Effects of pH, salinity, biomass concentration, and algal organic matter on flocculant efficiency of synthetic versus natural polymers for harvesting microalgae biomass

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Abstract

This study investigated the effects of pH, salinity, biomass concentration, and algal organic matter (AOM) on the efficiency of four commercial cationic flocculants. The tannin-based biopolymers Tanfloc SG and SL and the polyacrylamide polymers Flopam FO 4800 SH and FO 4990 SH were tested for flocculation of two microalgae models, the freshwater *Chlorella vulgaris* and the marine *Nannochloropsis oculata*. Both biomass concentration and AOM presence affected all polymers evaluated

whereas salinity and pH affected only Flopam and Tanfloc, respectively. A restabilization effect due to overdosing was only observed for Flopam polymers and increasing Tanfloc dose resulted in improved efficiency. Flopam polymers showed a significant decrease in the maximum quantum yield of photosystem II as function of polymer dose for *Chlorella*, which supported the need for toxicological studies to assess the potential toxicity of Flopam. In overall, Tanfloc was not affected by salinity nor presented potential toxicity therefore being recommended for the flocculation of both freshwater and marine species.

Keywords: Coagulation; Flocculation; Biopolymer; Photosystem II; Toxicity

Introduction

Harvesting is one of the major bottlenecks in large-scale microalgae production for biofuels and other low-value bioproducts [1]. According to Gudin and Therpenier [2], harvesting can comprise up to 30% of the total costs for biomass production. Harvesting costs are considered high, mainly because of the difficulty in concentrating the small-size microalgae cells (3–30 μm) present at relatively low concentrations in culture media (<0.5%) [3]. Therefore, huge amounts of water need to be processed in order to concentrate the microalgal biomass. Currently, harvesting is achieved for example by centrifugation [4], however, this technology is expensive because of its high energy requirements [5]. Flocculation is a promising low-cost technology to harvest even small amounts of microalgae from large volumes of liquid. In addition, flocculation can be used as a concentration step prior to centrifugation, thus reducing the volume to be manipulated and the production costs [6]. In general, flocculation is achieved by addition of coagulants-flocculants that destabilize and

aggregate particles in suspension, followed by a simple sedimentation step that results in separation of the biomass and liquid phase.

Traditionally, two broad classes of coagulants-flocculants are employed, namely hydrolyzing metal salts and organic polymers [7]. However, hydrolyzing metal salts are not recommended for microalgae harvesting as the presence of residual metal in the final biomass can interfere with downstream processing or cause toxicity [8]. Currently, synthetic polyacrylamide polymers form the majority of coagulants-flocculants in commercial use [7]. Previous studies have successfully applied synthetic polyacrylamide polymers for flocculating microalgae [9-11]. Although synthetic polyacrylamide polymers as such are not toxic, they may contain monomer residues that are presumably toxic [12]. Instead, natural polymers are biodegradable and non-toxic, which may be attractive if the microalgal biomass is to be used for animal feed, or in a biorefinery context [4]. A well-known natural cationic polymer is chitosan, a derivative of chitin obtained from shrimp shells. Studies have demonstrated that chitosan is quite effective at flocculating microalgae but too expensive for large-scale application [10, 13].

In a previous study, Roselet et al. [14] screened 25 commercial natural (Tanfloc) and synthetic (Flopam and Zetag) polymers of varying molecular weight and charge density for flocculation of the freshwater *Chlorella vulgaris* and marine *Nannochloropsis oculata*. The authors reported that flocculation was readily achieved for both species with Tanfloc, whereas Flopam and Zetag were most effective in freshwater *C. vulgaris*. In addition, the flocculation efficiency of Flopam and Zetag was largely influenced by charge density, with high charge density polymers

performing better. Overall, Tanfloc SL and Flopam FO 4990 SH were considered the most efficient polymers for microalgal flocculation. However, the flocculation efficiency of synthetic and natural polymers could be affected by several other factors as well. For example, culture pH affects flocculation due to changes in the surface charge of the microalgal cells, the extent of coiling, and the degree of ionization of polymers [15, 16]. Moreover, salinity reduces the chemical activity of polymers, masking their functional sites and changing their molecular structure [17]. Negatively charged algal organic matter (AOM) has been reported to interact with cationic polymers, resulting in increased polymer requirements [13, 18]. Finally, the polymer dose and biomass concentration ratio should not be too high, otherwise the microalgae surfaces will become covered to such an extent that charge reversal will occur, resulting in suspension restabilization [12].

Therefore, the main objective of this study was to compare and evaluate synthetic versus natural polymer flocculants both for freshwater and marine microalgae harvesting. The effects of pH, salinity, AOM, and biomass concentration on microalgal flocculation efficiency were studied We included two low-molecular-weight natural tannin polymers (Tanfloc SG and SL) and two high-molecular-weight synthetic polyacrylamide polymers (Flopam FO 4800 SH and FO 4990 SH). Experiments were performed on two microalgal model species, the freshwater *Chlorella vulgaris* and the marine *Nannochloropsis oculata*. In addition, the maximum quantum yield of photosystem II of *C. vulgaris* and *N. oculata* was quantified as function of polymer dose to detect first indications of toxicity.

Materials and methods

101 **Microalgae cultivation**

102 Two microalgal model species were studied: the freshwater *Chlorella vulgaris* (SAG
103 211-11b) and the marine *Nannochloropsis oculata* (SAG 38.85). Both microalgae
104 were cultured in Wright's Cryptophyte medium, which was prepared from pure salts
105 and deionized water except that for *Nannochloropsis*, synthetic sea salt (Homarsel,
106 Zoutman, Belgium) was added at a final concentration of 30 g L^{-1} [14]. The
107 microalgae were cultured for 6 days in 30-L Plexiglas bubble column
108 photobioreactors and mixed by bubbling with 0.2-µm filtered air (5 L min^{-1}) in a
109 temperature-controlled room (20ºC). The pH was maintained at 8.5 by addition of
110 CO_2 (2–3%) using a pH-controller system. Each photobioreactor was continuously
111 irradiated with daylight fluorescent tubes (100 µmol photons m^{-2} s^{-1}). Microalgal
112 biomass concentrations were monitored daily by measuring the absorbance at 750 nm.
113 These measurements were calibrated against dry weight measured gravimetrically on
114 pre-weighed GF/F glass fiber filters (R^2 = 0.99). The marine microalgae were washed
115 with 0.5 mol L^{-1} ammonium formate to remove salts adsorbed on the cell surface [19].
116

117 **General flocculation procedure**

118 Four commercial cationic polymers were selected for these experiments based on the
119 outcome of a previous screening study [14]. Tanfloc SG and SL are natural low-
120 molecular-weight quaternary ammonium polymers based on tannins extracted from
121 the black wattle tree (*Acacia mearnsii*) and manufactured by Tanac (Brazil). Flopam
122 FO 4800 SH and FO 4990 SH are high-molecular-weight synthetic copolymers of
123 acrylamide and quaternized cationic monomer polymers manufactured by SNF
124 Floerger (France) with charge densities of 80 and 100 mol%, respectively. All
125 polymers were kindly provided by the manufacturers. For each polymer, a 1 g L^{-1}

126 stock solution was prepared by adding 50 mg of polymer to 50 mL of deionized water
127 and by mixing them for 1 h. Thirteen concentrations (0, 2, 5, 10, 20, 30, 40, 50, 60,
128 70, 80, 90, and 100 mg L^{-1}) were selected to evaluate the flocculation behavior.
129
130 Standardized 100-mL jar tests were used to evaluate flocculation of *C. vulgaris* and *N.*
131 *oculata* using a 15-position digital magnetic stirrer and PTFE-coated magnetic stirring
132 bars. During addition of the previously mentioned polymers, the microalgal
133 suspensions were intensively mixed (350 rpm) for 10 min, to allow uniform polymer
134 dispersion, which was then followed by gentler mixing (250 rpm) for 20 min to allow
135 floc formation. Subsequently, the microalgal suspensions were allowed to settle for 30
136 min and samples were collected in the middle of the clarified zone. Optical density
137 was measured at 750 nm prior to polymer addition (OD_i) and after settling (OD_f), and
138 the flocculation efficiency (η_a) was calculated as:
139 $$\eta_a = \frac{OD_i - OD_f}{OD_i} \times 100$$
140
141 **Effect of pH, salinity, biomass concentration, and AOM**
142 After 6 days, when cultures achieved the stationary phase, the microalgae were
143 collected from the photobioreactors for use in the flocculation experiments. In a
144 previous study, Roselet et al. [20] cultured *N. oculata* year-round in an outdoor open
145 system without CO_2 addition and the culture presented a pH naturally ranging from 6
146 to 8. Therefore, to investigate the influence of pH on flocculant efficiency, three pH
147 values (5, 7, and 9) were tested 30 min after adjustment using 0.5 mol L^{-1} HCl or 0.5
148 mol L^{-1} NaOH solutions. Moreover, the same study observed that salinity in open
149 culture systems decreased from 30 g L^{-1} to salinity as low as 18 g L^{-1} during the rainy
150 seasons. On the contrary, during sunny seasons, salinity may reach values as high as

140 g L^{-1} [20]. Thus, it is relevant to study the effect of salinity variation on flocculant efficiency. Two concentrations of synthetic sea salt were added (15 g L^{-1} and 30 g L^{-1}) to fresh medium with *N. oculata* whereas no salt (0 g L^{-1}) was added to freshwater *C. vulgaris*. Higher concentrations were not tested because the elevation of salinity can be easily controlled by freshwater addition in large scale cultivation systems. The importance of biomass concentration on flocculation was also investigated. Three biomass concentrations (1, 2, and 4×) were prepared by resuspending different volumes of centrifuged microalgae in fresh medium. The final concentrations were further confirmed by dry-weight measurements (≈300, ≈600, and ≈1200 mg L^{-1}). The possible effect of AOM on flocculation of *C. vulgaris* and *N. oculata* was studied by comparing medium with and without AOM. To remove the AOM, microalgae were separated from the original medium by centrifugation at 4,000 g and resuspended in fresh medium without AOM [21]. The AOM comprises a broad spectrum of organic compounds including carbohydrates, amino acids, peptides, proteins, nucleic acids, lipids and various organic acids [22]. However, carbohydrates comprise 80–90% of the total extracellular released material, mainly in the stationary phase [23]. Therefore, despite the presence of other organic compounds, the AOM content was estimated through measurement of the total carbohydrates, using the phenol-sulfuric acid method [24]. Overall, the carbohydrate content in the fresh medium without AOM (AOM−) was reduced in comparison with the original medium with AOM (AOM+), from 10.6 and 58.5 µg mL^{-1} to 2.3 and 5.6 µg mL^{-1} for *C. vulgaris* and *N. oculata*, respectively. In general, experimental conditions were pH 7, salinity 0 g L^{-1} (for *C. vulgaris*) or 30 g L^{-1} (for *N. oculata*), and 300 mg L^{-1} of biomass resuspended in fresh medium without AOM (AOM−), except when otherwise stated. Previous

175 experiments demonstrated that centrifugation and subsequent resuspension in fresh
176 medium have no significant effect on flocculation [13, 14].
177
178 **Effect of polymer dose on the quantum yield of photosystem II**
179 The maximum quantum yield of photosystem II expressed as the ratio of variable
180 versus maximal fluorescence (Fv/Fm) was measured as function of polymer dose to
181 evaluate the effect on the photosynthetic performance of photosystem II. The
182 quantum yield of photosystem II is responsible for the conversion of light into
183 chemical energy, which makes it a sensitive indicator of stress in microalgae [25].
184 The quantum yield of photosystem II was measured in triplicate using the same batch
185 of microalgae, before and 1 h after the addition of cationic polymers and after 30 min
186 of dark adaptation of microalgae, using an AquaPen-C fluorometer (Photon Systems
187 Instruments, Czech Republic). Cells treated with 15% H_2O_2 for 30 min were used as a
188 negative control, whereas non-treated cells (no polymer addition) were used as
189 positive controls.
190
191 **Statistical analysis**
192 For each treatment, the polymer dose was log-transformed and a dose-response non-
193 linear regression analysis with least square iteration was performed to describe the
194 relationship among measured variables [26]. All dose-response curves were compared
195 by extra sum-of-squares F-test ($P < 0.05$) and D'Agostino-Pearson omnibus test was
196 performed to verify dataset normality [26]. Toxicity was analyzed by one-way
197 ANOVA, followed by Dunnett's multiple comparison tests [26].
198
199 **Results and Discussion**

Effect of pH

For *C. vulgaris*, the maximum flocculation efficiency (close to 100%) was achieved using 20 mg L^{-1} of Flopam FO 4800 SH and 30 mg L^{-1} of Flopam FO 4990 SH. For *N. oculata*, a flocculation efficiency of 80% was obtained using 20 mg L^{-1} of Flopam FO 4800 SH and 10 mg L^{-1} of Flopam FO 4990 SH. The dose-response curves showed no effect of pH between 5 and 9 using both types of Flopam, neither for *C. vulgaris* nor for *N. oculata* ($P > 0.05$; Fig. 1a–d). Both Flopam FO 4800 SH and FO 4990 SH are polymers substituted with quaternary ammonium functional groups with a degree of substitution of 80% and 100%, respectively. Cationic polymers with quaternary ammonium groups are salts of a strong base and therefore, according to Kam and Gregory [27], not subjected to loss of charge density, regardless of pH. Similarly, Graham et al. [28] reported that polyDADMAC, which has a degree of substitution of 90%, was permanently charged and insensitive to pH changes.

In contrast, the dose-response was significantly affected by pH using Tanfloc SG and SL ($P < 0.05$; Fig. 1e–h). Both for *C. vulgaris* and *N. oculata*, a maximum flocculation efficiency close to 100% was achieved using 15 mg L^{-1} of Tanfloc SG/SL at pH 5, while 70 mg L^{-1} was needed at pH 9. For *N. oculata*, the dose-response between pH 7 and 9 was not significantly different. According to the manufacturer, the point of zero charge of Tanfloc is at pH 8.17. Theoretically, above that point the surface of Tanfloc becomes neutral, losing its ability to neutralize the negative charge of the microalgal cells. The results of this study are in accordance with the manufacturer's data and with previous studies using Tanfloc in other applications and conditions such as for example for anionic surfactant removal [29]. Graham et al. [28] previously examined Tanfloc for kaolin clay coagulation and indicated that the

225 deprotonation of the secondary amine due to pH increase resulted in loss of charge
226 and efficiency. Recently, Gutiérrez et al. [30] employed Tanfloc to harvest freshwater
227 microalgae from urban wastewater and obtained 90.2% recovery at pH 7.9. Similarly,
228 Roselet et al. [31] employed Tanfloc at outdoor pilot scale cultures (1200 L) and
229 reported that reducing the pH from 8 to 6 increased *N. oculata* flocculation efficiency
230 from 33% to 95%, corroborating the results from the present work. However, this
231 study shows additionally the strong pH dependency of Tanfloc efficiency as opposed
232 to Flopam.
233
234 The observed variation amongst polymer type can be related to variations in polymer
235 charge densities. For example, Flopam FO 4800 SH and FO 4990 SH have charge
236 densities of 80 and 100 mol%, respectively, which explains why FO 4990 SH
237 performed better at optimal pH. No information is available for Tanfloc, but SL
238 performed better suggesting that it may have a higher charge density than SG.
239
240 **Effect of salinity**
241 In general, all flocculants evaluated were more efficient at freshwater (0 g sea salt L^{-1})
242 than at marine (15 and 30 g sea salt L^{-1}) conditions, clearly showing that flocculation
243 was hindered in marine medium (Fig. 2a and c). Flopam efficiency was significantly
244 affected by salinity, as an increase in synthetic sea salt concentration from 15 g L^{-1} to
245 30 g L^{-1} decreased flocculation ($P < 0.05$; Fig. 2b and d). The optimum dosages for
246 Flopam were 2 mg L^{-1} for *N. oculata* and 5–10 mg L^{-1} for *C. vulgaris*, suggesting that
247 Flopam efficiency may have been improved by the compression of the double layer
248 near the microalgal cell surface, reducing the required polymer dosage [7]. According
249 to the DLVO theory, salinity will influence the thickness of the double layer of

250 counter ions, also called electrical double layer (EDL), around a charged particle [32].
251 An increase of salinity, by for example the addition of an indifferent electrolyte like
252 sodium chloride, leads to a compression of the EDL. This compression will cause a
253 reduction in the electrostatic repulsion forces and will result in easier coagulation.
254 However, restabilization was observed for *N. oculata* when Flopam was overdosed
255 (Figs. 1–4). This phenomenon was even strongly observed with increasing salinity
256 (Fig 2 b and d). It has been reported before that Flopam, which is a linear polymer,
257 was susceptible to severe polymer coiling in high ionic strength marine media [17].
258 Further fundamental study is needed to fully understand the observed restabilisation
259 phenomenon of Flopam in marine conditions.
260
261 In contrast to Flopam, Tanfloc was not affected by salinity, as its flocculation
262 efficiencies were described by the same dose-response curves (Fig. 2e–h). The fact
263 that the flocculation efficiency of Tanfloc does not differ between salinity conditions
264 may be due to the different secondary structure of Tanfloc in comparison to Flopam,
265 as Tanfloc is a branched rather than a linear polymer [33]. As a result, it may suffer
266 less from coiling at high ionic concentrations than Flopam. This property makes
267 Tanfloc attractive for harvesting marine microalgae as in open aquaculture systems,
268 salinity can vary largely due to evaporation or rainfall [20], impacting further large-
269 scale flocculation. This shows that the behavior of polymers as function of salinity is
270 crucial to study further in detail to find novel natural polymers that could serve as
271 efficiency flocculants for marine microalgae.
272
273 **Effect of biomass concentration**

In general, the polymer dosage increased as a function of biomass concentration (Fig. 3). For *C. vulgaris*, Flopam FO 4800 SH and FO 4990 SH exhibited 100% flocculation efficiency at 5 mg L^{-1} for low biomass concentrations (1×), whereas increasing the biomass concentration to 2× required a dosage increase to 10 mg L^{-1} and 4× a dosage increase to 20 mg L^{-1} (Fig. 3a and c). Tanfloc SG and SL exhibited 100% flocculation efficiency at 10 mg L^{-1} at low biomass concentrations (1×), whereas increasing the biomass concentration to 2× required a dosage increase to 20 mg L^{-1} and 4× a dosage increase to 40 mg L^{-1} (Fig. 3e and g). For *N. oculata*, similar results were observed using Tanfloc SG (Fig. 3f). This suggests a linear correlation between polymer dosage and biomass concentration. According to Bolto and Gregory [12], polymer absorption should not be too low, otherwise charge neutralization or bridging will not be effective. Conversely, polymer absorption should not be too high either, otherwise the particle surfaces will become so highly covered that charge reversal will occur, resulting in restabilization. Therefore, half surface coverage should result in optimum adsorption. Tenney et al. [15] tested several biomass concentrations (100, 200 and 350 mg L^{-1}) and reported a linear relationship between the cationic polyamine and the *Chlorophyta* concentration studied. Recently, Henderson et al. [34] also observed a reasonable log–log relationship between cell surface area and aluminum dose, demonstrating that the cell surface area can be used to predict an approximate coagulant dose for metal salts. However, actual demand varies depending other characteristics as well, primary that of AOM character [34]. Wyatt et al. [35] studied ferric chloride-induced flocculation of *Chlorella zofingiensis* and reported that the linear relationship breaks down at algae concentrations above 500 mg L^{-1}. For higher algal concentrations (up to 1500 mg L^{-1}) the volume of ferric chloride required for effective flocculation becomes stable (≈200 mg L^{-1}). This was

299 explained by changes in the flocculation mechanisms as increasing the ferric chloride
300 dose resulted in a switch from charge neutralization and bridging to sweep
301 flocculation [35]. The same behavior can be observed for Tanfloc (Fig. 3e-h) where,
302 at higher doses, no restabilization is observed and flocculation efficiency achieves its
303 maximum. Graham et al. [28] reported that, in an analogous behavior to ferric
304 chloride, flocculation mechanism may involve both charge neutralization and
305 enmeshment by precipitated Tanfloc. However, where charge neutralization and
306 bridging are the prevailing mechanisms, a linear relationship is established (Fig. 3e-
307 h). It should be emphasized that AOM may impact this relationship, as it reduces
308 flocculation efficiency and increases polymer demand (see next section). Moreover,
309 AOM concentration will increase with the increase in biomass concentration,
310 affecting the optimum dose requirement in higher biomass density cultures. In this
311 study regarding Flopam, a linear relationship between polymer dosage and biomass
312 concentration was not clearly observed for *N. oculata* because of the severe
313 restabilization that occurred for all biomass concentrations (Fig. 3b and d).
314
315 **Effect of AOM**
316 The presence of AOM negatively affected both Flopam and Tanfloc efficiencies,
317 resulting in an increase of the required dosage (Fig. 4). The AOM concentrations were
318 10.6 mg L^{-1} (AOM+) and 2.3 mg L^{-1} (AOM–) for *C. vulgaris*, and 58.5 mg L^{-1}
319 (AOM+) and 5.6 mg L^{-1} (AOM–) for *N. oculata*. For *C. vulgaris,* the dosage of
320 Flopam FO 4800 SH doubled from 10 mg L^{-1} to 20 mg L^{-1} for maximum flocculation
321 in the presence of AOM (Fig. 4a). The optimum dosage of Flopam FO 4990 SH
322 increased from 5 to 30 mg L^{-1} in the presence of AOM (Fig. 4c). For *N. oculata*, the
323 required dosage of Flopam FO 4800 SH for maximum flocculation efficiency

increased from 2 mg L^{-1} to 20 mg L^{-1} when AOM was present (Fig. 4b). The optimum dosage of Flopam FO 4990 SH increased from 2 to 10 mg L^{-1} when AOM was present (Fig. 4d). A similar trend was noted for both types of Tanfloc. For *C. vulgaris,* the dosage of Tanfloc SG and SL required for maximum flocculation efficiency increased from 10 mg L^{-1} to 50 mg L^{-1} and from 5 to 40 mg L^{-1}, respectively, in the presence of AOM (Fig. 4e and g). For *N. oculata,* the required dosage of Tanfloc SG and SL increased from 20 mg L^{-1} to 50 mg L^{-1} and from 10 to 50 mg L^{-1}, respectively, in the presence of AOM (Fig. 4f and h). In general, the presence of AOM resulted in a larger increase in required flocculant dose for Tanfloc compared to Flopam. However, it is clear that there is a difference between both algae species and the interaction with each polymer type.

Some studies have also demonstrated that AOM has a negative impact on flocculation. For instance, according to Henderson et al. [18], AOM has a negative zeta potential in the pH range from 2 to 10, and consequently, it interacts with cationic polymers. Vandamme et al. [13] studied the flocculation of *C. vulgaris* and, similarly, reported that AOM affected five different flocculation methods and increased the required flocculant dosage up to 9-fold, depending on the type of flocculant used. These findings are similar with those by Garzon-Sanabria et al. [10], who employed Flopam to flocculate *N. salina* and observed that 20 mg L^{-1} (a 7-fold increase) in polymer dose was required in medium with 100 mg L^{-1} of AOM measured as total carbohydrates. In the present study, high doses of Tanfloc (~60 mg L^{-1}) were required to achieve a flocculation efficiency of more than 90% in medium with AOM (Fig. 4e–h), suggesting that it is more strongly affected by AOM than Flopam especially for *Chlorella vulgaris*.

349

350 According to Pivokonsky et al. [22], AOM comprises a broad spectrum of organic
351 compounds including carbohydrates (monosaccharides and polysaccharides),
352 nitrogen-containing compounds (amino acids, peptides, proteins, nucleic acids), lipids
353 and various organic acids released as by-products of microalgal photosynthesis and
354 secondary metabolism [22]. Nevertheless, Vandamme et al. [21] recently reported for
355 *C. vulgaris* that flocculation was mainly inhibited by high-molecular-weight
356 carbohydrate structures, rather than with proteins. This finding is in agreement with
357 Myklestad [23], who quantified that carbohydrates comprise 80–90% of the total
358 extracellular polymeric substances released by microalgae, mainly in the stationary
359 phase. Moreover, Pivokonsky et al. [36] reported that, for green algae, proteins
360 contents in AOM are usually low (less than 19%) whereas those produced by
361 cyanobacteria have the appropriate characteristics for protein-flocculant
362 complexation, hindering microalgal flocculation. In general, this study shows that the
363 flocculation performance of the tested polymers is indeed negatively affected by the
364 presence of AOM. A detailed fractionation of AOM based on size and
365 hydrophobicity, followed by additional flocculation tests would be required to fully
366 elucidate the impact of AOM on the flocculation process.
367

368 **Effect of polymer dose on the quantum yield of photosystem II**

369 The maximum quantum yield of photosystem II (expressed as Fv/Fm ratio) of *C.*
370 *vulgaris* and *N. oculata* was measured as function of polymer dose (Fig. 5). Tanfloc
371 addition had no effect on the maximum quantum yield of photosystem II for *C.*
372 *vulgaris* or *N. oculata* ($P > 0.05$; Fig. 5e–h). Similarly, Gutiérrez et al. [30] assessed
373 Tanfloc's potential toxicity based on quantum yield measurements for freshwater

374 microalgae and reported that doses up to 50 mg L^{-1} presented no adverse effects. The
375 addition of Flopam to *N. oculata* had no effect either on the maximum quantum yield
376 of photosystem II ($P > 0.05$). However, in this study, a significant effect of FO 4800
377 SH and FO 4990 SH was observed for *C. vulgaris* as concentrations above 10 mg L^{-1}
378 and 5 mg L^{-1}, respectively, resulted in a consistent decrease of the maximum quantum
379 yield as function of polymer dosage ($P < 0.05$; Fig. 5a and c). This experiment
380 showed that Flopam did affect the efficiency of photosystem II of *C. vulgaris* and
381 therefore appeared to have a stress effect on the cells. According to Costa et al. [37],
382 the toxicity of polyacrylamides on aquatic organisms depends on the chain
383 architecture, with a decreased toxicity for branched polymers due to their lower cell
384 coverage. Thus, as FO 4800 SH and FO 4990 SH are both linear polymers, this may
385 result in a higher cell coverage and hence in increased toxicity. Furthermore, FO 4990
386 SH seems to be more toxic than FO 4800 SH, probably due to its higher charge
387 density, which increases the affinity of the cationic polymer chains to the algal cells
388 [37]. A recent study reported no lethal toxicity for *Chlamydomonas reinhardtii*, a
389 freshwater species, co-cultivated with both Flopam FO 4800 SH and FO 4990 SH at
390 concentrations up to 2 mg L^{-1} [38]. Similarly, in the present study no effect on the
391 quantum yield was observed for *C. vulgaris* at 2 mg L^{-1} of Flopam ($P > 0.05$; Fig. 5a
392 and c). However, Flopam may present potential toxicological issues at higher
393 concentrations and it would therefore be recommended to conduct a detailed toxicity
394 essay in a subsequent study. Evaluating the potential toxicity of flocculants is relevant
395 if the algal biomass is planned to be used as feed or food whereas, for other
396 biotechnological applications, it does not matter if the algae is viable or not [4,8].
397 Nevertheless, if the medium is planned to be reused or discharged, and contains a
398 significant concentration of flocculant, it may affect the cycle production or the

surrounding environment and its microbial community. Therefore, forthcoming studies must also focus on the safety of using flocculants and not only on their efficiencies.

Conclusions

This study demonstrated the need for detailed screening of novel natural polymers based on multiple parameters to replace synthetic flocculants. Our results showed that the overall efficiency of synthetic versus natural polymers depended on several factors. The flocculation efficiency of Flopam mainly depends on salinity, biomass concentration, and the presence of AOM. Overdosing resulted in severe restabilization of this flocculant. The flocculation efficiency of Tanfloc is mainly dependent on the pH, the presence of AOM, and biomass concentration, while its performance is independent of salinity. Tanfloc appears to be a promising non-toxic and efficient polymer for flocculation of freshwater and marine microalgae that produce low amounts of AOM.

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541

542 **Figures**

543 **Fig. 1** Effect of pH (5, 7, and 9) on Flopam (FO 4800 SH and FO 4990 SH) and
544 Tanfloc (SG and SL) efficiencies. Experiments were run in freshwater (0 g sea salt L^{-1}
545 for *C. vulgaris*) and marine (30 g sea salt L^{-1} for *N. oculata*) conditions with 300 mg
546 L^{-1} of algal biomass resuspended in medium without AOM

547

548 **Fig. 2** Effect of salt concentration (0, 15 and 30 g sea salt L^{-1}) on Flopam (FO 4800
549 SH and FO 4990 SH) and Tanfloc (SG and SL) efficiencies. Experiments were run in
550 freshwater (0 g sea salt L^{-1} for *C. vulgaris*) and marine (15 and 30 g L^{-1} for *N.*
551 *oculata*) conditions, at pH 7 with 300 mg L^{-1} of algal biomass resuspended in medium
552 without AOM

553

554 **Fig. 3** Effect of algal biomass concentration (1, 2, and 4×) on Flopam (FO 4800 SH
555 and FO 4990 SH) and Tanfloc (SG and SL) efficiencies. Experiments were run in
556 freshwater (0 g sea salt L^{-1} for *C. vulgaris*) and marine (30 g sea salt L^{-1} for *N.*
557 *oculata*) conditions, at pH 7 and biomass resuspended in medium without AOM

558

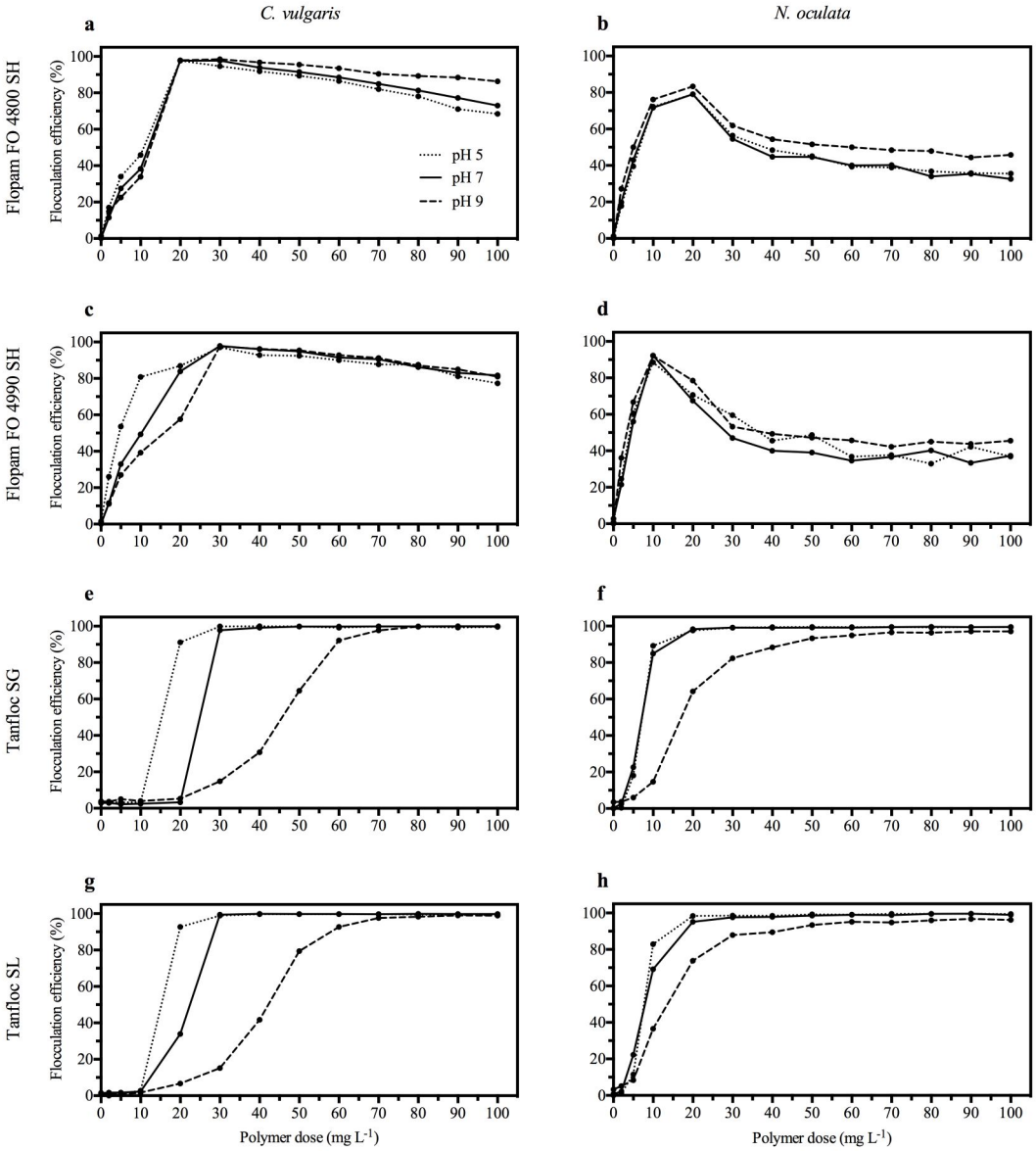
559 **Fig. 4** Effect of algal organic matter presence (AOM+) and absence (AOM−) on
560 Flopam (FO 4800 SH and FO 4990 SH) and Tanfloc (SG and SL) efficiencies.
561 Experiments were run in freshwater (0 g sea salt L^{-1} for *C. vulgaris*) and marine (30 g
562 sea salt L^{-1} for *N. oculata*) conditions, at pH 7 with 300 mg L^{-1} of algal biomass

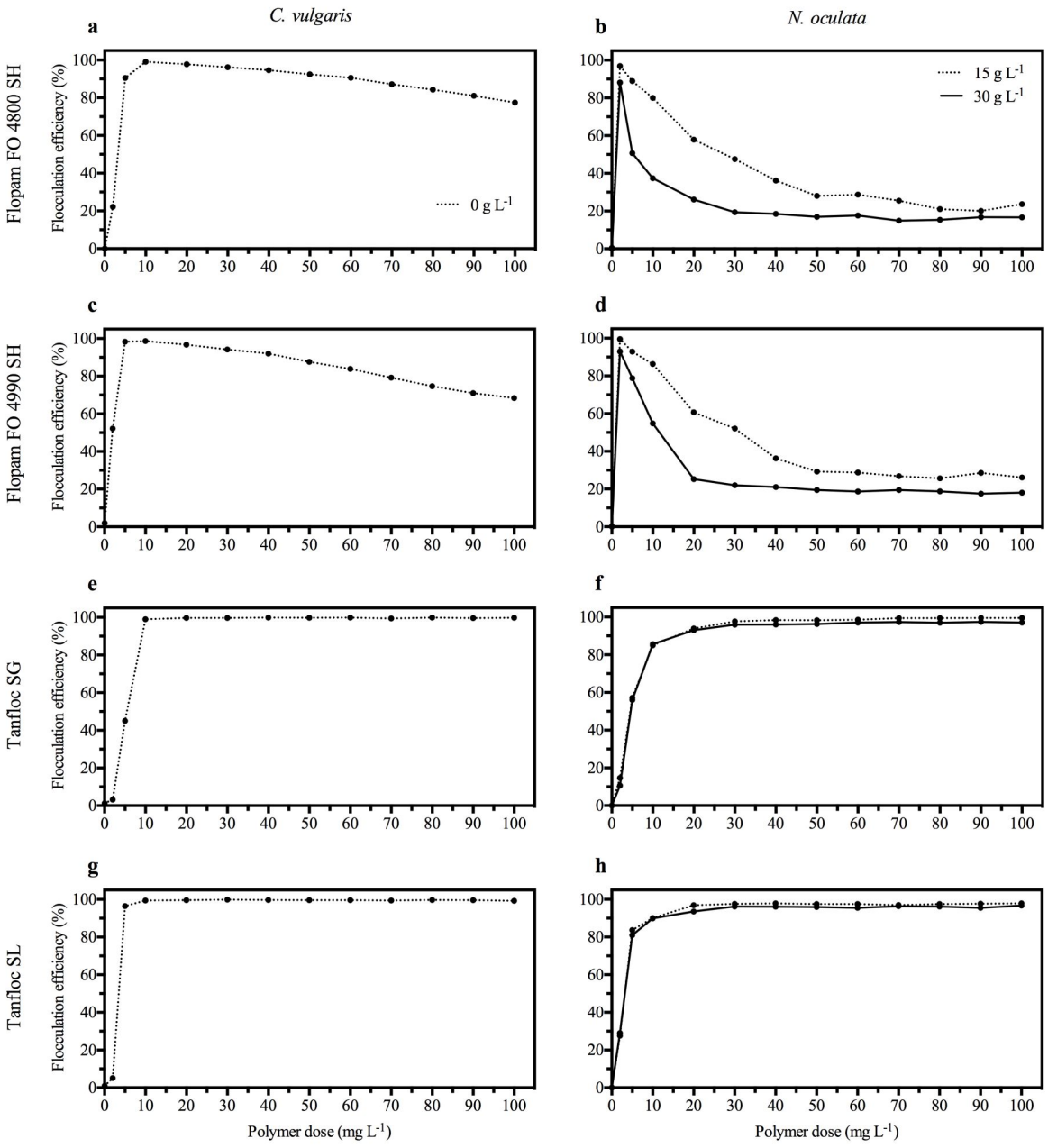
563

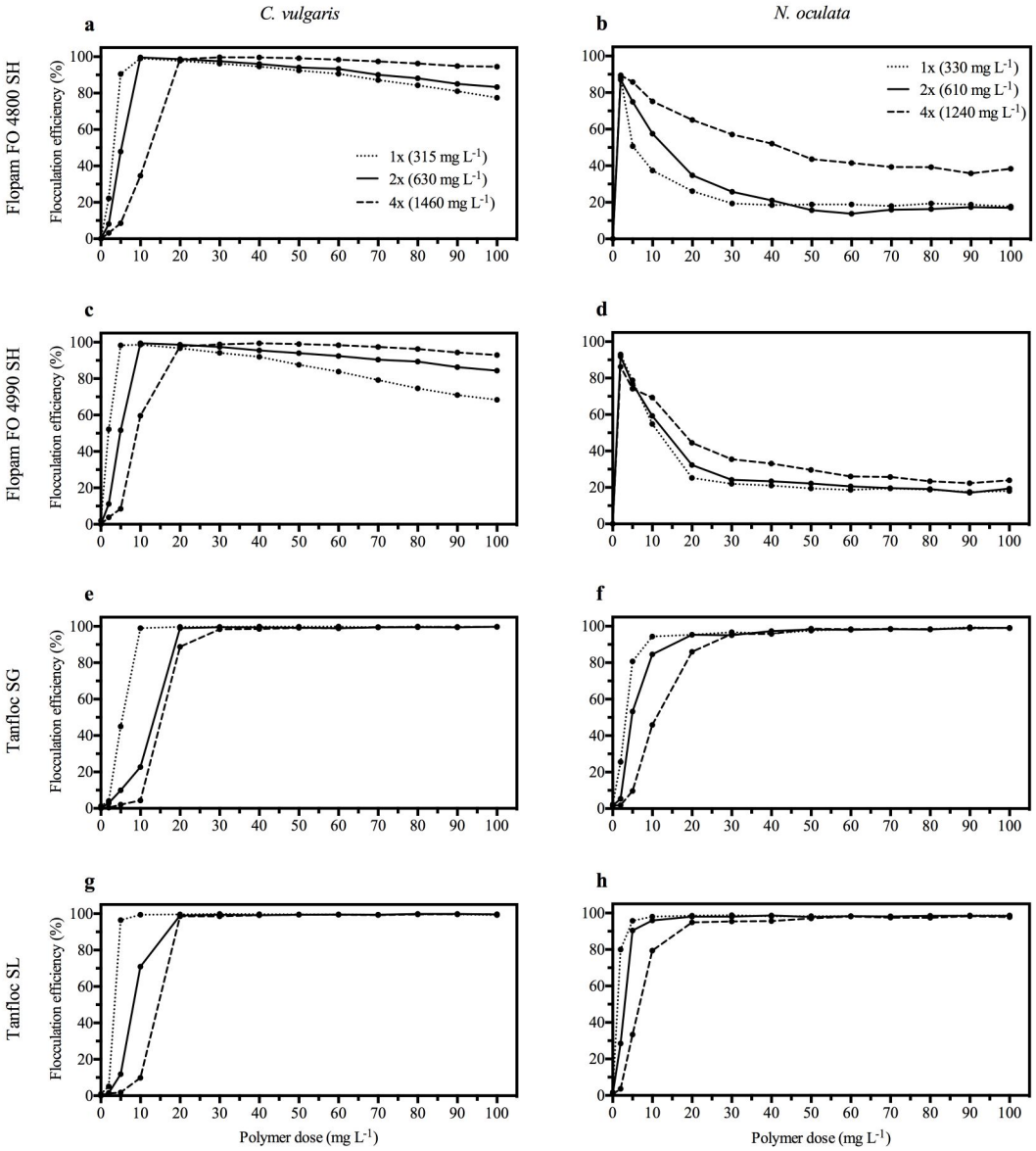
564 **Fig. 5** Effect of Flopam (FO 4800 SH and FO 4990 SH) and Tanfloc (SG and SL)
565 dose on the maximum quantum yields of photosystem II of *C. vulgaris* and *N.*

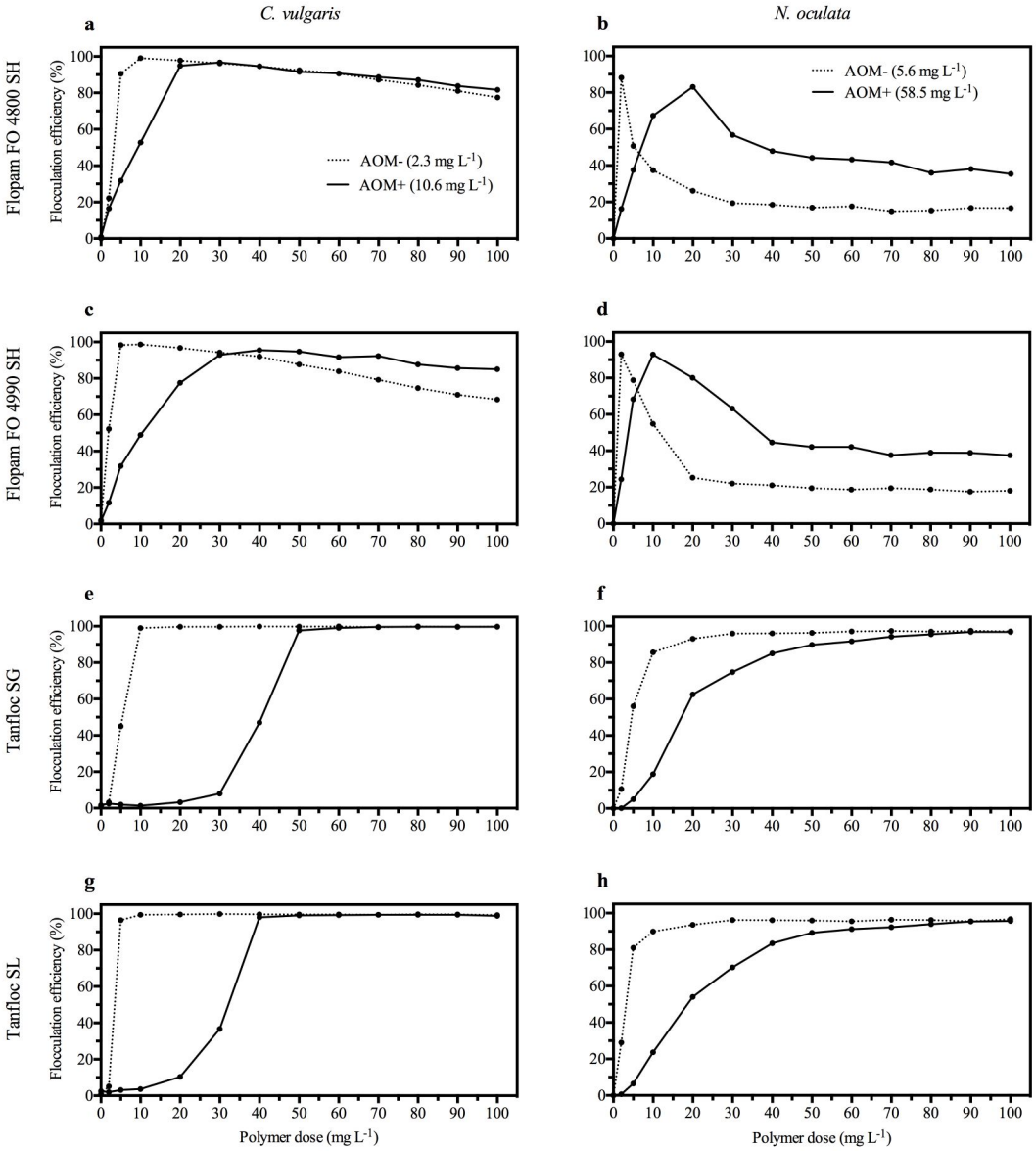
566 *oculata*. (−) Cells treated with 15% H_2O_2 for 30 min, (+) cells without addition of

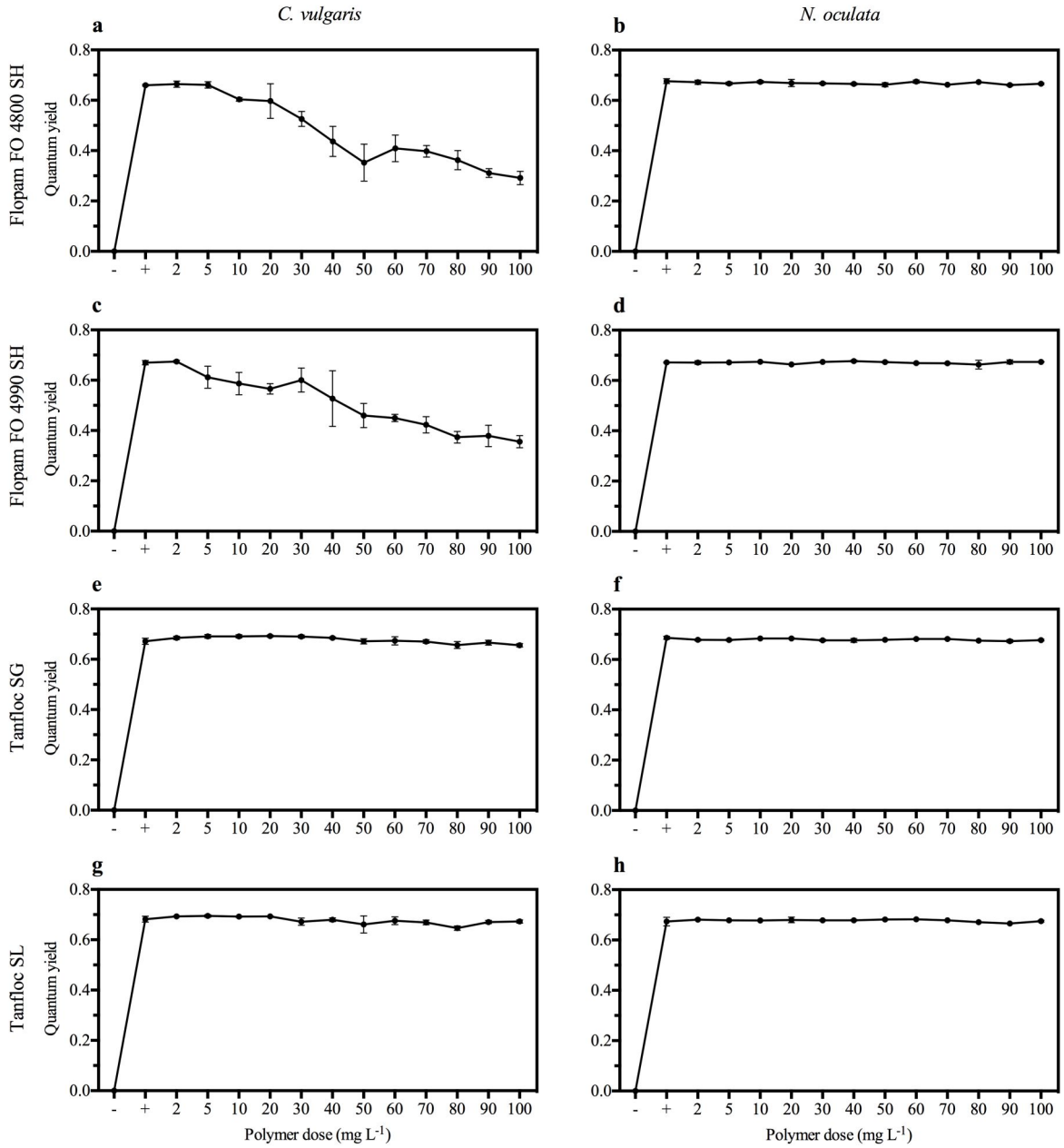
567 polymer


C. vulgaris
N. oculata
a
b
c
d
e
f
g
h
Flopam FO 4800 SH
Flopam FO 4990 SH
Tanfloc SG
Tanfloc SL
Flocculation efficiency (%)
Polymer dose (mg L-1)
pH 5
pH 7
pH 9


C. vulgaris
N. oculata
a
b
c
d
e
f
g
h
Flopam FO 4800 SH
Flopam FO 4990 SH
Tanfloc SG
Tanfloc SL
Flocculation efficiency (%)
Polymer dose (mg L-1)
0 g L-1
15 g L-1
30 g L-1


C. vulgaris
N. oculata
a
b
c
d
e
f
g
h
Flopam FO 4800 SH
Flopam FO 4990 SH
Tanfloc SG
Tanfloc SL
Flocculation efficiency (%)
Polymer dose (mg L-1)
1x (315 mg L-1)
2x (630 mg L-1)
4x (1460 mg L-1)
1x (330 mg L-1)
2x (610 mg L-1)
4x (1240 mg L-1)


C. vulgaris
N. oculata
a
b
c
d
e
f
g
h
Flopam FO 4800 SH
Flopam FO 4990 SH
Tanfloc SG
Tanfloc SL
Flocculation efficiency (%)
AOM- (2.3 mg L-1)
AOM+ (10.6 mg L-1)
AOM- (5.6 mg L-1)
AOM+ (58.5 mg L-1)
Polymer dose (mg L-1)


C. vulgaris
N. oculata
a
b
c
d
e
f
g
h
Flopam FO 4800 SH
Flopam FO 4990 SH
Tanfloc SG
Tanfloc SL
Quantum yield
0.0
0.2
0.4
0.6
0.8
- + 2 5 10 20 30 40 50 60 70 80 90 100
Polymer dose (mg L-1)