mg L^{-1}. Regarding charge density, flocculation was most efficient when using FO 4990 SH, the highest charge density polymer. Udom et al. [30] compared several Zetag polymers (8846, 8848, 8814, 8816 and 8819), with molecular weight ranging from high to very high. Zetag 8819 was selected for further study because it provided the highest harvesting efficiency (98%) at the lowest optimal dose (34 mg L^{-1}).

3.3. Cost analysis

Polymer cost is an important factor to be considered as biomass recovery can contribute 20-30% to the total budget of the produced biomass [34]. Thus, a cost analysis based on dose and efficiency among hydrolyzing metal salts ($Al_2(SO_4)_3$ and $AlCl_3$), synthetic (Flopam and Zetag) and natural polymers (Chitosan and Tanfloc) can be found in Table 3. Hydrolyzing metal salts were the least expensive, costing ~US$ 34 metric ton^{-1} of biomass harvested, thought the quantity needed was higher (~86 kg metric ton^{-1} of biomass) comparing to polymers (~21 kg metric ton^{-1} of biomass). Furthermore, hydrolyzing metal salts are not recommended for harvesting microalgae due to biomass contamination with residual metal [11]. On the other hand, synthetic polymers, like Zetag and Flopam, were highly efficient at a very low dosage although they were much more expensive than metal salts, at ~US$ 171 metric ton^{-1}. Moreover, dispersion of toxic acrylamide oligomers to the environment may happen, which may also present a health hazard [18]. Regarding Zetag, the manufacturer recommends it to be moistened with 3% acetone prior to dissolving with water, what may increase not only costs but also environmental risks. For these reasons, alternative natural polymers like chitosan have been considered for environmental applications [18]. However, the costs for employing chitosan vary greatly, depending on the studies. For example, in table 3, the calculated cost for harvesting *N. salina* was only US$ 44 metric ton^{-1} whereas for *C. vulgaris* it increased to US$ 376 employing concentrations lower than 10 mg L^{-1}. Nonetheless, Rashid et al. [19] reported 120 mg L^{-1} as being the optimal dosage for chitosan, removing 92% of *C. vulgaris*, what would cost prohibitive US$ 1,860 metric ton^{-1} of biomass. In addition, the bulk price for chitosan varies between US$ 10,000 and 100,000 metric ton^{-1}. Instead, Tanfloc presented both performance and cost advantages, costing about US$ 37 for harvesting one ton of *C. vulgaris* and *N. oculata* in the present study. Sánchez-Martín et al. [35] also employed Tanfloc though to reduce turbidity in surface waters. Applying a dose of 10 mg L^{-1} resulted in 99% removal what, using the same calculations from Table 3, would cost ~US$ 73 metric ton^{-1} of biomass produced. More recently, Wang et al. [32] employed 10 mg L^{-1} of tannin to harvest 97% of *M. aeruginosa* which would cost ~US$ 75 metric ton^{-1}. Even at this high costs, having in mind that Tanfloc is a natural biopolymer, it is not only a much more economical but also a more ecological option for flocculating microalgae than potentially toxic hydrolyzing metal salts or synthetic polymers.

4. Conclusions

The result of this screening of a broad range of synthetic and natural polymers showed that flocculation of *N. oculata* and *C. vulgaris* was readily achieved using Tanfloc. On the other hand, Flopam and Zetag were most effective in freshwater. In addition, for synthetic polymers, data indicates that flocculation is largely influenced by charge density. Contrarily to synthetic polymers, restabilisation was not observed for Tanfloc. In overall, Tanfloc is a promising low cost and environmentally friendly polymer for both freshwater and marine flocculation.

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Tables

Table 01: Summary of screened polymers (0.55, 1.66 5, 15 and 45 mg L^{-1}), molecular weight (10^6 Da), charge density (mole %) and flocculation efficiency (%) for *C. vulgaris* (260 mg L^{-1}) and *N. oculata* (290 mg L^{-1}). Efficiencies above 90% threshold are highlighted in bold.

Table 02: Summary of different flocculants (hydrolyzing metal salts, synthetic and natural polymers) reported for harvesting *Chlorella* and *Nannochloropsis* species.

Table 03: Cost analysis of harvesting *C. vulgaris* and *N. oculata* with hydrolyzing metal salts ($Al_2(SO_4)_3$ and $AlCl_3$), synthetic (Flopam and Zetag) and natural (chitosan and Tanfloc) polymers. All costs are in US$. Hydrolyzing metal salts and chitosan data were obtained from [9, 10].

Figures

Figure 01: Effect of mean molecular weight (10^6 Da) and charge density (mole %) on flocculation efficiency of *N. oculata* (A, B) and *C. vulgaris* (C, D). All polymers from the Flopam series were dosed at 0.55 mg L^{-1}.

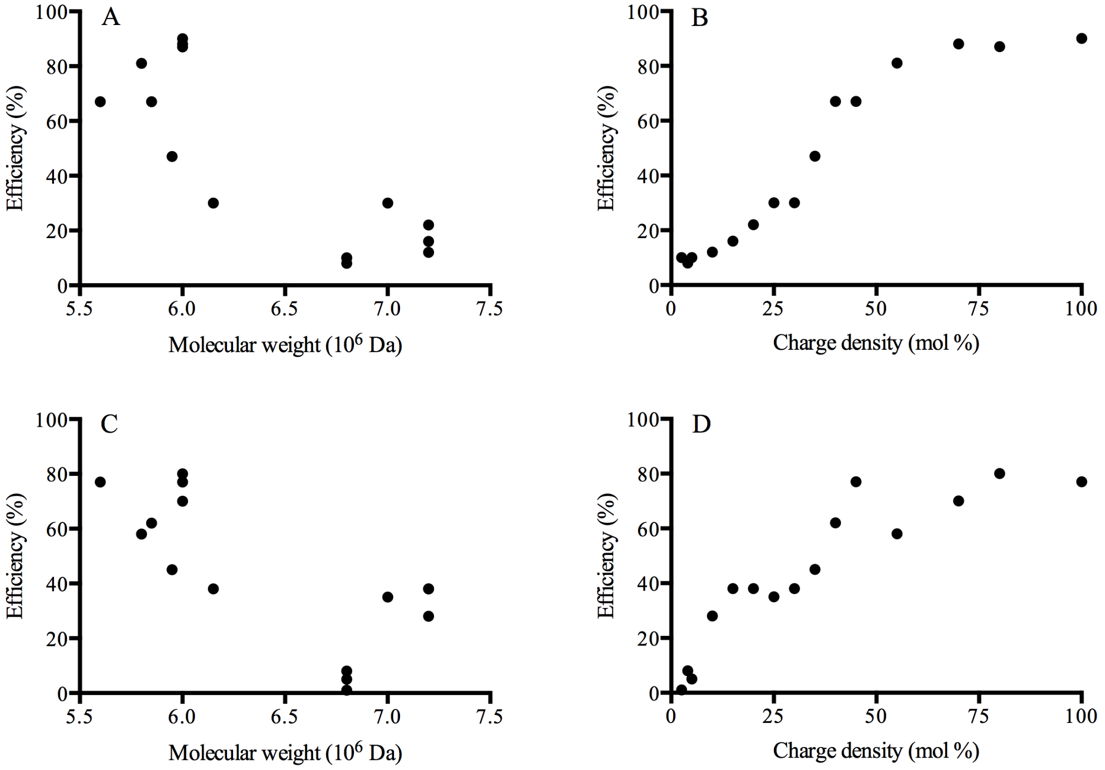

Figure 01: Effect of mean molecular weight (10^6 Da) and charge density (mole %) on flocculation efficiency of *N. oculata* (A, B) and *C. vulgaris* (C, D). All polymers from the Flopam series were dosed at 0.55 mg L^{-1}.

			Chlorella vulgaris (260 mg L^{-1})					*Nannochloropsis oculata* (290 mg L^{-1})				
Polymer	Molecular weight	Charge density	0.55 mg L^{-1}	1.66 mg L^{-1}	5 mg L^{-1}	15 mg L^{-1}	45 mg L^{-1}	0.55 mg L^{-1}	1.66 mg L^{-1}	5 mg L^{-1}	15 mg L^{-1}	45 mg L^{-1}
TANFLOC												
SG 1500	Low	Low-medium	2	5	81	**98**	**99** ^{a}	8	37	**90**	**95**	**98** ^{a}
POP	Low	Low-medium	4	11	**99**	**100**	**100** ^{a}	14	71	**92**	**96**	**99** ^{a}
SG	Low	Low-medium	4	12	**99**	**100**	**100** ^{a}	22	76	**90**	**97**	**99** ^{a}
SL	Low	Low-medium	8	**90**	**100**	**100**	**100** ^{a}	70	**94**	**97**	**98**	**99** ^{a}
FLOPAM												
FO 4115 SH	5.9-7.7	2.5	1	7	57	**90**	**94** ^{a}	10	13	16	27	45 ^{d}
FO 4125 SH	5.9-7.7	4	8	13	**98**	**96**	86 ^{a}	8	8	12	15	38 ^{d}
FO 4140 SH	5.9-7.7	5	5	84	**98**	**99**	**95** ^{a}	10	10	11	13	36 ^{d}
FO 4190 SH	6.3-8.1	10	28	**99**	**99**	**99**	**97** ^{a}	12	12	27	33	54 ^{d}
FO 4240 SH	6.3-8.1	15	38	**99**	**100**	**100**	**98** ^{a}	16	16	41	38	32 ^{c}
FO 4290 SH	5.9-8.5	20	38	**99**	**99**	**99**	**98** ^{a}	22	23	50	41	36 ^{c}
FO 4350 SH	5.5-8.5	25	35	**98**	**98**	**100**	**98** ^{a}	30	28	56	42	38 ^{c}
FO 4400 SH	4.9-7.4	30	38	**99**	**100**	**100**	**97** ^{a}	42	37	63	52	47 ^{b}
FO 4440 SH	4.8-7.1	35	45	**99**	**99**	**100**	**97** ^{a}	47	42	66	54	47 ^{b}
FO 4490 SH	4.6-7.1	40	62	**99**	**98**	**100**	**98** ^{a}	67	66	66	56	47 ^{b}
FO 4550 SH	4.1-7.1	45	77	**100**	**98**	**99**	**95** ^{a}	67	66	72	68	53 ^{b}
FO 4650 SH	4.5-7.1	55	58	**99**	**99**	**99**	**94** ^{a}	81	72	75	61	48 ^{a}
FO 4700 SH	4.9-7.1	70	70	**99**	**99**	**98**	**93** ^{a}	88	72	78	70	50 ^{a}
FO 4800 SH	4.9-7.1	80	80	**99**	**99**	**98**	87 ^{a}	87	72	73	63	45 ^{a}
FO 4990 SH	4.9-7.1	100	77	**99**	**98**	**98**	**92** ^{a}	**90**	79	74	60	44 ^{a}
ZETAG												
8125	High	Low	5	**99**	**99**	**99**	**96** ^{a}	10	15	25	27	44 ^{c}
8160	High	Medium-high	30	**94**	**99**	**99**	**96** ^{a}	47	48	47	39	33 ^{b}
8180	High	High	**94**	**95**	**99**	**98**	**91** ^{a}	70	60	48	41	35 ^{a}
7652	Very High	Medium	3	63	**99**	**99**	**96** ^{a}	10	10	27	25	25 ^{c}
8165	Very High	Medium-high	6	75	**99**	**99**	**97** ^{a}	43	33	51	43	35 ^{b}
8185	Very High	High	12	**90**	**100**	**99**	**95** ^{a}	75	60	64	53	42 ^{a}

^{a} Different letters indicate significant differences between dose-response curves within each polymer series ($P < 0.05$).

Table 1: Summary of screened polymers (0.55, 1.66 5, 15 and 45 mg L^{-1}), molecular weight (10^{6} Da), charge density (mole %) and flocculation efficiency (%) for *C. vulgaris* (260 mg L^{-1}) and *N. oculata* (290 mg L^{-1}). Efficiencies above 90% threshold are highlighted in bold.

Flocculant	Microalgae species	Biomass (mg L^{-1})	DOM [a]	Efficiency (%)	Dosage (mg L^{-1})	Reference
Hydrolyzing metal salts						
$AlCl_3$	*N. salina*	700	–	90	50	[10]
$Al_2(SO_4)_3$	*C. vulgaris*	250	–	85	20	[9]
Synthetic						
Flopam FO 4550 SH	*N. salina*	700	–	73	3	[10]
	N. oculata	290	–	67	0.55	This study
	C. vulgaris	260	–	100	1.66	This study
Flopam FO 4650 SH	*N. salina*	700	–	73	3	[10]
	N. oculata	290	–	81	0.55	This study
	C. vulgaris	260	–	99	1.66	This study
Flopam FO 4800 SH	*N. salina*	700	–	88	3	[10]
	N. oculata	290	–	87	0.55	This study
	C. vulgaris	260	–	99	1.66	This study
Flopam FO 4990 SH	*N. salina*	700	–	94	3	[10]
	N. oculata	290	–	90	0.55	This study
	C. vulgaris	260	–	99	1.66	This study
Zetag 7570	*N. salina*	414	+	10	20	[31]
Zetag 8819	*Chlorella sp.*	700	+	98	34	[30]
Zetag 8185	*N. oculata*	290	–	75	0.55	This study
	C. vulgaris	260	–	100	5	This study
Natural						
Chitosan	*C. vulgaris*	250	–	85	8	[9]
Chitosan	*N. salina*	700	–	98	3	[10]
Tannin	*M. aeruginosa*	n/a	+	97	10	[32]
Tanfloc SL	*N. oculata*	290	–	97	5	This study
	C. vulgaris	260	–	100	5	This study

[a] – medium without DOM, + medium with DOM

Table 02: Summary of different flocculants (hydrolyzing metal salts, synthetic and natural polymers) reported for harvesting *Chlorella* and *Nannochloropsis* species.

	Chlorella vulgaris					*Nannochloropsis oculata*				
	$Al_2(SO_4)_3$	Flopam	Zetag	Chitosan	Tanfloc	$AlCl_3$	Flopam	Zetag	Chitosan	Tanfloc
Initial biomass (mg L^{-1})	250 [a]	260	260	250 [a]	260	700 [b]	290	290	700 [b]	290
Flocculant dosage (mg L^{-1})	20 [a]	5	5	8 [a]	5	50 [b]	5	5	3 [b]	5
Flocculant efficiency (%)	85 [a]	98	100	85 [a]	100	90 [b]	74	64	98 [b]	97
Biomass harvested (mg L^{-1})	213	255	260	213	260	630	215	186	686	281
Flocculant needed per ton of biomass harvested (ton)	0.094	0.020	0.019	0.038	0.019	0.079	0.023	0.027	0.004	0.018
Flocculant cost (US$ ton^{-1})	300	8,000	8,000	10,000	2,000	500	8,000	8,000	10,000	2,000
Flocculant cost per ton of biomass harvested (US$)	28	157	154 [c]	376	38	40	186	216 [c]	44	36

[a] [9]

[b] [10]

[c] Cost of wetting with 3% acetone not included

Table 03: Cost analysis of harvesting *C. vulgaris* and *N. oculata* with hydrolyzing metal salts ($Al_2(SO_4)_3$ and $AlCl_3$), synthetic (Flopam and Zetag) and natural (chitosan and Tanfloc) polymers. All costs are in US$. Hydrolyzing metal salts and chitosan data were obtained from [9, 10].