# Understanding the salinity effect on cationic polymers in inducing flocculation of the microalga *Neochloris oleoabundans*

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## A B S T R A C T

A mechanistic study was performed to evaluate the effect of salinity on cationic polymeric flocculants, that are used for the harvesting of microalgae. The polyacrylamide Synthofloc 5080H and the polysaccharide Chitosan were employed for the flocculation of *Neochloris oleoabundans*. In seawater conditions, a maximum biomass recovery of 66% was obtained with a dosage of 90 mg/L Chitosan. This recovery was approximately 25% lower compared to Synthofloc 5080H reaching recoveries greater than 90% with dosages of 30 mg/L. Although different recoveries were obtained with both flocculants, the polymers exhibit a similar apparent polymer length, as was evaluated from viscosity measurements. While both flocculants exhibit similar polymer lengths in increasing salinity, the zeta potential differs. This indicates that polymeric charge dominates flocculation. With increased salinity, the effectivity of cationic polymeric flocculants decreases due to a reduction in cationic charge. This mechanism was confirmed through a SEM analysis and additional experiments using flocculants with various charge densities.

## 1. Introduction

The low energy requirements for flocculation establishes it as a promising technique for concentrating microalgae (Uduman et al., 2010; Vandamme et al., 2013). Flocculation of seawater cultivated microalgae, however, is still very challenging. In sea-water, ionic hindrance occurs which inhibits the interaction of the flocculant molecules with the microalgae (Bilanovic et al., 1988; Uduman et al., 2010; Vandamme et al., 2010, 2013). Unfortunately, only a small number of techniques are reported to be successful for flocculation of marine species: i.e. pH-increase, inorganic flocculation, and polymeric flocculation (Wu et al., 2012; Chatsungnoen and Chisti, 2016; 't Lam et al., 2014). A pH-increase induces the precipitation of salts. Those precipitates will settle and, meanwhile, will sweep the biomass (Wu et al., 2012). In their study, several microalgae have been succesfully flocculated by increasing the pH, resulting in a precipitation of the divalent ion magnesium. The use of inorganic flocculants in seawater salinities has also been reported (Chatsungnoen and Chisti, 2016). However, as mentioned by Uduman et al. (2010), the use of inorganic flocculants in seawa-

ter salinities commonly requires high dosages that are about 5–10 times higher compared to polymeric flocculants. With polymeric flocculation, polymeric bridges between individual cells are formed and, subsequently, aggregates of biomass evolve (Vandamme et al., 2013; 't Lam et al., 2014).

Among polymeric flocculants, cationic polymers are regarded as successful, though not all are equally efficient in inducing flocculation of marine microalgae. Currently, only polyacrylamides are reported to be successful ('t Lam et al., 2014; König et al., 2014; Roselet et al., 2015).

Despite the success of cationic polyacrylamides in harvesting marine microalgae, 't Lam et al. (2015) reported that, when commercially available cationic polymers are applied as flocculants, the required flocculant dosage is quite high ($40–100\,mg_{flocculant}/g_{biomass}$), resulting in a lower economic feasibility. Additionally, the use of polyacrylamides is forbidden for food and feed applications as several of these flocculants are reported to be toxic and non-food grade petroleum processing techniques are commonly used to manufacture them (Lee et al., 2014). To overcome these limitations, other flocculants that preferably have an equal of even better performance and that are allowed in the food and feed industry should be selected or designed. To allow the rational selection or design of novel flocculants, the mechanism that is

responsible for successful flocculation of marine microalgae needs to be revealed.

One of the mechanisms that was proposed by Tenney et al. (1969) and by Bilanovic et al. (1988) is that adsorption is mainly due to charge attraction between flocculant and cells. These studies suggest that repulsive forces within the polymer decrease in elevated salinities due to high ionic strength, resulting in the coiling of the flocculants. Due to this coiling, flocculants lose their ability to form polymeric bridges between individual cells and this subsequently results in decreased flocculation efficiency (Tenney et al., 1969; Bilanovic et al., 1988). The lack of polymeric coiling in elevated salinities could thus be an explanation for the success of charged polymeric flocculants such as cationic polyacrylamides.

However, recent studies of Roselet et al. (2015) showed that the cationic charge of the polymeric flocculants had a positive effect on the biomass recovery where the polymer length was of minor importance and that is not in accordance with the previously specified explanation of polymeric coiling. It is, therefore, still difficult to explain why certain cationic polymers are successful in inducing flocculation in seawater salinities while others are not.

The goal of this study was to provide further information to better understand cationic polymeric flocculation in seawater salinities and possibly reveal why cationic polyacrylamides remain functional in high salinities while other cationic polymers do not. This gained insight also provided information that can be applied in optimizing the design of flocculants.

In this study, Synthofloc 5080H and Chitosan were exploited as flocculants. Synthofloc 5080H is a cationic polyacrylamide that is reported to be successful in flocculating marine microalgae ('t Lam et al., 2014). Chitosan is a natural polysaccharide which is recognized as being successful in inducing flocculation under freshwater conditions but becomes less successful in seawater salinities and in neutral pH (Bilanovic et al., 1988). The apparent polymer length and nett cationic charge of both flocculants were compared with each other as a function of salinity.

The used microalga in this study was *Neochloris oleoabundans* which is able to grow in both fresh and salt water conditions. It.has been reported to contain a high protein content and, under stressed conditions, a high lipid content. This makes *N. oleoabundans* an interesting species for several applications (Popovich et al., 2012; Breuer et al., 2012). In addition, *N. oleoabundans* is a spherical Chlorophyta, hence, its shape eliminates possible side-effects of the cell shape during flocculation.

## 2. Material and methods

### 2.1. Biomass cultivation

The microalgal strain *N. oleoabundans* UTEX1185 was cultivated in artificial seawater medium with various salinities: NaCl: 15 g/L (brackish), 25 g/L (seawater), 35 g/L (saline); $KNO_3$: 1.7 g/L; $Na_2SO_4$: 0.5 g/L; 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES): 23.83 g/L; $MgSO_4·7H_2O$: 0.73 g/L; $CaCl_2·2H_2O$: 0.36 g/L; $K_2HPO_4$: 0.43 g/L; $Na_2EDTA·2H_2O$: 0.03 g/L; $MnCl_2·4H_2O$: 0.004 g/L; $ZnSO_4·7H_2O$: 0.0012 g/L; $CoCl_2·6H_2O$: 0.0003 g/L; $CuSO_4·5H_2O$: 0.0003 g/L; $Na_2MoO_4·2H_2O$: 0.00003 g/L; NaFeEDTA: 0.01 g/L.

Biomass was cultivated in 100 mL shake flasks in an Infors Multitron incubator (Infors AG, Bottmingen, Switzerland). The cultures were continuously illuminated at 120 $\mu mol\,m^{-2}\,s^{-1}$ in atmospheric air enriched with 2.5% $CO_2$ at a temperature of 25° C. The flasks were orbitally shaken at 90 rpm.

Part of the cultured biomass was harvested using pipetting two days after inoculation. On the seventh day, new cultures were inoculated for further cultivation. By re-inoculating a new flask every seven days and taking biomass after two days of cultivation, we prevented using stressed biomass in the flocculation experiments. Prior to the flocculation experiments, two cultivation cycles of nine days were performed to allow the biomass to adapt to their salinity.

### 2.2. Flocculants

1000 ppm stock solutions were prepared according to 't Lam et al. (2014) whereby the low charged flocculant 'Synthofloc 5025H', the moderately charged flocculant 'Synthofloc 5040H', and the highly charged flocculant 'Synthofloc 5080H' were dissolved in de-ionized (Milli-Q®) water. The flocculants of the 'Synthofloc'-series were generously provided by Sachtleben Wasserchemie GmbH, Germany. All flocculants are large polyacrylamides with various cationic charges and are commonly used in wastewater applications.

Chitosan (purchased from Sigma-Aldrich, product nr.: 448869-50G) was dissolved overnight in 0.1% (v/v) acetic acid after which the pH was adjusted to pH 7 ± 0.2. Flocculants were stored at 4 °C in a dark environment and were never stored longer than seven days.

### 2.3. Biomass recovery

After harvesting the biomass, the initial optical density at 750 nm was established at 0.8 ± 0.01 using culture medium (corresponds with a dry weight of 0.24 ± 0.07 g/L). After setting the $OD_{750}$, 10 mL of the sample was transferred to a beaker glass and stirred at 500 rpm. From a stock solution, flocculant was added until the desired dose was achieved (ranging between 0 and 90 ppm). After five minutes of mixing at 500 rpm followed by a ten minute period of mixing at 100 rpm, samples were transferred to 4 mL polystyrene cuvettes. The mixing protocol that was used first involved a severe mixing followed by a gentle mixing time and is in accordance with protocols reported in other studies (Bilanovic et al., 1988). Using the photometric method of (Salim et al., 2012), the gradual biomass recovery was followed in a Beckman Coulter DU730 photometer. After two hours of sedimentation, the biomass recoveries were determined and calculated according to (Salim et al., 2012). All experiments were performed in duplicate:

$$Recovery\,(\%) = \frac{OD_{750(t0)} - OD_{750(t_{supernatant})}}{OD_{750(t0)}} \times 100$$

### 2.4. Viscosity

The viscosity of a polymeric solution is correlated with the apparent polymer length. To study the effect of the salinity on the apparent polymer length of the flocculants, the viscosity of the flocculant solutions in various salinities was measured. The flocculant concentrations ranged between 0 and 100 ppm. The viscosity was measured using a Physica MCR 301 Rheometer. Polymeric solutions were made with various salinities by varying the NaCl-concentration (0–10 g/L NaCl). After the addition of the flocculant solution in the rotational cylinder, the viscosity was measured at shear rates ranging from 1 to 100 $s^{-1}$.

### 2.5. ζ-Potential

ζ-Potential measurements were performed to determine the effect of salinity on the net cationic charge of the flocculant. Several flocculant solutions with different NaCl concentrations were prepared. Flocculant dosages ranged between 30 and 200 ppm. The salinity ranged between 0 and 4 g/L of NaCl. The charge was measured using a Malvern Zetasizer Nano.
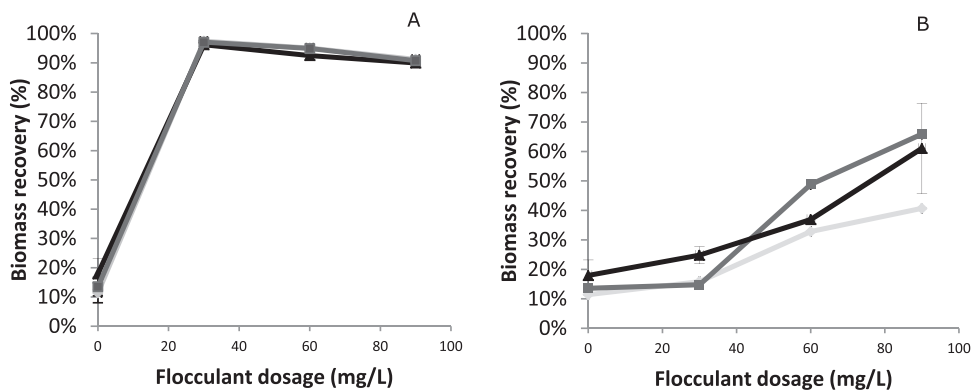
**Fig. 1.** Biomass recovery as a function of the flocculant dosage at salinities of 25 g/L (♦), 35 g/L (■) and 45 g/L (▲). Recoveries obtained with Synthofloc 5080H in figure A, Chitosan in Figure B. All samples represent biological duplicates.
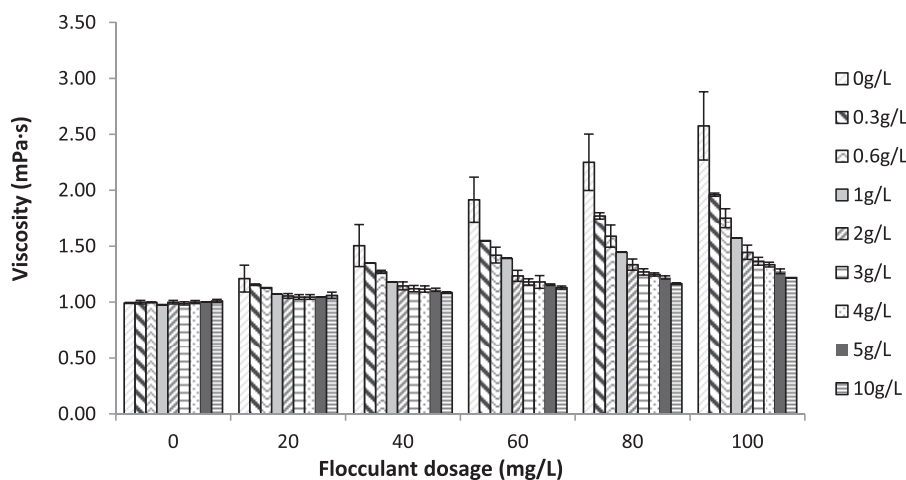


**Fig. 2.** Viscosity of Synthofloc 5080H measured at a share rate of $100 \, s^{-1}$. Every cluster of bars represents a flocculant dosage. Within every cluster, the salinity was increased, corresponding with the legend at the right site of the figure.

**Table 1**
Comparison of obtained biomass recoveries with Chitosan at neutral pH in various studies.

| Species | Cx (g/L) | pH | dosage (mg/L) | fresh/marine | recovery | Reference |
|---|---|---|---|---|---|---|
| C. sorokiniana | $0.27 \pm 0.07$ | 7 | 5 | fresh | >90% | Xu et al. (2013) |
| C. vulgaris | 1 | 7 | 120 | fresh | $92\% \pm 0.4$ | Rashid et al. (2013) |
| N. oleoabundans | 0.5 | 7.2 | 100 | fresh | 95% | Beach et al. (2012) |
| S. obliquus | 0.54 | 7 | 80 | fresh | 95% | Cheng et al. (2011) |
| N. salina | – | 8 | 8 | marine | >90% | Garzon-Sanabria et al. (2013) |
| N. oleoabundans | $0.24 \pm 0.07$ | 7 | 90 | marine | 66% | this study |

## 2.6. SEM imaging

The scanning electron microscopy objects were prepared according to the protocol described in Salim et al. (2014). In this protocol, aliquots of the microalgae were mixed with the flocculant for ., five minutes of severe mixing (500 rpm) followed by ten minutes of gentle mixing (100 rpm). Immediately after the mixing, a drop of suspended flocs was transferred to a poly-L-lysine coated microscopy cover slip. After one hour, the cover slip was rinsed, and the remaining cells on the cover slip were fixated in a 3% a glutaraldehyde solution in a PBS-buffer for one hour. The cells were post-fixated in a 1% $OsO_4$ solution for another hour. Afterwards, the fixated cells were rinsed and dehydrated using ethanol. They were subsequently, dried using critical point $CO_2$ drying. After drying, the cover slips were coated with a 10 nm Iridium layer using sputter-coating.

## 3. Results and discussion

### 3.1. Flocculation

The biomass recoveries were measured at various dosages of Synthofloc 5080H (Fig. 1A) and Chitosan (Fig. 1B) at three different salinities: 25, 35, and 45 g/L of NaCl.

With Synthofloc 5080H, the biomass recovery is always higher than 90% regardless of the salinity. A lower biomass recovery is recorded when Chitosan is applied as a cationic polymeric flocculant using a similar dosage.

At elevated dosages, the biomass recovery in all three salinities decreases with $7\%_{recovery}$ when using Synthofloc 5080H as a flocculant. This is in agreement with the model presented in previous work ('t Lam et al., 2015) in which there is an optimum flocculant-
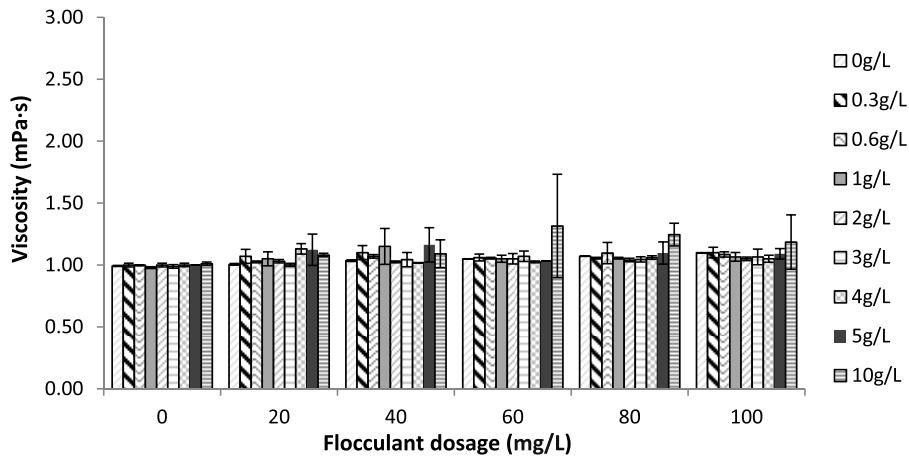
**Fig. 3.** Viscosity of Chitosan measured at a share rate of $100\,s^{-1}$. Every cluster of bars represent a flocculant dosage. Within every cluster, the salinity was increased, corresponding the legend at the right site of the figure.

biomass ratio. When this ratio is exceeded, flocculation becomes inhibited due to restabilization.

The successful use of Chitosan in freshwater conditions has previously been reported (Table 1), and the obtained results of Fig. 1B were compared with these studies. In all of the studies mentioned in Table 1, the biomass was cultivated in nutrient replete conditions. Possible biological effects such as the formation of extracellular polymeric substances due to nutrient stress (Salim et al., 2013), were thus eliminated.

The comparison between the biomass recoveries obtained with Chitosan in this study and other studies demonstrated that, in seawater salinities, a considerably lower biomass recovery is obtained using merely chitosan (Table 1). Although Garzon-Sanabria et al. (2013) did incite elevated biomass recoveries by using Chitosan in seawater salinities, it is not known if there was a possible pH effect involved as the pH after flocculant addition was adjusted to 8 in their study. In addition to the lower biomass recovery, other studies in Table 1 used substantial lower flocculant dosages. The use of lower flocculant dosages with higher biomass recoveries implies that, in other studies in freshwater conditions, Chitosan was a more efficient flocculant.

The differences in polymeric properties that were observed between Synthofloc 5080H and Chitosan in increasing salinities have been attributed to the degree of polymeric coiling (Bilanovic et al., 1988). They concluded that, as a function of the salinity, a polymer shrinks until it reaches it smallest dimensions.

### 3.2. Viscosity measurements

To verify if polymeric coiling provides an explanation for the lower biomass recovery observed with Chitosan compared to Synthofloc 5080H, viscosity measurements of both flocculants dissolved in water with different salinities were performed.

The viscosity of a polymeric solution is proportional to the apparent length of the polymers (Yamakawa, 1971; Tricot, 1984; Bilanovic et al., 1988).

In Figs. 2 and 3, the two bar diagrams illustrate the viscosity as a function of the flocculant dosage and as a function of the salinity.

In Fig. 2, the decrease in viscosity obtained with Synthofloc 5080H is in agreement with the trend described by Bilanovic et al. (1988). In their study, also a decrease in viscosity as a function of the medium salinity was observed. But despite the observed substantial viscosity decrease of the Synthofloc 5080H suspension in high salinities, it still induces flocculation (Fig. 1). Moreover, the viscosity of Synthofloc 5080H drops dramatically to values close to the viscosity of water already in medium with salt concentrations
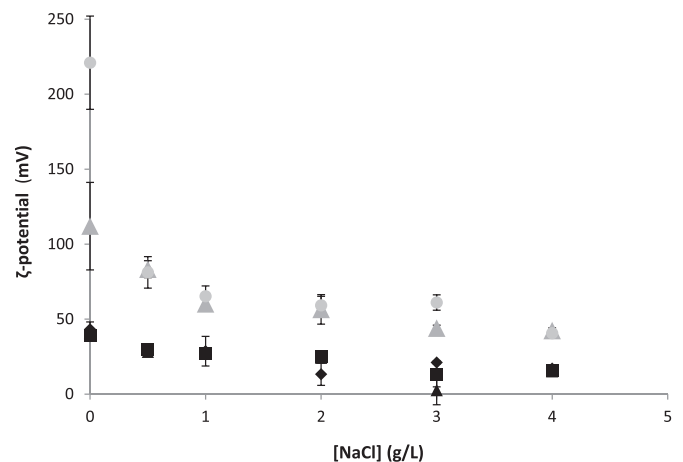


**Fig. 4.** ξ-potential as a function of [NaCl] (g/L). Synthofloc 5080H, potentials measured at: 100 mg/L ( ) and 200 mg/L ( ). Chitosan, potentials measured at: 30 mg/L ( ♦ ), 60 mg/L ( ▲ ) and 90 mg/L ( ■ ). Error bars are duplicates.

lower than 1 g/L of NaCl. This illustrates that Synthofloc 5080H polymer is very sensitive to surrounding ionic forces and becomes coiled.

With Chitosan (Fig. 2), the viscosity remains similar to the viscosity of water regardless of the flocculant dosage and salinity that is applied. These results demonstrate that no coiling occurred to explain the lower biomass recoveries obtained in Fig. 1 with Chitosan compared to Synthofloc 5080H. In addition, both flocculants had a viscosity similar to water in salinities of 10 g/L NaCl and a flocculant dosage lower than 100 ppm. This result illustrates that both flocculants had a similar apparent polymer length in these conditions.

Although polymeric coiling obviously occurs in elevated salinity, it does not explain the success of Synthofloc 5080H in high salinity and the decreasing functionality of Chitosan with increasing salinity as the salinity of seawater is approximately 35 g/L. These results illustrate that another characteristic of the flocculants should be responsible for the degree of success of flocculants in high salinities.

### 3.3. ζ-Potential

In addition to the apparent length of the polymeric chain, the charge of cationic polymers may be an important feature. With increasing salinity, the nett cationic charge of polymers should
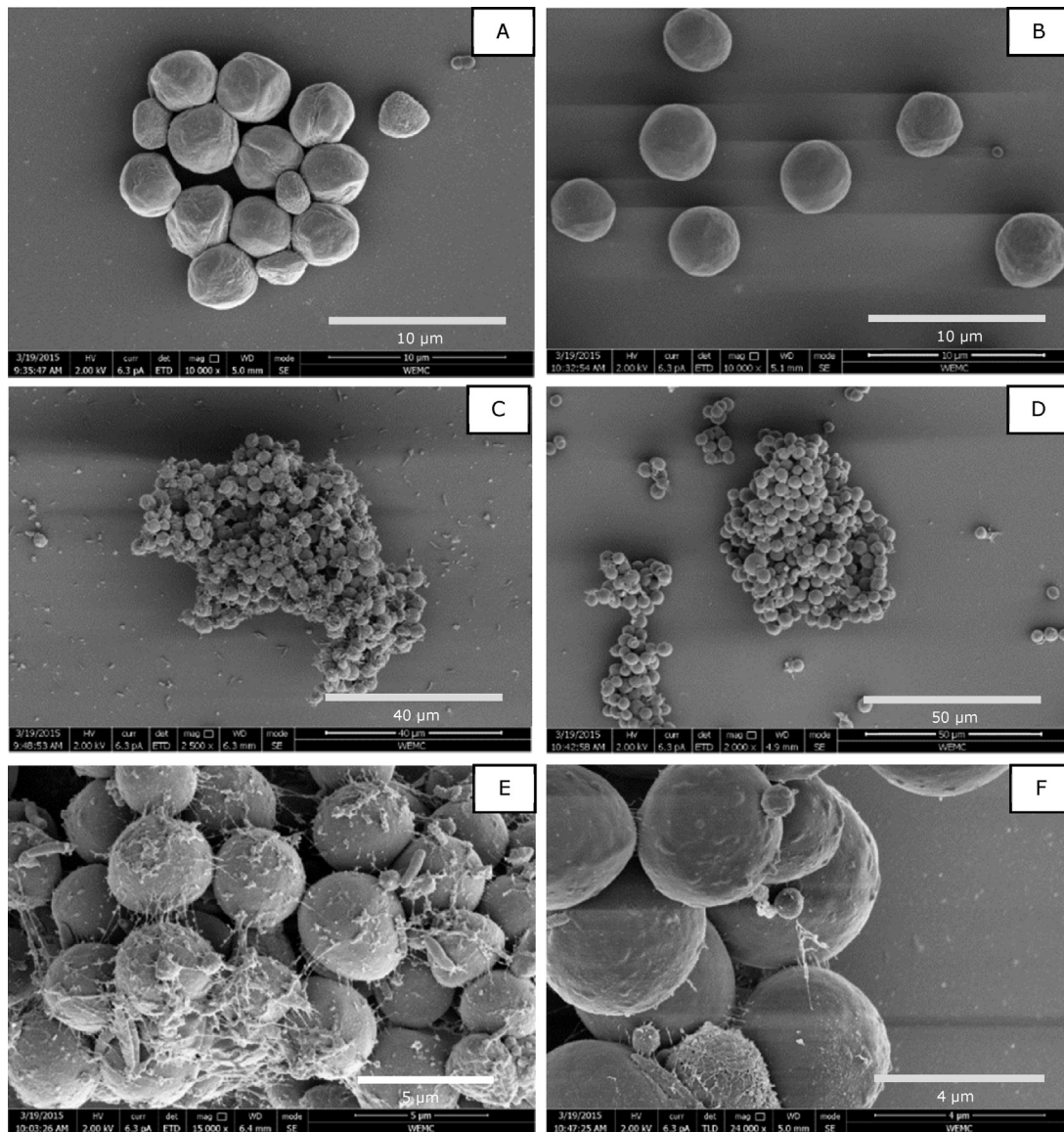
**Fig. 5.** SEM imaging, A: control at 25 g/L salinity. B: control at 45 g/L salinity. C: Floc with Synthofloc at 25 g/L. D: floc with Synthofloc at 45 g/L. E: zoom in on the bridges with Synthofloc at 25 g/L. F: zoom in on the bridges with Synthofloc at 45 g/L. Used flocculant concentration was 60 mg/L.

decrease due to the surrounding of anions. ζ-Potential measurements were performed to measure the impact of increasing salinity on the nett charge of the cationic polymers (Fig. 4). For both flocculants, the polymeric potential was measured as a function of salinity. The salinity was increased by an addition of NaCl. These measurements were performed with various dosages (Fig. 4).

With both flocculants, the ζ-potential decreases as a function of the salinity. When the ζ-potential as a function of salinity of Synthofloc 5080H is compared with the ζ-potential of Chitosan (Fig. 4), it appears that the ζ-potential of Synthofloc 5080H is generally more than twice as high regardless of the salinity. Both flocculants demonstrate an initial sharp decrease in ζ-potential with salinity, but Synthofloc 5080H always has at least a 20 mV or higher charge than Chitosan.

The combination of the observed difference in cationic charge for both flocculants with the observed similarities in viscosity with salinity suggests that the cationic charge is a predominant parameter influencing the flocculation efficiency of *N. oleoabundans* under saline conditions.

### 3.4. SEM imaging

In addition to viscosity- and ζ-potential measurements, Scanning Electron Microscopy (SEM) was performed to verify if a difference between the two flocculants and any effect of salinity on the structure of the flocculated microalgae could be observed. The intention was to visualize if the flocculant is indeed adsorbed to the cell wall. In addition, the pictures can also reveal how individual cells are attached to each other: bridging, patching, a combination, or another possibility.

In Fig. 5, the cells and formed aggregates are depicted at brackish salinity (25 g/L, Fig. 5A, C and E) and at high salinity (45 g/L, Fig. 5B, D and F) after adding 60 mg/L of Synthofloc 5080H.

Fig. 5A illustrates the cells without flocculant in brackish salinity. According to the figure, the cells are clustered which may be caused from the dehydration of the samples during the preparation. However, despite this clustering, the cells have smooth surfaces and are not bound to each other by a fibrous network of flocculants. After addition of the flocculant in brackish conditions, Synthofloc 5080H was strongly interacting with the single cells
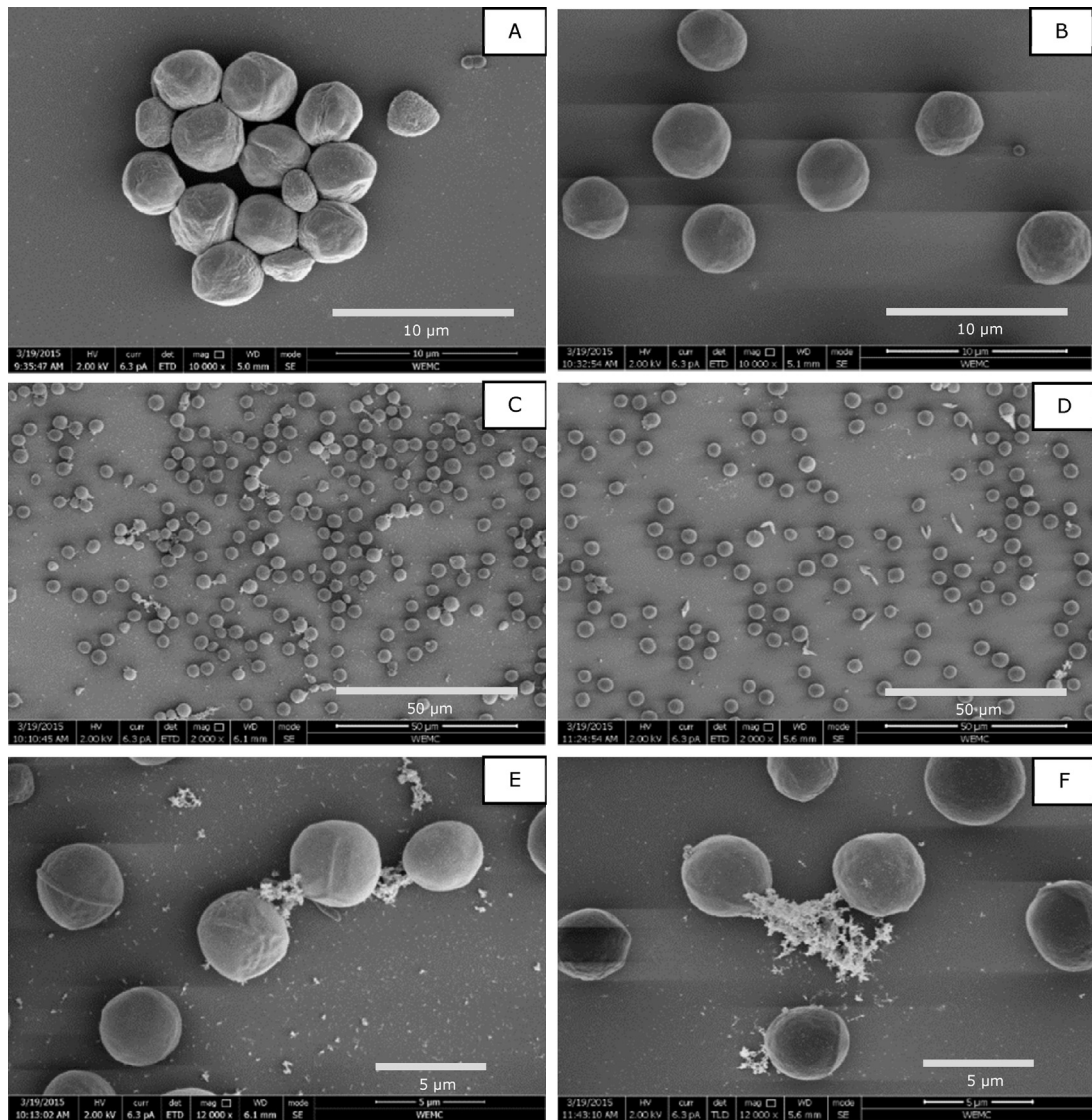
**Fig. 6.** SEM imaging A: control at 25 g/L. B: control at 45 g/L. C: Floc with Chitosan at 25 g/L. D: floc with Chitosan at 45 g/L. E: zoom in on the bridges with Chitosan at 25 g/L. F: zoom in on the bridges with Chitosan at 45 g/L. Used flocculant concentration was 60 mg/L.

(Fig. 5C and E). The polymers adsorb to the surfaces and form a fibrous network between the single cells. As a result, large aggregates of flocs are formed. In addition, all of the flocculants appear to be adsorbed to the cells as no non-absorbed flocculants are observed.

Fig. 5B shows that the single cells also have a smooth surface in very saline conditions. According to Fig. 5D and F, large agglomerates are formed just as those in brackish conditions. However, in this high salinity, Synthofloc 5080H appears to experience a weaker interaction with the cells as the large polymeric fibrous networks were not observed between individual cells. It appears that the flocculants are still adsorbed to the surface (Fig. 5F), however, they locally cover the cell surface which allow cells to interact and form small bridges.

In Fig. 6, the floc formation after an addition of 60 mg/L of Chitosan is shown. Fig. 6A, C, and E are pictures taken in brackish salinity (25 g/L), and Fig. 6B, D, and F are taken in very saline conditions (45 g/L).

The control picture in Fig. 6A is the same control picture that was taken in brackish salinities for Fig. 5. Fig. 6C exhibits that, although 60 mg/L of Chitosan was added, no large aggregates are formed in brackish conditions. There are several small aggregates

formed, but those contain no more than approximately three to four cells. In comparison with Fig. 6C a relatively large amount of non-adsorbed flocculant was observed in the form of white small aggregates between the algal cells.

There were similar observations in very saline conditions. In Fig. 6B, the same control that was depicted in Fig. 5 is shown. Also, small algal flocs are depicted in Fig. 6D and F. Just as was observed in brackish conditions, a relatively large amount of non-absorbed flocculant remains next to the small flocs.

In both salinities, the cationic polymers of Chitosan appear to be more entangled with each other than those of Synthofloc 5080H. Despite this entanglement, the polymers were adsorbed to the cell wall. This is in accordance with the observed biomass recoveries obtained with Chitosan (Fig. 1B).

The observations (Figs. 5 and 6) correspond well with the results of the $\zeta$-potential measurements. It was hypothesized that polymeric flocculants must be absorbed to the cell wall before inducing flocculation. After 15 min of mixing, all of the Synthofloc 5080H polymers appear to be adsorbed since white aggregates are no longer detected. However, with Chitosan, a relatively large amount of non-absorbed polymers are still observed outside the flocs.
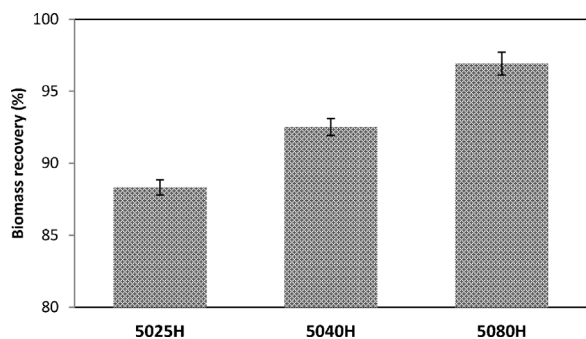
**Fig. 7.** Biomass recoveries as a function of the charge density (Control, 5025H; 5040H and 5080H). All experiments are performed in biological duplicates. Synthofloc 5080H is adapted from Fig. 1.

Our previous work ('t Lam et al., 2015) mathematically confirmed a proposed floc forming mechanism that, just as in other, earlier studies, assumes polymeric adsorption (Vandamme et al., 2013). The SEM analysis in this study supports the proposed mechanism of adsorption of a flocculant on a cell wall.

Polymeric adsorption to a surface can be enhanced by charge differences (Bolto and Gregory, 2007). The larger the charge difference between polymers and the cell wall, the quicker the polymer will be adsorbed (Al-Hashmi and Luckham 2010; Tekin et al., 2010). These results obtained in other studies suggest the necessity of a high charge difference between polymer and surface (in this case, the microalgal cell wall). Ensuing from this conclusion, the results reported in Fig. 4 suggest that the decrease in cationic charge caused a decreased efficiency of cationic polymers in elevated salinities.

In addition to a lower degree of adsorption of polymers on the cell wall, Tenney et al. (1969) suggested that charge neutralization plays a role in inducing floc formation. When charge neutralization is actually taking place during floc formation, a polymer with a higher cationic charge will be more efficient in locally neutralizing the charge of individual cells.

The decrease in cationic charge that caused a lower degree of adsorption in combination with a decreased ability to neutralize cell wall charges plausibly caused the decreased flocculation of Chitosan in elevated salinities (Fig. 1). It may also explain the remaining amount of polymers that were observed after 15 min of mixing (Fig. 6).

### 3.5. Flocculation at various cationic charge densities

To confirm that a decrease in cationic charge due to an increasing salinity is causing a decrease in flocculation, additional tests were performed with flocculants from the Synthofloc 50-series. By keeping the polymeric structure (and size) constant and varying the charge density from a low charge (5025H) through a moderate cationic charge (5040H) up to a highly charged cationic polymer (5080H), the effect of cationic charge could be confirmed (Fig. 7). The applied salinity in this experiment was 35 g/L.

According to Fig. 7, with a flocculant dosage of 30 mg/L, the flocculant with the highest charge density (5080H) was the most efficient in harvesting the biomass in marine conditions. On average, a 9% higher biomass recovery was obtained with 5080H compared to 5025H. These results demonstrate that a higher charge density results in greater biomass recoveries. The combination of the results presented in Fig. 7 with the observed decrease in ζ-potential as a function of medium salinity (Fig. 4) and apparent independence of the biomass recovery on the degree of coiling of a flocculant suggest that, due to a decrease in cationic charge in elevated salinities, flocculants become less functional.

A change in biomass recovery as a function of the charge density, similar to the results in Fig. 7, was previously observed by Roselet et al. (2015). In their study, the freshwater microalga *Chlorella vulgaris* and the seawater microalga *Nannochloropsis oculata* were flocculated with cationic poly(acryl) amides of the 'Flopam' series. By maintaining a constant polymeric size and varying the charge density from 0% to 100%, the effect of the cationic charge on the biomass recovery was determined. The biomass recovery increased from recoveries lower than 10% to recoveries higher than 90% with both microalgae as a function of the charge density.

## 4. Conclusion

The decrease in nett cationic charge in elevated salinities incites decreased functionality of cationic polymers and induces flocculation of *N. oleoabundans*. In high salinities, the resulting lower charge caused diminished efficiency in forming polymeric bridges between individual cells. This insight resulted in the conclusion that the cationic charge is an important criterion in selecting cationic polymers as a flocculant for marine applications where the apparent polymer length is of minor significance. This study also revealed that, in both brackish and marine conditions, polymeric bridging is a dominant mechanism in floc formation for cationic polymers.

### Acknowledgements

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