Synthesis and evaluation of cationic polyacrylamide and polyacrylate flocculants for harvesting freshwater and marine microalgae

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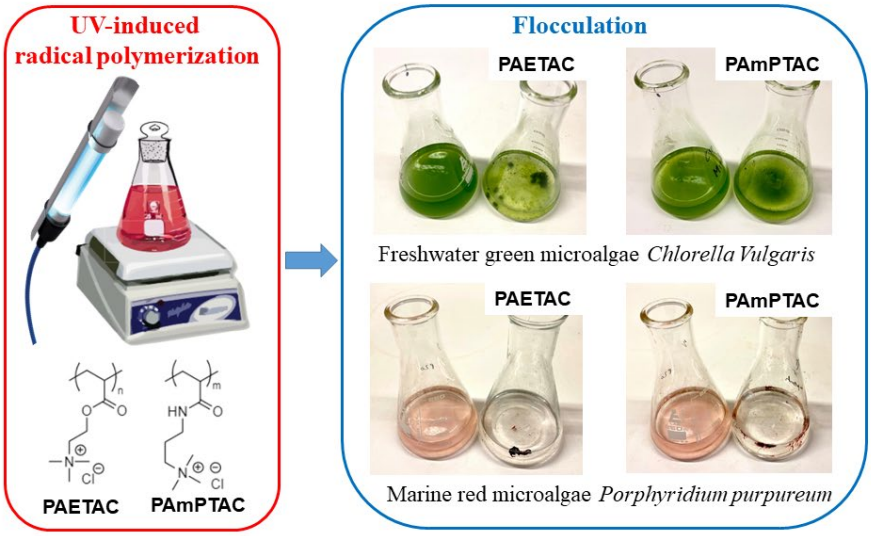
Abstract

This study addresses the challenge of microalgae harvesting through the development of flocculants. Two positively charged cationic polymers including poly[2 (acryloyloxy)ethyl]trimethylammonium chloride (PAETAC) and poly(3 acrylamidopropyl)trimethylammonium chloride (PAmPTAC) were synthesized using the UV-induced radical polymerization, for harvesting both freshwater and marine microalgae. The results show that the synthesized polymers have excellent flocculation performance for both freshwater green microalgae (*Chlorella vulgaris)* and marine red microalgae (*Porphyridium purpureum).* PAETAC outperformed PAmPTAC for both *Chlorella vulgaris* and *Porphyridium purpureum* microalgae. The optimal PAETAC doses for *Chlorella vulgaris* and *Porphyridium purpureum* microalgae were 50 and 4.8 mg/g of dry biomass while the optimal PAmPTAC doses were 252 and 35 mg/g of dry biomass respectively. Additionally, the floc formation with the PAETAC was more stable than PAmPTAC, which supported the dewatering step via sieving. The superior performance can be attributed to the higher molecular weight of the PAETAC polymer when compared to the PAmPTAC polymer. In comparison to commercially available polydiallyldimethylammonium chloride (PolyDADMAC), the newly synthesised PAETAC and PAmPTAC polymers demonstrated superior flocculation efficiency at a lower dose. The findings of this study established a platform technology for designing and synthesising cationic flocculants for use in microalgae harvesting.

Keywords: *Chlorella vulgaris*; *Porphyridium purpureum*; Polymer synthesis; Microalgae harvesting; Polyacrylamide; Polyacrylate.

Graphical abstract



Highlights

- Cationic PAETAC & PAmPTAC were synthesized by UV-induced radical polymerization
- New polymers showed good flocculation performance of freshwater & marine microalgae
- Optimal doses of PAETAC were 50 and 4.8 mg/g for *C. vulgaris* & *P. purpureum*
- PAETAC and PAmPTAC produced stable flocs, supporting simple dewatering step

1. Introduction

Microalgae are photosynthetic microorganisms that grow at a rapid rate, doubling their body cells every 4 to 8 hours at the optimal conditions. They are 400 times more efficient at CO_2 uptake than terrestrial plants [1, 2] and can be utilised to produce high-value biochemicals for a variety of industrial purposes, including food supplements, cosmetics, and biofuel. Microalgal production at an industrial scale could support several Sustainable Development Goals by the United Nations including Zero Hunger, Clean Water and Sanitation, Affordable and Clean Energy, Responsible Consumption and Production, Life Below Water and Life on Land [1]. Microalgal biomass has already had a long history in the human diet [3], animal feed [4], biofuel [5, 6], and biochemical feedstock [7]. Microalgal biomass production involves algal cultivation, harvesting, and downstream extraction of specific product compounds from harvested biomass. Microalgae harvesting is a crucial stage in the cultivation process, as it serves as a link between the cultivation and downstream processes. Microalgal harvesting techniques have long been a focus of algal research and industry [8, 9, 10].

Microalgae harvesting is a significant challenge due to the inherent characteristics of algal cells and their culture conditions. Microalgae are tiny with cell sizes in the range of 4 to 100 μm [11, 12]. They are cultivated in freshwater or seawater to a mature suspension of less than 1 g/L in dry biomass [13]. Liquid-solid separation methods, including centrifugation [14], membrane filtration [15], and flocculation have been utilized in commercial microalgal production facilities. Among these, flocculation has emerged as the most versatile microalgae harvesting method considering key selection criteria such as scalability, biomass quality, operating cost, processing time, and intended biomass applications.

In the flocculation process, flocculant is added to aggregate small microalga cells to form flocs that can subsequently be separated by gravity or flotation via sedimentation or dissolved air flotation, respectively. Inorganic salts (e.g. $AlCl_3$ and $FeCl_3$) [16], synthetic polymers (e.g.

polyacrylamide and polyethyleneimine) [17, 18], and natural polymers (e.g. chitosan) [18, 19] are common flocculants for microalgae harvesting from natural water. Microalgae cells are usually suspended particles with a negative surface charge. Thus, polymers for microalgae harvesting are usually cationic (or positively charged) to neutralise the negative charge of the microalga cells. They also have a high molecular weight for inter-particle bridging and floc formation. Once they have been formed, the microalgae flocs can be separated from the water phase by settling or floatation [17, 20] .

Previous studies have shown superior performance at a low dose of synthetic polymers over traditional inorganic [13, 21] and natural flocculants [22]. Vu et al. [13] observed inorganic flocculant doses of up to 0.5 g/g of dry biomass was required for effective flocculation, resulting in high metal content in the harvested biomass. Natural flocculants (e.g. chitosan, cationic starch, and acacia tannin) have been used at doses of up to 0.3 g/g of dry biomass with culture pH adjustment [23]. Synthetic polymers doses can be below 0.1 g/g of dry biomass [13, 22]. High flocculation efficiency at the low dose of synthetic polymers also allows culture media recycling. Labeeuw et al. [17] demonstrated the feasibility to recycle culture media after harvesting freshwater *Synechocystis sp, Chlorella vulgaris*, and the marine *Phaeodactylum tricornutum* using synthetic polymer without treatment. Culture media recycling could save up to 80% of the water footprint in microalgal biomass production.

Previous studies [24, 25] have also achieved promising results in designing and producing new polymers for microalgae harvesting in order to reduce the cost of microalgal biomass production. Gupta et al. [25] utilised the ring-opening condensation method to synthesize a high molecular weight polyamine and reported that the obtained polymer provide more than 90% flocculation efficiency of *Scenedesmus sp.* at a dose of 8 mg/L. They also reported that the new polymer did not affect lipid recovery from harvested biomass. Recently, Kumar et al. [26] developed a new cationic polymer dextrin-g-PMEATC using conventional

grafting with 97% flocculation efficiency for three freshwater microalgae species. However, these studies utilised traditional polymerization methods such as ring-opening condensation, grafting, and cyclisation which requires high temperature (e.g. 85-90 °C [25, 26]), multi-step reactions [25], high dose of initiator [26] and solvents for precipitation [26].

Recently, free-radical polymerization in aqueous solution has emerged as a facile synthesizing process due to simple operation and ability to precisely control the polymer properties [24]. Ultrasound, plasma, microwave and ultraviolet (UV) light can be used as initiators in the free radical polymerization process [27]. Zhao et al. [27] synthesized a cationic and hydrophobic polymer for oily wastewater treatment via ultrasound-induced free-radical polymerization. Sun et al. [28] also produced a chitosan-based flocculant via the plasma-induced free-radical polymerization. This new polymer provided above 99% and 96% turbidity and chemical oxygen demand removal efficiency, respectively. Photo-induced radical polymerization has been considered a versatile technology for the synthesis of polymers possessing pre-designed composition, functionalities, and structures. This platform technology can tolerate a variety of monomers, solvents, and initiators. Photo-induced radical polymerization has gain its application in different industry such as protective coatings (i.e. paint), adhesives, inks, nanocomposites, and polymer gels [29]. UV light as an initiator source is considered an environmentally friendly and economic process, which occur at low temperature, less initiator, and higher polymerization efficiency [24, 30].

In this study, we utilized recent developments in the photo-polymerization technique to develop two cationic polymer flocculants by polymerizing two types of monomers for harvesting *Chlorella vulgaris* (*C. vulgaris*) and *Porphyridium purpureum* (*P. purpureum*), which are freshwater and marine microalgae species, respectively. The charge-density and molecular weight of the synthesised polymers were measured and assessed against flocculation performance. Flocculation efficiency of the newly synthesized polymers was systematically

evaluated to identify the optimal dose and compared with commercially available flocculants. This study demonstrated the importance of flocculant properties as well as the new capability of tuning the properties of polymer flocculants for specific microalgae species.

2. Materials and Methods

2.1 Materials

Monomers [2-(acryloyloxy)ethyl]trimethylammonium chloride solution (80 wt% in H_2O, Sigma) and (3-acrylamidopropyl)trimethylammonium chloride solution (75 wt% in H_2O, Sigma) were stirred with inhibitor remover prior to use. Photo-initiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (>95%) and deuterium oxide (D_2O at 99.9% purity) were purchased from Sigma Aldrich, Australia.

Poly(diallyldimethylammonium chloride) (PolyDADMAC) of different molecular weights was purchased from Sigma Aldrich (Australia) and used as reference commercial polymers. These polymers are at 20% wt/v in H_2O with an average molecular weight in the range of <100 KDa, 200 – 350 KDa, and 400 – 500 KDa. These polymer solutions were used to prepare standards at 1, 2.5, 5, 7.5 and 10% wt/v to quantify the average viscosity molecular weight of the polymers synthesized in this study. They were also used in the microalgae harvesting experiments for comparison.

Freshwater green microalgae *C. vulgaris* (CS-41) was from the algae culture bank at the University of Technology Sydney. Marine red microalgae *P. purpureum* was from the Australian National Algae Collection at CSIRO Microalgae Research (Hobart, Tasmania, Australia). Both species were cultivated in a 350 L pilot photobioreactor. *C. vulgaris* was cultivated in MLA, while *P. purpureum* was cultivated in f/2 medium with a salinity of 33-35 g/L. Details of the cultivation process can be found in our previous publications [13, 17]. Microalgae cultures in the stationary growth phase were used for the assessment of flocculation

efficiency. The dry biomass concentration, optical density, and pH of the *C. vulgaris* culture were 0.31 g/L, 1.71, and 8.6, respectively. The dry biomass concentration, optical density, and pH of the *P. purpureum* culture were 0.25 g/L, 0.21, and 8.9, respectively.

2.2 Synthesis of cationic flocculants

Poly[2-(acryloyloxy)ethyl]trimethylammonium chloride (PAETAC) and poly(3-acrylamidopropyl)trimethylammonium chloride (PAmPTAC) was synthesized by UV-induced free-radical polymerization in an aqueous solution([M] = 1.2M). The reaction mixture was sealed in a glass vial, degassed by bubbling N_2 at 0.5 L/min and placed above a magnetic stirrer plate. An UV lamp (9W × 4 with λ_{max} = 365 nm and 3.5 mW/cm^2) was used for radical polymerisation (Figure 1). After 30 min of irradiation, polymer gels were obtained.

In this system, the initiators will be decomposed under UV irradiation with low light intensity. The generated radical species first attack the unsaturated C=C bonds of the monomers, making the start of polymerization. With the consumption of the monomers, the polymer chains grow and the viscosity of the mixture increases. Finally, the propagating chains are terminated through recombination and or disproportionation mechanisms.

Monomer conversion was estimated using ^{1}H NMR by comparing the integrals of the peaks corresponding to the polymer backbone to those of unsaturated acrylate double bond.

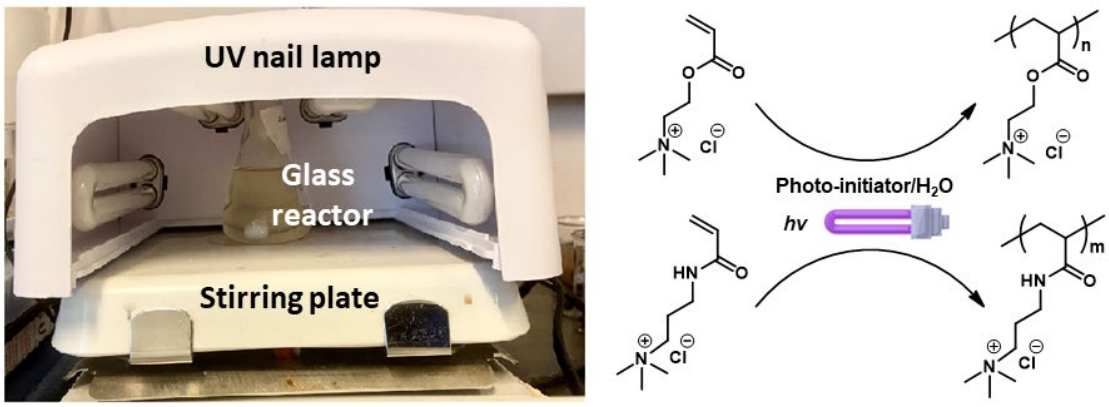


Figure 1: A photograph of UV-induced radical polymerization set-up.

2.3 Flocculation experiments

The obtained PAETAC and PAmPTAC polymer gels were further dissolved in Milli-Q water stirred for 30 min to 0.4 % wt/v, stored at room temperature, and used within 1 day of preparation. PolyDADMAC solutions were diluted with Milli-Q water to get a final concentration of 0.4 % wt/v. The resultant solution was prepared and used within 1 day to avoid any potential hydrolysis.

Flocculation efficiency was accessed using a 4G Platypus Jar Tester (Australia Scientific, Kotara NSW Australia). The microalgal suspension (200 mL) was added to a 600 mL flask. A specified polymer solution volume was introduced to the microalgal suspension mixed at 200 rpm. Then, the mixing rate was reduced to 50 rpm for 15 min, followed by 1 h settling. Optical density was measured before and after the experiment to determine the flocculation efficiency.

The optimal dose of the polymer was determined by a dose-response relationship experiment. Polymer doses (1 – 200 mg/L) were added to the microalgal suspension following flocculation experiments. The polymer doses were subsequently normalized against dry biomass concentration.

2.4 Analytical methods

2.4.1 Characterization of polymers

The charges of all polymers were measured using a Zetasizer nano instrument (Nano ZS Zen 3600, Malvern, UK). Charge density and monomer conversion were measured using nuclear magnetic resonance spectroscopy (^{1}H NMR) (Varian Unity 400 MHz spectrometer). The polymers were dissolved in D_2O at 0.2% w/v.

Polymer rheology was examined using a Brookfield DV2T with a small sample adapter unit (Part SC4-45Y), a removable sample chamber with embedded RTD temperature probe (SC4-13RPY) and a coaxial cylinder spindle SC4-18. The sample chamber has a diameter of

19.05 mm, a depth of 64.77 mm, and an effective volume of 16.1 mL. During each test, polymer samples were mixed thoroughly by handshaking and transferred 16 mL into the sample chamber. Then the sample chamber was hooked on the holder. The polymer temperature was kept at 25 °C. The spindle speed was from 1 to 250 rpm. Plastic viscosity, shear rate, shear stress, and torque were recorded for rheology analysis.

The Bingham Plastic model is used to describe the shear stress and shear rate relation. Polymer sample behaves as Bingham plastic fluid, meaning that fluid will not flow until the shear stress applied exceeds the yield stress of the fluid. The Bingham Plastic model is as follows:

$$\tau = \tau_y + \mu\gamma \qquad \text{Eq. (1)}$$

Where: τ is shear stress (Pa), τ_y is yield stress (Pa), μ is plastic viscosity (Pa) and γ is the shear rate (sec^{-1}).

The intercept on the shear stress axis represents the yield stress and the slope of the line represents the plastic viscosity. Likewise, the yield stress and the plastic viscosity of three PolyDADMAC with known molecular weights were also determined at different polymer concentrations. The obtained plastic viscosity of three PolyDADMAC was plotted against the molecular weight using a logarithmic scale (Figure S1). This correlation was then used to calculate the viscosity average molecular weight of the obtained PAETAC and PAmPTAC polymers.

2.4.2 Flocculation efficiency

After flocculation, an aliquot from half the height of the 200 mL bottle was taken to evaluate the flocculation effect using a UV spectrophotometer at OD 750 nm. The flocculation efficiency was calculated based on the change in the optical density at a wavelength of 750 nm before and after each polymer addition, as shown in equation (2).

$$\text{Flocculation efficiency (\%)} = \left(\frac{OD_i - OD_f}{OD_i}\right) \times 100 \quad \text{Eq. (2)}$$

Where: OD_i and OD_f is the optical density of the culture before and after flocculant addition. All experiments were conducted with three technical replicates using one biological replicate of the microalgae culture.

A light microscopy system was used to estimate floc size after the flocculation experiment. The floc size was then used to determine sieve pore size for microalgal biomass harvesting. Initially, the *C. vulgaris* and *P. purpureum* cells have an average size of less than 30 µm (Figure S2).

The biomass concentration was measured following the standard method. A 100 mL aliquot microalgal culture was filtered through a 0.22 µm pre-weighed glass fibre filter paper. In case of *P. purpureum*, the aliquot culture was centrifuged at 3219 g for 15 min at room temperature. The supernatant was decanted. Then a 100 mL of Milli-Q water was used to mix the biomass in centrifuge tube before filtration through a 0.22 µm pre-weighed glass fibre filter paper. The retained biomass on the filter paper was dried at 60 °C and overnight. The weight of the final filter paper was used to calculate the dry biomass.

3. Results and Discussion

3.1 Polymer characterisation

The PAETAC and PAmPTAC polymers synthesized here are highly positively charged with zeta potential values of 86 ± 9 and 80 ± 8 mV, respectively. In comparison, a 100% charge PolyDADMAC solution has zeta potential value of 11.9 mV [31]. The high-charge (>80% charge) commercially available cationic polyacrylamide (i.e. FO3801) has a zeta potential value of 64 ± 5 mV [32].

^{1}H NMR spectrum (Figure 2) of the raw polymers indicated successful polymerization with our method. The monomers C=C groups showed a peak between 6.2 to 5.6 ppm. This peak disappeared after the polymerization of PAETAC. Consequently, a new peak of –CH_2-CH(C=O)– group was observed between 2.2 to 1.2 ppm, indicating the successful preparation of polycations with high monomer conversion. Likewise, successful polymerization of PAmPTAC was also confirmed via ^{1}H NMR spectrum with the appearance of a peak belonging to –CH_2-CH(C=O)– group. Based on these analyses, the charge density of the PAETAC and PAmPTAC are above 95%.

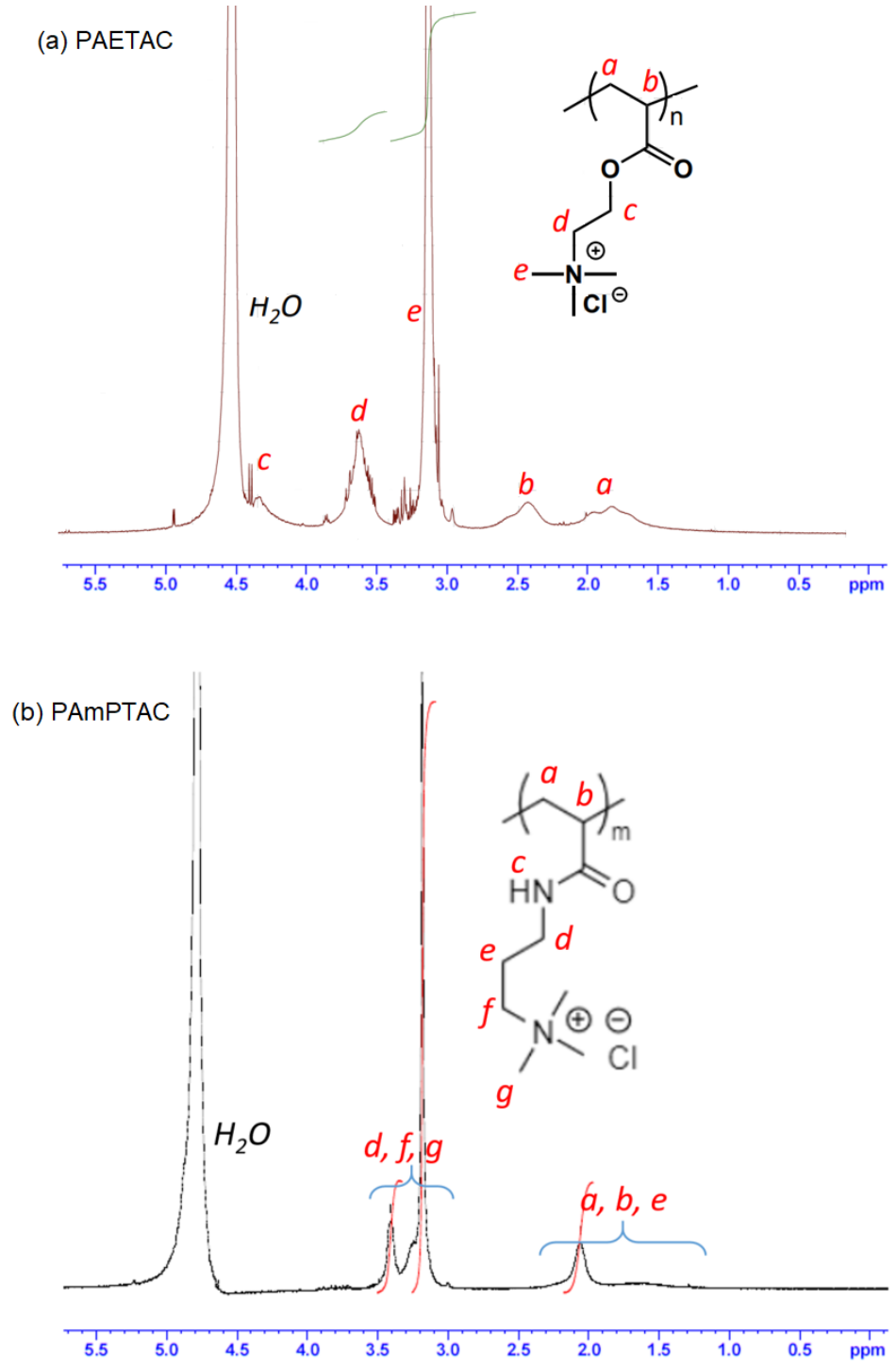


Figure 2: ^{1}H NMR spectra of polymer (a) PAETAC and (b) PAmPTAC.

Viscosity measurement was used to calculate the polymer average molecular weight. Viscosity average molecular weight of PAETAC was in the range of 340 to 560 KDa and PAmPTAC was 180 to 190 KDa. These ranges can be classified as medium and high molecular

weight, respectively. Polymer charge, charge density, and molecular weights are important properties for flocculation of negative charge microalgal cells and thus increase harvesting efficiency (Section 3.2).

3.2 Flocculation efficiency with PAETAC and PAmPTAC

3.2.1 Flocculation efficiency with freshwater microalgae

The flocculation efficiency and optimal dose of the PAETAC and the PAmPTAC for flocculation of *C. vulgaris* were obtained from the dose-response relationship experiments. The flocculation efficiency increased from 40 to 84% with PAETAC dosing of 30 and 50 mg/g of dry biomass, respectively (Figure 3). No significant change in the flocculation efficiency (i.e. 84 – 86%) at higher PAETAC doses (i.e. 50 to 102 mg/g of dry biomass) was observed. The flocculation efficiency decreased slightly at PAETAC dose of 125 mg/g of dry biomass. The obtained results indicated that the optimal dose of PAETAC for *C. vulgaris* was 50 mg/g dry biomass and over-dosing polymer can be counterproductive.

A similar trend was observed with the PAmPTAC polymer (Figure 3). However, the PAmPTAC was less effective when compared to the PAETAC. The PAmPTAC polymer had an optimal dose at 252 mg/g dry biomass. The difference in flocculation efficiency between two polymers is likely due to the differences in molecular weight. In the flocculation process by cationic polymers, charge neutralization of microalgal cells initiates floc formation. High molecular weight polymer (i.e. very long chain of monomers) bridges destabilised microalgae flocs [17, 22, 32]. The PAETAC has viscosity average molecular weight in the range of 340 to 560 KDa compared to 180 to 190 KDa of the PAmPTAC. Commercially available polymers with higher molecular weight have shown better flocculation efficiency for both freshwater and marine microalgae [22, 32]. The outperformance of the PAETAC over the PAmPTAC was also observed from the floc size. The PAETAC formed larger and denser flocs compared to

that of the PAmPTAC (Figure S3). The formation of large and dense flocs can be clarified by direct filtration via a 100-µm pore-size stainless steel sieve.

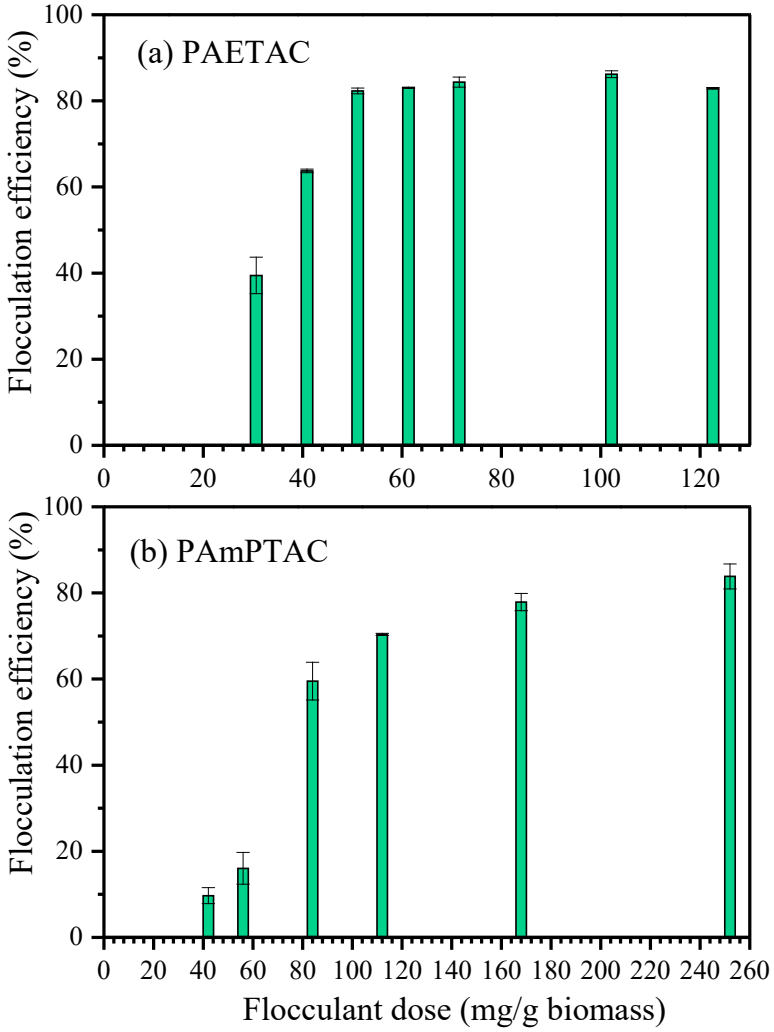


Figure 3: The effect of (a) the PAETAC and (b) the PAmPTAC doses on flocculation efficiency of freshwater green microalgae *C. vulgaris* indicating by the change in optical density of microalgae suspension before and after flocculation experiment. Value and error bars are the mean and standard deviation of 3 replicate experiments.

3.2.2 Flocculation efficiency with marine microalgae

The PAETAC and PAmPTAC achieved high flocculation efficiency (i.e. > 85%) with marine microalgae *P. purpureum* (Figure 4). The optimal doses of the PAETAC and the

PAmPTAC were 4.8 and 35 mg/g dry biomass, respectively. High flocculation efficiency at a low polymer dose would indicate the feasibility of this method for harvesting *P. purpureum*. *P. purpureum* is a red marine microalgae species with valuable biochemicals such as phycoerythin, phycobiliproteins, polyunsaturated fatty acids, and exopolysaccharides. The phycoerythin is a water-soluble bioactive compound with anti-inflammatory and antioxidant properties [33, 34]. Both polymers induced a dense floc formation with *P. purpureum*, which is stable even under vigorous mixing conditions (Figure S3). The observed flocs stability is a major advantage of cationic polymer flocculants. Inorganic flocculants formed a foamy and powdery layer of flocculated biomass on the bottom of the flask in the experiment [13]. The flocculated biomass can be disintegrated during the biomass separation step (e.g. dewatering), resulting in a decrease in the overall harvesting efficiency [21]. On the other hand, it was observed during the experiment that the flocculation of 100 mL microalgal suspension formed dense flocs. The flocs size ranged from 5 to 10 mm. The stable flocs by our polymers were due to both charge neutralisation and subsequent bridging during the flocculation process. Whereas, inorganic flocculants result in a foamy and powdery layer of biomass on the container due to the sweeping mechanism [13]. These flocs easily separate from the supernatant via a 100-µm stainless steel sieve at 100% harvesting efficiency. The high flocculation efficiency of *P. purpureum* by the new polymers would provide a new way to harvest this high-value biomass at a commercial scale. Polymer flocculation and sieve are likely effective methods for *P. purpureum* biomass harvesting. High flocculation efficiency would also support the feasibility of residual culture recycling [13, 17] to reduce water footprint of microalgal biomass production. Future studies will explore the toxicity of new polymers on biomass quality and ability to recycle culture media.

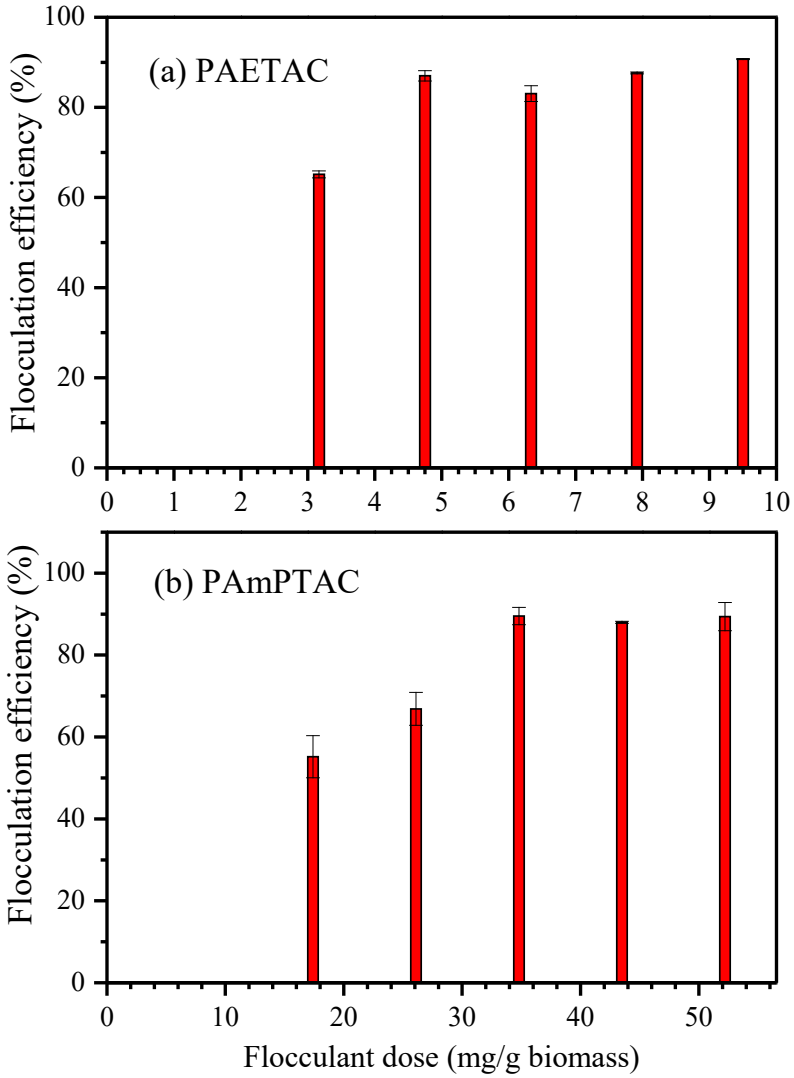


Figure 4: The effect of (a) the PAETAC and (b) the PAmPTAC doses on flocculation efficiency of marine red microalgae *P. purpureum* indicating by the change in optical density of microalgae suspension before and after flocculation experiment. Value and error bars are the mean and standard deviation of 3 replicate experiments.

Results from this study suggest that the flocculation efficiency by cationic polymers is both water matrix and species dependent. Marine red microalgae *P. purpureum* flocculated better with both the PAETAC and the PAmPTAC compared to freshwater green microalgae *C. vulgaris*. Several factors (i.e. cell membrane morphology, integrity, culture media, growth phase, and algal organic matters) could induce different flocculation performances amongst

microalgal species [9, 35, 36, 37]. Algae organic matter appears to be the dominant factor. For example, Gonzalez-Torres et al. [36] observed different floc properties of the same species and similar morphology with variable algal organic matters properties. Recently, Vu et al. [9] differentiated the role of residual phosphorus and microalgal extracellular polymeric substances concentrations (i.e. main composition of algal organic matters) in the culture media at different microalgae growth phase on the flocculation by cationic polymers. Their finding highlighted negatively charged and high extracellular polymeric substance concentration interact with positively charged polymer and enhance the flocculation efficiency via charge neutralization and bridging. In this study, both species were at the mid-stationary growth phase, which is usually the harvesting point in the most batch microalgal culture.

Seawater medium also has high ionic strength due to the presence of high concentration of cations and anions, which could interact with the cationic polymer and impact the flocculation efficiency. However, the extent of impact is different amongst microalgae species. For example, no different observation in flocculation efficiency and polymer dose between the initial and a diluted culture of *P. purpureum* (i.e. a ratio of 1:1 v/v with Milli-Q water) in this study. On the other hand, cationic polymers are less effective to flocculate marine specie *Isochrysis galbana* (i.e. flocculation efficiency <20%). The flocculation efficiency increased to above 90% with a 1:1 v/v diluted culture.

3.3 Role of molecular weight

The newly synthesized PAETAC showed better flocculation efficiencies for both freshwater microalgae *C. vulgaris* and marine microalgae *P. purpureum* at lower doses compared to the three commercially available PolyDADMAC (Figure 5). For example, the optimal dose of PAETAC was 50 mg/g of dry biomass of *C. vulgaris*, which is significant lower than that of the PolyDADMAC with a molecular weight of 400 – 500 KDa (i.e. 202 mg/g of dry biomass). Likewise, the optimal dose of PAETAC for *P. purpureum* was 4.8 mg/g of

dry biomass compared to the optimal dose of high molecular weight PolyDADMAC (i.e. 24 mg/g of dry biomass). The PAmPTAC with a molecular weight of 180 – 190 KDa showed comparable flocculation efficiencies for marine microalgae *P. purpureum* with PolyDADMAC of 200 – 350 and 400 – 500 KDa molecular weight (Figure 5).

Figure 5 shows the relationship between polymer molecular weight and flocculation efficiency. Low molecular weight PolyDADMAC (i.e. < 100 KDa) provided flocculation efficiency below 20% for freshwater microalgae *C. vulgaris* at 224 mg/g dry biomass and below 80% for marine microalgae *P. purpureum* at 140 mg/g dry biomass. High flocculation efficiency of both freshwater microalgae *C. vulgaris* and marine microalgae *P. purpureum* was observed with medium and high molecular weight PolyDADMAC (Figure 5). The observed relationship between molecular weight and flocculation efficiency using PolyDADMAC is consistent with the obtained data using the newly synthesised PAETAC and PAmPTAC. Low molecular weight polymers induce floc formation mainly by surface charge neutralization with less contribution from bridging [38]. The three PolyDADMAC have a similar charge with zeta potential values of 12.3, 11.9 and 12.9 mV for very low, medium, and high molecular weights. This study, therefore, suggests that new polymer synthesis could aim to achieve a molecular weight of highly charge polymer at above 350 KDa to ensure high flocculation efficiency of microalgae.

Results in Figure 5 also confirmed the dependency of flocculation efficiency on microalgal species. Similar to the PAETAC and the PAmPTAC flocculants, PolyDADMAC provided better flocculation efficiency for *P. purpureum*. This is an interesting observation since *P. purpureum* was cultivated in seawater with high ionic strength. In a high ionic strength solution, polymeric coiling could occur to deteriorate polymer properties and its performance [37]. König et al., [37] observed the negative impact of culture salinity on the flocculation of *Chlorella weissflogii*. The ionic strength also affected natural-based polymers (e.g. chitosan

and cationic starch) [39, 40]. However, Nguyen et al. [32] observed high flocculation efficiency of marine microalgae *Phaeodactylum tricornutum* with a cationic polyacrylamide flocculant (FO3801). The discrepancy in the literature suggested that a future flocculation study using one type of polymer with multiple marine species.

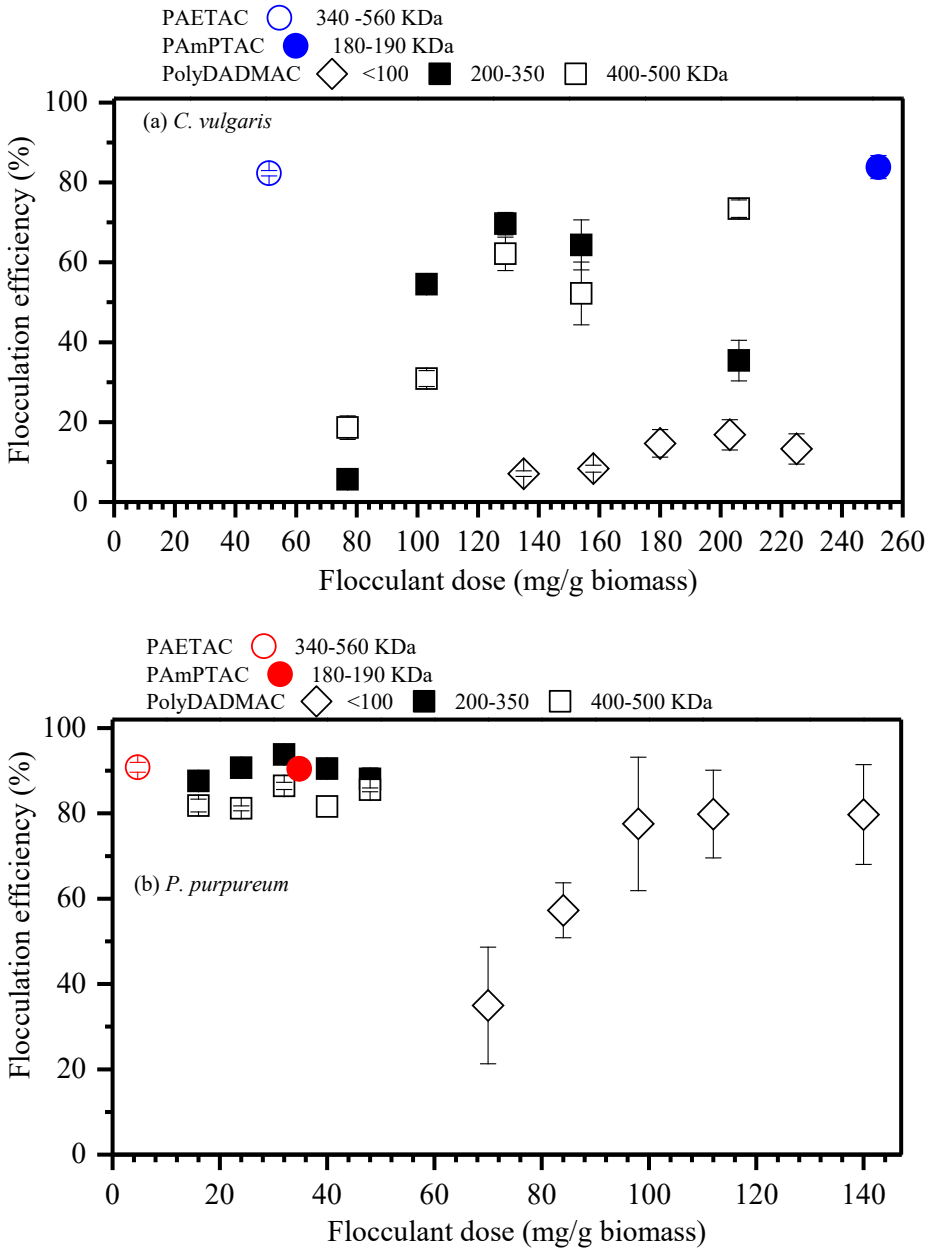


Figure 5: Comparison of flocculation efficiency of the PAETAC and the PAmPTAC with three different molecular weight PolyDADMAC (a) *C. vulgaris* and (b) *P. purpureum*. Optimal

doses of the PAETAC and the PAmPTAC were showed for comparison. Value and error bars are the mean and standard deviation of 3 replicate experiments.

4. Conclusions

This study demonstrated facile synthesis of two cationic flocculants via UV-induced polymerization of monomers [2-(acryloyloxy)ethyl]trimethylammonium chloride and (3-acrylamidopropyl)trimethylammonium chloride for harvesting freshwater green microalgae (*C. vulgaris)* and marine red microalgae (*P. purpureum*). The synthesized flocculants are cationic and highly charged with a viscosity average molecular weight of 340 – 560 KDa and 180 – 190 KDa. The high molecular weight of PAETAC provided better flocculation efficiency for both microalgal species. A dose-response relationship showed the optimal dose of the PAETAC is 50 and 4.8 mg/g of dry algal biomass for *C. vulgaris* and *P. purpureum*, respectively. Whereas, the optimal doses of PAmPTAC were 252 and 35 mg/g of dry algal biomass for *C. vulgaris* and *P. purpureum*, respectively. The newly synthesized flocculants also outperformed the commercially available PolyDADMAC in terms of flocculation efficiency and optimal doses. Finally it can be highlighted that the performance of newly synthesized flocculants is influenced by their molecular weight as well as the type of microalgae species for harvesting.

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Supplementary data

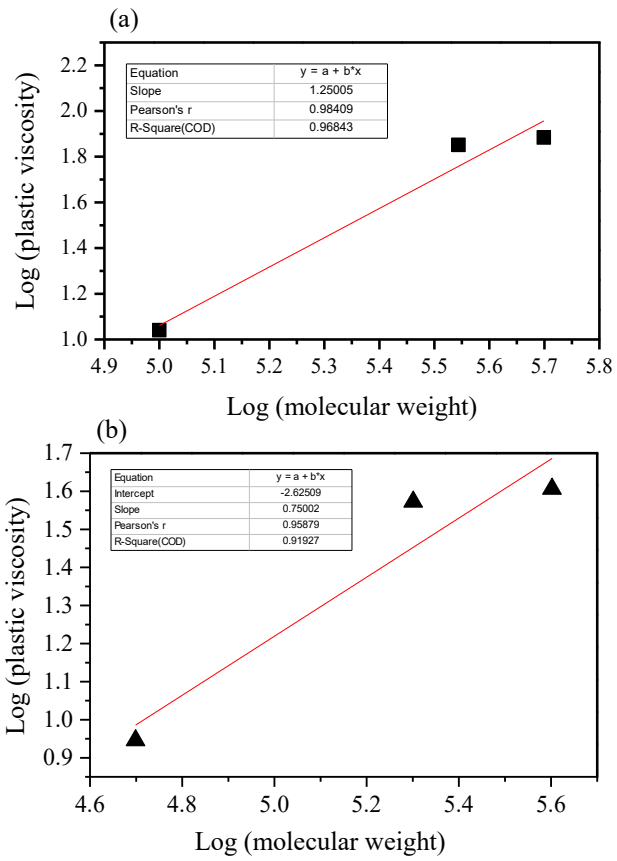
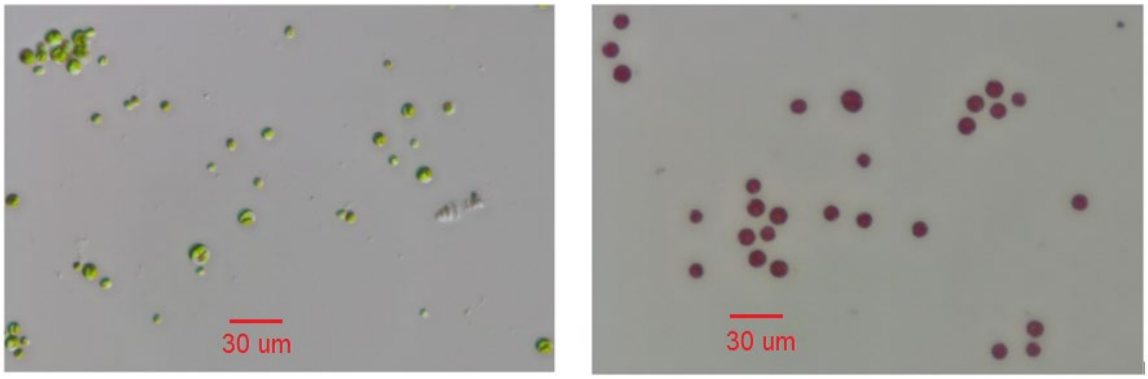


Figure S1: Calibration curves to calculate the viscosity average molecular weight of PAETAC and PAmPTAC. The plots are linear functions of the plastic viscosity and molecular weight of PolyDADMAC. Molecular weights of PolyDADMAC was 100, 350 and 500 KDa in Figure a and 50, 200 and 400 KDa in Figure b.



C. vulgaris at 40X

P. purpureum at 40X

Figure S2: Light microscopy images of *C. vulgaris* and *P. purpureum* at 40x in the flocculation experiments.

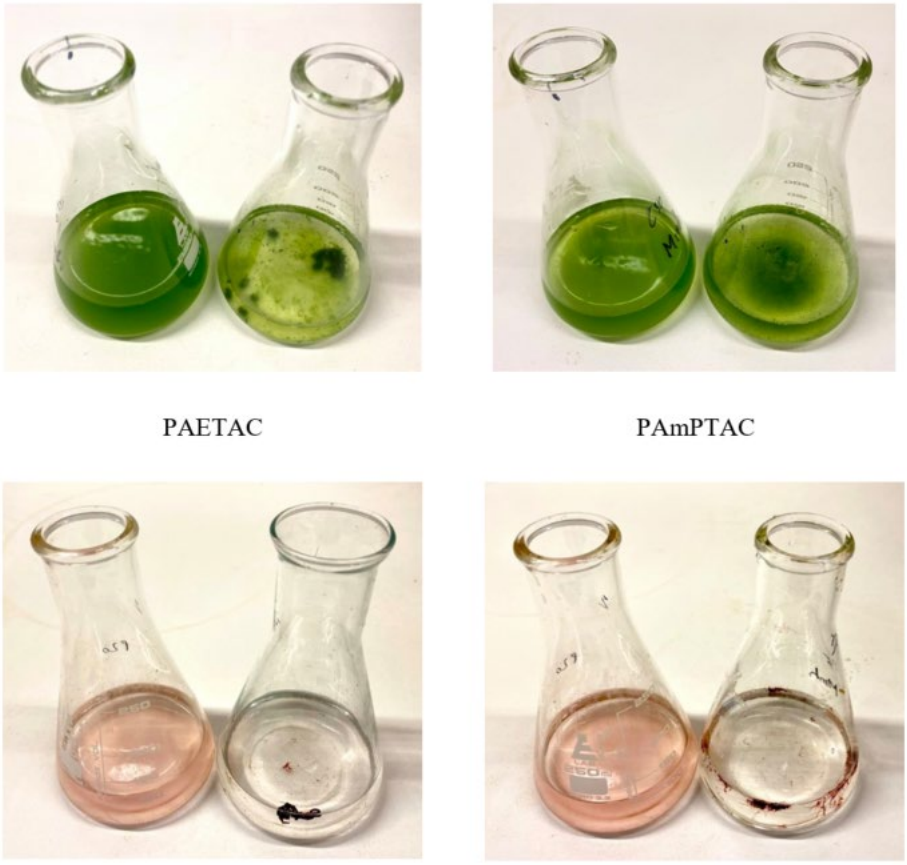


Figure S3: Photographs of freshwater green algae *C. Vulgaris* and marine red algae *P. purpureum* before and after flocculation experiments with PAETAC and PAmPTAC.

Table S1: Plastic viscosity (m.Pas) of the PAETAC and the PAmPTAC in comparison to PolyDADMAC.

Concentration (%wt/v)	**Plastic viscosity (m.Pas)**		
	PAETAC	**PAmPTAC**	**PolyDADMAC**
5	58.2	27.4	36.4
7.5	90.5	30.6	77.5
10	112.6	36.3	146.3