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Effective harvesting of microalgae: Comparison of different polymeric flocculants

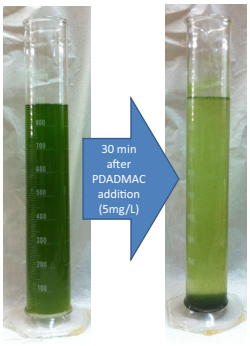
Yoram Gerchman [a,*,1], Barak Vasker [b,1], Mordechai Tavasi [a,1], Yael Mishael [c], Yael Kinel-Tahan [b], Yaron Yehoshua [b]

[a] Department of Biology and Environment, Faculty of Natural Sciences, University of Haifa-Oranim, Tivon 3600600, Israel
[b] The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 5290002, Israel
[c] Department of Soil and Water Sciences, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 7610001, Israel

HIGHLIGHTS

- Microalgae are promising feed stock for biofuels but harvesting is a major hurdle.
- PDADMAC, used for water treatment, can be used for sedimentation of such algae.
- This polymer was effective at 5 mg/L, with fast kinetics and stable sedimentation over wide pH range.
- Achieving zeroing of Zeta potential is not needed for effective sedimentation.
- This flocculant could be used for effective low-cost microalgae flocculation.

GRAPHICAL ABSTRACT



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ABSTRACT

Microalgae harvesting is a major hurdle in the use of microalgae for oil production. Here we describe the use of a standard cationic polymer used for water treatment, Polydiallyldimethylammonium chloride (PDADMAC), for sedimentation of *Chlorella vulgaris* and comparison of its flocculation properties with two other polymers, chitosan and Superfloc®. We found PDADMAC to be the most effective flocculant with 90% of the algae flocculating at concentrations as low as 5 mg/L within 60 min, and good activity even at pH = 10. Interestingly, with both PDADMAC and chitosan maximum flocculation was achieved much before zeroing of zeta potential. PDADMAC flocculation was also very effective in enhancing harvest by filtration and somewhat at flocculation and sedimentation of marine algae, *Nannochloropsis salina*.

1. Introduction

In recent years, much interest has risen in the utilization of oil-accumulating microalgae as feedstock for biodiesel production, mainly due to their ability to accumulate high levels of lipids (Hu et al., 2008; Liu et al., 2012; Dixon, 2013). To date, microalgal

* Corresponding author.
E-mail address: gerchman.yoram@gmail.com (Y. Gerchman).
[1] These authors contributed equally to this manuscript.

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feedstock is not economically feasible, among other reasons, due to the high cost (in infrastructure and energy consumption) of harvesting (Uduman et al., 2010) and low organic content, usually around 1% w/v for autotrophic growth and up to 10% w/v for heterotrophic growth (Wu and Shi, 2007; Gouveia and Oliveira, 2008). Harvesting and dewatering are necessary prior to biomass use. Indeed, the cost of energy needed for microalgae harvesting was estimated to be ~30% of the total biomass production cost (Gudin and Therpenier, 1986; Grima et al., 2003).

Several methods have been developed for harvesting microalgae: centrifugation, foam fractionation, filtration, flocculation, and others [reviewed in Chen et al. (2011) and Milledge and Heaven (2013)]. Currently, flocculation is considered to be the most cost-effective and convenient process since it allows the rapid treatment of large volumes of microalgal cultures (Vandamme et al., 2013). Polymers have been demonstrated to be an effective means of algal flocculation. Chitosan (Divakaran and Pillai, 2002) and cationic starch (Vandamme et al., 2010) were suggested, as well as synthetic polymers (Granados et al., 2012), but the search for better flocculants continues. Here, we compare three cationic polymers: chitosan, made from shrimp shells treated with alkali, Polydiallyldimethylammonium chloride (PDADMAC), a synthetic polymer commonly used for pretreating drinking and wastewater, and Superfloc® 496, for their ability to harvest the freshwater microalga *Chlorella vulgaris* under different pH flocculant dosage. Our results suggest that low concentrations of PDADMAC could result in effective flocculation under a wide range of pHs, and that zeta potential neutralization is a poor predictor of flocculant efficiency.

2. Materials and methods

2.1. Algae growth

The microalga *Chlorella vulgaris* (*C. vulgaris*) was axenically grown in a Bristol medium and kept under constant illumination at 24 °C in a 1 L Erlenmeyer flask with air bubbling. Analytical-quality chemicals (Sigma-Aldrich, Israel) were used for Bristol-medium preparation. *Nannochloropsis salina* was grown in F/2 media based on NeoMarine (Brightwall Aquatics) artificial sea water preparation and kept at 22 °C in a 1 L Erlenmeyer flask with air bubbling.

2.2. Materials

PDADMAC was purchased as 20% w/v solution in H_2O from Sigma–Aldrich (Cat# 409014) and diluted in deionized (DI) water to 1 g/L stock solution. Superfloc® 496 was purchased from Kemira and dissolved in DI water to 20% w/v stock solution before use. Chitosan (Sigma; medium viscosity; MW 400,000; 80% deacetylation) stock solution was prepared by dissolving 5 g/L of flakes in 1% aqueous acetic acid, final pH = 3.5.

2.3. Testing flocculant concertation and flocculant/algae ratio

Flocculant-concentration and pH-effect experiments were conducted on 5 ml culture samples from 4-day-old batch cultures, with algal concentrations of ~1.5 g/L wet weight (0.3 g/L dry weight). Algal samples were placed into 10 ml glass tubes, and polymers were added to the designated final concentration from the stock solution (volume change was negligible). Tubes were capped momentarily, mixed by inversion, and left at room temperature for 1 h for settling. Cell settling was evaluated by measuring the optical density of the upper liquid at 680 nm (Genesis 20, Thermo Scientific, USA). The percentage of flocculation was calculated as OD_{680} of upper liquid divided by the original suspension OD and multiplied by 100.

2.4. Testing the effect of pH

To test for the influence of pH, phosphate buffer from a 0.5 M stock solution at the desired pH was added to the algal culture to reach a final concentration of 25 mM. For pH = 4, a few drops of 0.1 N HCl were added, while for pH 10, a few drops of 0.1 N NaOH were added until the desired pH was achieved. Flocculants were prepared at 1 g/L stock solution and added to the buffered algal suspension to a final concentration of 10 mg/L (1% added volume). The suspensions was mixed immediately with a glass rod, allowed to set for one hour, and measured as above.

2.5. Settling kinetics

Settling kinetics was established using 'kinetics' mode in a Shimadzu UV-1650PC spectrophotometer. Four ml of algal suspension was placed in a 4.5 ml cuvette in which the bottom one cm was covered with black masking tape. Flocculants were added at time zero, and the cuvette rapidly mixed and placed back in the spectrophotometer, with the whole process taking roughly one minute. Optical density at 680 nm was measured every minute. The experiments were done in triplicate.

2.6. Zeta potential measurements

One ml of algal suspension at a concentration equal to OD680 ~1.39 was mixed with polymer (PDADMAC or chitosan) to give the designated concentration. The sample was rapidly mixed and the zeta potential measured immediately using a Zetasizer Nano-ZS instrument (Malvern Instruments, Westborough, MA).

2.7. Algae growth in reused water (RW)

The *C. vulgaris* culture (OD_{680nm} = 1.19; 250 ml volume) was treated with 5 mg/L PDADMAC and allowed to settle for 120 min. The upper 200 ml was collected and transferred to a new vessel. Clay was added, and the culture was mixed and allowed to settle. Then, the upper 150 ml was transferred to a new vessel, and 16 ml of 10× concentrated Bristol medium and 16 ml *C. vulgaris* inoculum added. Growth was measured by following absorbance at 680 nm. Algal culture vessels were placed on an MRC TOS-4030FD shaker, 150 rpm, under constant LED light 85 μmol q m^{-2} s^{-1} at a temperature of 24 ± 1 °C.

3. Results and discussion

3.1. Effect of pH, polymer concertation, and polymer/algae ratio

While very little sedimentation was apparent without polymer addition (Fig. 1A, 0 mg/L; Fig. 3; Supplementary information) all three polymers tested demonstrated the ability to flocculate and sediment *C. vulgaris* (Fig. 1). Out of the three, PDADMAC and Superfloc® showed better performance, reaching a maximum at 5 mg/L vs. 10 mg/L for chitosan, and keeping performance up to 50 mg/L (Fig. 1A) in a wide pH range (4–10; Fig. 1B). Chitosan, on the other hand, showed reduced sedimentation performance at the higher concentrations (Fig. 1A, C), and lost its flocculation ability at pH > 8, in line with the loss of charge at this pH (Fig. 1B; Table 1). The effects of algal culture concentration and PDADMAC/algal cell ratio on sedimentation efficiency were studied using two approaches: (1) varying algal cell concentration while keeping polymer concentration constant (Fig. 1C, empty symbols); and (2)

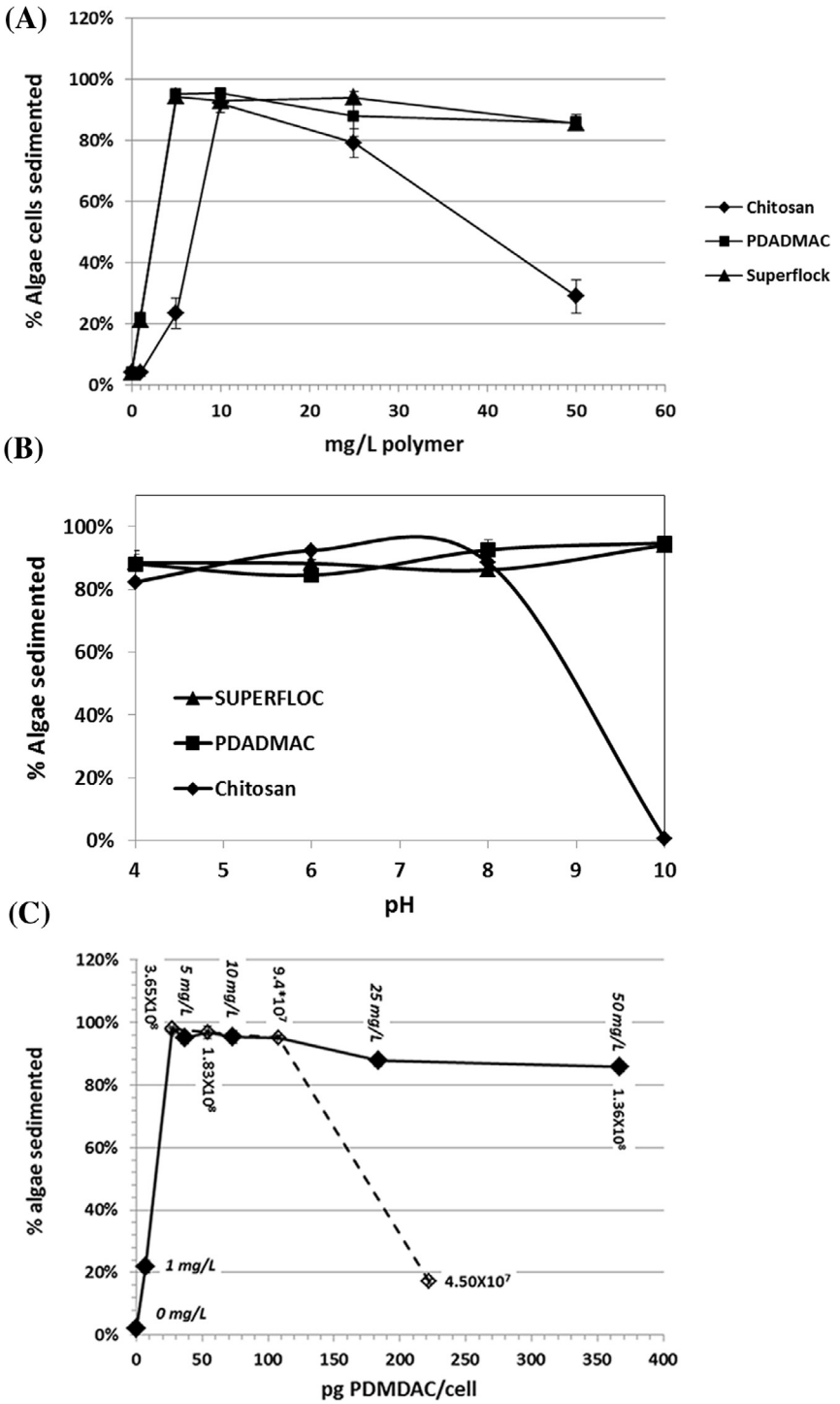

Fig. 1. (A) *C. vulgaris* sedimentation along range of polymer concentration (all three polymers). All experiments were done at pH = 6.5, and sedimentation was allowed to take place for one hour. Starting cell concentration was $1.36 \times 10^8 \pm 1.79 \times 10^6$ cells/ml. (B) Effect of pH on sedimentation of *C. vulgaris* by the three flocculants (n = 3; error bars are one standard deviation; all polymers at a final concentration of 10 mg/L; initial algal concentration $2.3 \times 10^8 \pm 2.1 \times 10^6$ cells/ml; sedimentation was allowed to take place for one hour). (C) Effect of algal concentration and cell/PDADMAC ratio on sedimentation of *C. vulgaris*. Data presented as% algal sedimentation vs. cell/PDADMAC ratio (all data point average of triplicates ± standard deviation). Closed symbols indicate a constant algal concentration (1.36×10^8 cells/ml) and varying polymer concentration (0–50 mg/L, as indicated in italics); open symbols indicate a constant polymer concentration (10 mg/L) with varying algal cell concentration (4.5×10^7 to 3.65×10^8 cells/ml, as indicated).

keeping algal concentration constant while varying polymer concentration (Fig. 1C, closed symbols). The results suggest a complex connection, where at high algae concentration (1.36×10^8), all ratios of 27 pg/cell and above gave sedimentation above 95%, but when this ratio was higher than 10^8 pg/cell, mixed behavior was observed. When algal concentration was high (1.36×10^8), no ill effect was seen, even at ratios as high as 367 pg/cell, but when it was low (4.50×10^7 cells/ml), sedimentation efficiency dropped, even at a lower ratio of 222 pg/cell (Fig. 1C). These results fit well with the supposed flocculation mechanism of PDADMAC, i.e., not

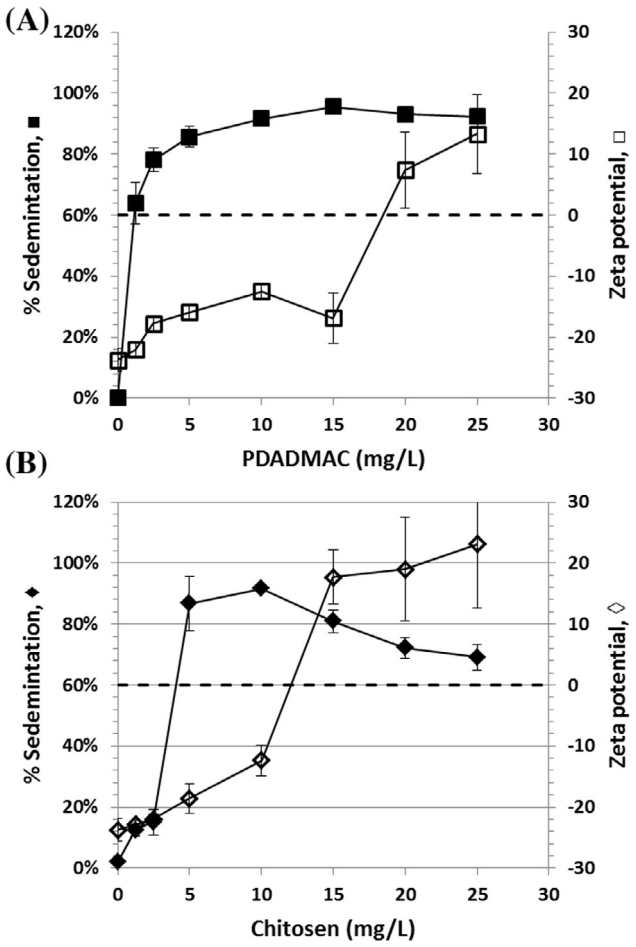

Fig. 2. Zeta potential and sedimentation of *C. vulgaris* cell suspension as a function of organic cation concentration. A. PDADMAC; B. Chitosan. Sedimentation took place at Zeta potential ~(−20) mV (n = 3 for all experiments; all data point average ± standard deviation; initial algal concentration $1.36 \times 10^8 \pm 1.79 \times 10^6$ -cells/ml for all experiments).

enough bridging events can occur to produce flocculation at a low polymer concentration or a low algal concentration.

3.2. Zeta potential and sedimentation

As sedimentation is often correlated to the neutralization of zeta potential, we measured the zeta potential of the polymers (Table 1) and the algal suspension in the presence of different concentrations of PDADMAC and chitosan. The results are presented in Fig. 2. The algae, without any polymer, showed a zeta potential of −25 mV, supporting the claim that electrostatic stabilization is part of the mechanism preventing agglomeration (Vandamme et al., 2015). Interestingly, for both PDADMAC and chitosan, maximum sedimentation was achieved at polymer concentrations much lower than those needed to establish full neutralization of the zeta potential (Fig. 2), with sedimentation happening as soon as ~−20 mV was achieved (i.e., ~1 mg/mL PDADMAC or ~3 mg/mL chitosan). The further addition of polymers (i.e., ⩾10 mg/L chitosan or ⩾20 mg/L PDADMAC) pushed the system beyond the isoelectric point making the polymer–alga complex positively charged and electrostatically restabilize the colloidal dispersion, leading to a reduction in sedimentation efficiency (Fig. 2B). Curiously, the effect is much less profound for PDADMAC, suggesting that chitosan holds much more positive charges than PDADMAC per weight. The different sedimentation behavior of chitosan and PDADMAC at the onset of the isoelectric point suggests that the mechanism of flocculation is different in these two polymers.

3.3. Kinetics of sedimentation

The kinetics of sedimentation with PDADMAC was studied (Fig. 3). Rapid sedimentation occurred in the presence of the polymer, with 80% sedimentation achieved after only ~20 min and almost no sedimentation observed without polymer addition (Fig. 3), and similar sedimentation behavior observed for large volume of algae culture (1 L; see Supplementary information). When cells were collected by course filtration (Whatman 4 paper; pore size ~22 μm) with or without pre-mixing with 10 mg/L PDADMAC, a clear difference was visible, with 9-fold more biomass trapped on

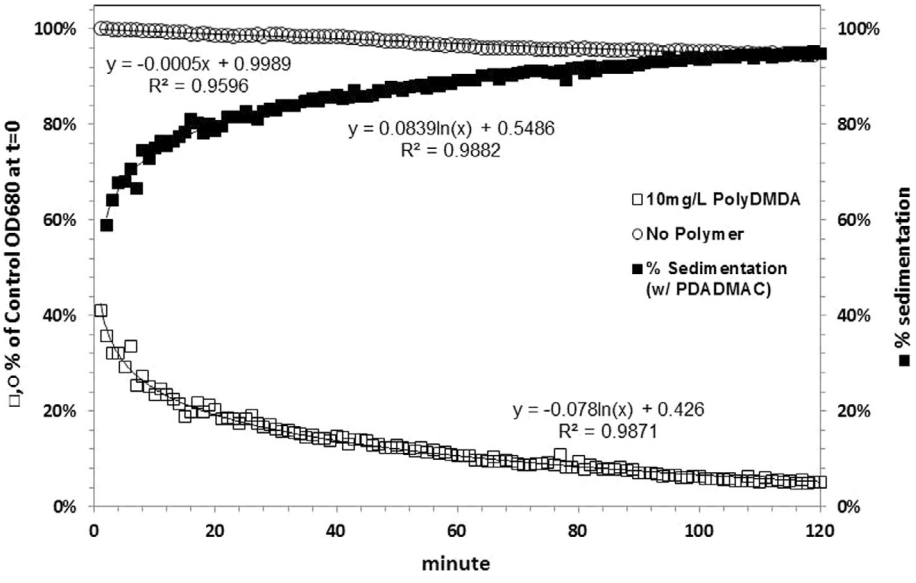

Fig. 3. Detailed kinetics of the sedimentation of *C. vulgaris* (n = 3; points are average ± standard deviation; initial algal concentration $2.3 \times 10^8 \pm 2.1 \times 10^6$ cells/ml).

Table 1
Zeta potential of chitosan and PDADMAC at pH = 4 and pH = 10, respectively.

Polymer	Zeta potential at pH = 4	Zeta potential at pH = 10	Comments
Chitosan	30 mV	0	Chitosan starts to lose its charge at pH > 4, and, therefore, precipitates out of solution
PDADMAC	60 mV	60 mV	PDADMA stays positively charged at pH range of 2–11

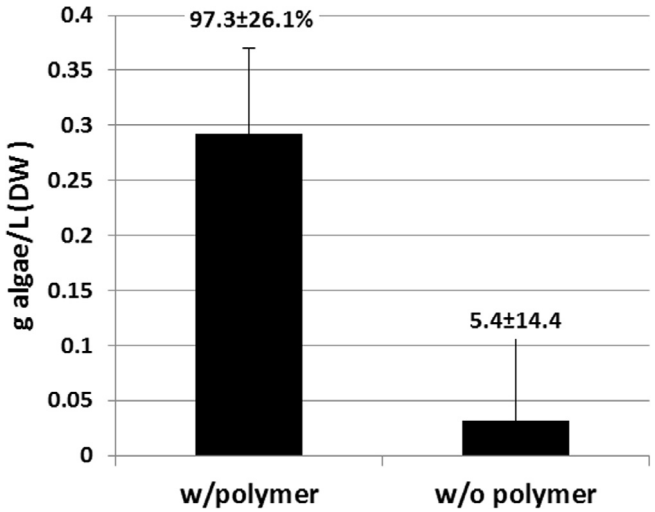

Fig. 4. Net dry weight of the 50 ml algae culture collected on Whatman 4 (pore size ~22 μm) filter (n = 3; data average ± standard deviation). Numbers above bars represent% of biomass collected (compared to centrifugation without PDADMAC).

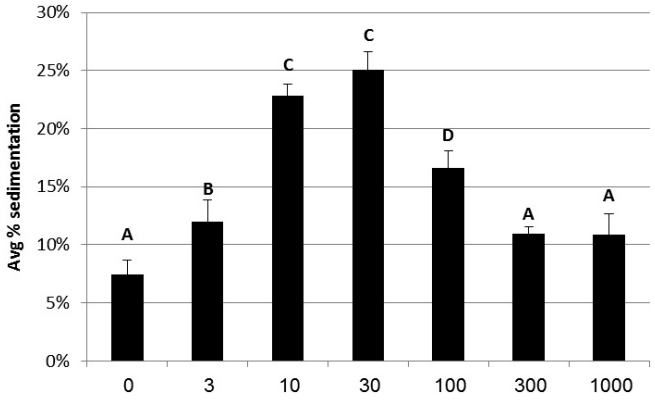

Fig. 5. Marine microalga *N. salina* sedimentation by different PDADMAC concentrations. Settling time 4 h; bars are average ± standard deviation of three experiments; one-way ANOVA $F(6,14) = 64.369$, $p = 0.0000$. Different letters denote significant differences in Tukey's post hoc test.

the filter after adding the polymer, resulting in the collection of 97% and 5% of total biomass, respectively (Fig. 4 and filter images in Supplementary information). These results correlate well with the polymer-induced aggregation observed by scanning electron microscopy (see SEM images in Supplementary information).

3.4. Water recycling

Growing algae for biomass requires large volumes of water (Wigmosta et al., 2011), although the exact amount varies according to growth strategy (open raceways or photobioreactors) and geographical region (Quinn and Davis, 2015). Accordingly, water reuse is desirable. To test water reuse feasibility, we flocculated *C. vulgaris* using 5 mg/L PDADMAC and, after cell sedimentation (2 h), collected the upper water and used it as a base for refreshed medium. Algal inoculum was added, and algal growth was monitored. For purposes of comparison, we tested the upper water after clay filtration as means for removing possible residual PDADMAC. Medium based on distilled water was used as a control. The results demonstrated little difference in growth rate with no statistical significance difference between the treatments (Doubling times in days: 2.53 ± 0.118, 2.56 ± 0.063 and 2.62 ± 0.162 averages for medium based on DW, recycled medium after sedimentation with 5 mg/L PDADMAC and recycled medium after treatment with clay respectively; n = 3 for all experiments), suggesting that the water can indeed be reused after PDADMAC flocculation.

3.5. Sedimentation of marine microalgae

Since marine microalgae are of emerging interest, we have tested the suitability of PDADMAC for sedimentation of the marine microalga *Nannochloropsis salina (N. salina)* [which usually grows in 3% NaCl (w/v)]. The maximal effect of PDADMAC was achieved at 30 mg/L (much higher than for *C. vulgaris*) and although statistically significant not satisfactory, as only ~25% of the biomass was sedimented even after four hours (Fig. 5). This effect was probably due to the adsorption of salt anions on the polymer or on the algae, as the addition of 3% NaCl to *C. vulgaris* in Bristol medium resulted in dramatic decrease in the sedimentation effect of the PDADMAC (data not shown).

4. Conclusions

PDADMAC was the most suitable flocculant for *C. vulgaris*, requiring low concentrations (5 mg/L) and sustaining wide range of pHs and concetrations, while chitosan lost effectivity at basic pHs and higher concetrations. Flocculation and sedimentation were very rapid – (80% algal cells within 20 min). Coarse filtration after flocculation allowed collecting ~97% of the biomass, making this a promising approach. Flocculating of marine microalga *N. salina* was much lower (25%). The use of such polymers could reduce biomass costs, although care should be taken if ozonation process is following, as PDADMAC ozonation could result in production of N-Nitrosodimethylamine, a suspected hepatotoxic (Padhye et al., 2011).

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2016.12.040.

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