Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2014.970581

57 (2016) 881–887
January

Taylor & Francis
Taylor & Francis Group

Chitosan-graft-polydiallyldimethyl ammonium chloride for microalgae harvesting from wastewater

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Received 1 April 2014; Accepted 19 September 2014

ABSTRACT

Harvesting microalgae is considered as a bottleneck to the process of microalgal biofuel production. Coagulation and flocculation of microalgae is shown to be the most suitable method of large-scale harvesting of microalgae. This study focused on synthesizing chitosan-g-polyDADMAC by grafting polydiallyldimethyl ammonium chloride onto the chitosan molecule to remove microalgae and total phosphorus (TP) from wastewater. Chitosan-g-polyDADMAC showed higher positive zeta potential than chitosan at all values of pH tested. Chitosan-g-polyDADMAC exhibited no isoelectric point characteristic of unmodified chitosan. Flocculant to algae mass ratio of 1:1 was required for chitosan-g-polyDADMAC to achieve 70% total suspended solids removal whereas, chitosan was required in the ratio 2:1 for 50% suspended solids removal. TP removal of nearly 20–25% was achieved with chitosan and chitosan-g-polyDADMAC at flocculant to algae ratio of 3:1. The use of chitosan-g-polyDADMAC as compared with unmodified chitosan has the potential to reduce material costs of microalgae harvesting.

Keywords: Chitosan modification; Harvesting; Microalgae; Phosphorus; Polydiallyldimethyl ammonium chloride

1. Introduction

Microalgae have triggered great interest in recent years as a source of sustainable energy, nutritional supplements, and specialized chemicals. High lipid content of microalgae coupled with high growth rates, low freshwater requirements, and high photosynthetic efficiency have all contributed to the emergence of microalgae as the sustainable biofuel crop of the future [1]. The ability of microalgae to thrive in wastewater or even brackish water has been employed in some communities to treat wastewater. Microalgae in these wastewater systems uptake phosphorus and nitrogen as growth nutrients, and achieve tertiary treatment [2]. Wastewater treatment in Logan, Utah is achieved in a 465 acre facultative lagoons system, wherein the dissolved nutrients and natural conditions facilitate the growth of microalgae. This symbiotic relationship between microalgae growth and nutrient removal from the wastewater can be harnessed to obtain a sustainable supply of biomass and achieve complete biological treatment of wastewater [3].

However, harvesting microalgae is a challenge due to small cell size, low culture concentration, and colloidal stability provided by electrostatic surface charges on microalgae, entailing high energy input

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and associated costs [3]. Researchers have tested several techniques of microalgae harvesting [4], and have concluded that only chemical precipitation or coagulation–flocculation is most suited for large-scale harvesting of microalgae [5]. Chemical precipitation or coagulation, which has been employed by conventional wastewater treatment plants (WWTPs) for solids and nutrient removal [6] is achieved by the addition of inorganic electrolytes such as aluminum sulfate or ferric chloride.

McGarry tested aluminum sulfate and four other synthetic polyelectrolytes to achieve about 90% removal with algal to coagulant ratio of up to 5:1 [7]. Aluminum sulfate and Pestan, a novel flocculant biopolymer tested on 1 g/L initial concentration of *Botryococcus braunii* showed an optimum dosage of 200 mg/L and 100 mg/L, respectively, to achieve 90% algae removal [5]. Papazi et al. [8] performed elaborate flocculation studies with 12 salts including chloride and sulfate salts of aluminum, iron, zinc, calcium, magnesium, and ammonium and concluded that aluminum salts were the most effective followed by ferric and zinc [8]. One particular study also tested commercially available organic flocculants such as Greenfloc and Cargill C*bond to achieve nearly 80–90% removal of microalgae (0.5 g/L initial concentration) with a coagulant dose of 30 mg/L [9]. As an organic coagulant, unmodified chitosan was used to harvest a culture of 1 g/L of *Chlorella vulgaris*, and achieved about 80% removal of microalgae with 100 mg/L of dosage [10].

Although effective, inorganic coagulants are required in high dosages resulting in increased sludge volume and high costs of harvesting microalgae [9]. Besides, the biomass harvested is contaminated with metal hydroxides, which makes it unsuitable for downstream processes producing bioproducts [8]. To achieve sustainability in microalgae harvesting, organic coagulants must be tested and utilized. Organic coagulants such as cationic starch, chitosan, and guar gum have been to a certain extent researched for microalgae harvesting [9,11–13]. Organic coagulants are naturally available, inexpensive, and can provide a source of carbon for downstream processes such as in fermentation or anaerobic digestion of the harvested biomass. This study focused on chitosan and its derivatives to harvest microalgae and treat wastewater.

Chitosan is a linear polyaminosaccharide produced by the deacetylation of chitin obtained from shells of crustaceans. Chitosan has been studied in the harvesting of freshwater [14] and marine microalgae [15]. However, high costs of chitosan about $50/kg [16], and the requirement of pH adjustment of colloids to pH ≤ 7 to obtain significant solids removal has limited the large scale use of chitosan for microalgae harvesting [14]. Polyelectrolytes such as polydiallyldimethyl ammonium chloride (polyDADMAC) have also been used in wastewater treatment to remove particulates through a combined mechanism of coagulation and flocculation [17]. PolyDADMAC is a homopolymer of diallyldimethyl ammonium chloride having a high charge density [17]. High costs of polyDADMAC (~160$/kg) (Sigma-Aldrich, St. Louis, MO) and no large or pilot scale demonstrations have restricted the use of polyDADMAC, as the sole method of microalgae harvesting. This study focused on grafting polyDADMAC onto chitosan, thereby incorporating the superior flocculant properties of polyDADMAC of high charge density and long polymer chain length into chitosan to synthesize modified chitosan effective at all values of pH. The objectives of this study was to synthesize chitosan-graft(g)-polyDADMAC by grafting polyDADMAC onto chitosan, and test its total suspended solids (TSS) and total phosphorus (TP) removal efficiency on wastewater from the Logan city wastewater lagoons.

2. Materials and methods

All chemicals including chitosan, polyDADMAC (20% in H_2O), ceric ammonium nitrate (CAN), hydrochloric acid, ethanol, and nitric acid were obtained from Sigma-Aldrich (St. Louis, MO) and used as received. To synthesize chitosan-g-polyDADMAC, 5 g of chitosan was dissolved in 100 mL solution of 1% hydrochloric acid. After complete dissolution, 0.5 g of CAN was added and heated for 30 min at 80˚C. The pH of solution was adjusted to pH 3.0 by nitric acid after which 7.5 mL of polyDADMAC solution (20% in H_2O) was added to the mixture and heated for 3 h at 80˚C. After the reaction, chitosan from the mixture was precipitated out by pH neutralizing with NaOH. The precipitated chitosan was washed in a Soxhlet apparatus with ethanol for 8 h to remove any unreacted components. The chitosan-g-polyDADMAC was then dried at room temperature (25˚C) and pulverized for further use.

The average degree of substitution of chitosan was calculated using Eq. (1) by measuring the total nitrogen content of chitosan and chitosan-g-polyDADMAC with the Hach Test 'N Tube (Loveland, CO), which employs the 4500-N C Standard Methods [18] and subtracting the nitrogen content of chitosan from that of chitosan-g-polyDADMAC.

$$\text{Degree of substitution, DS} = \frac{165.2 \times \text{N}\%}{[1,400 - (161.5 \times \text{N}\%)]} \quad (1)$$

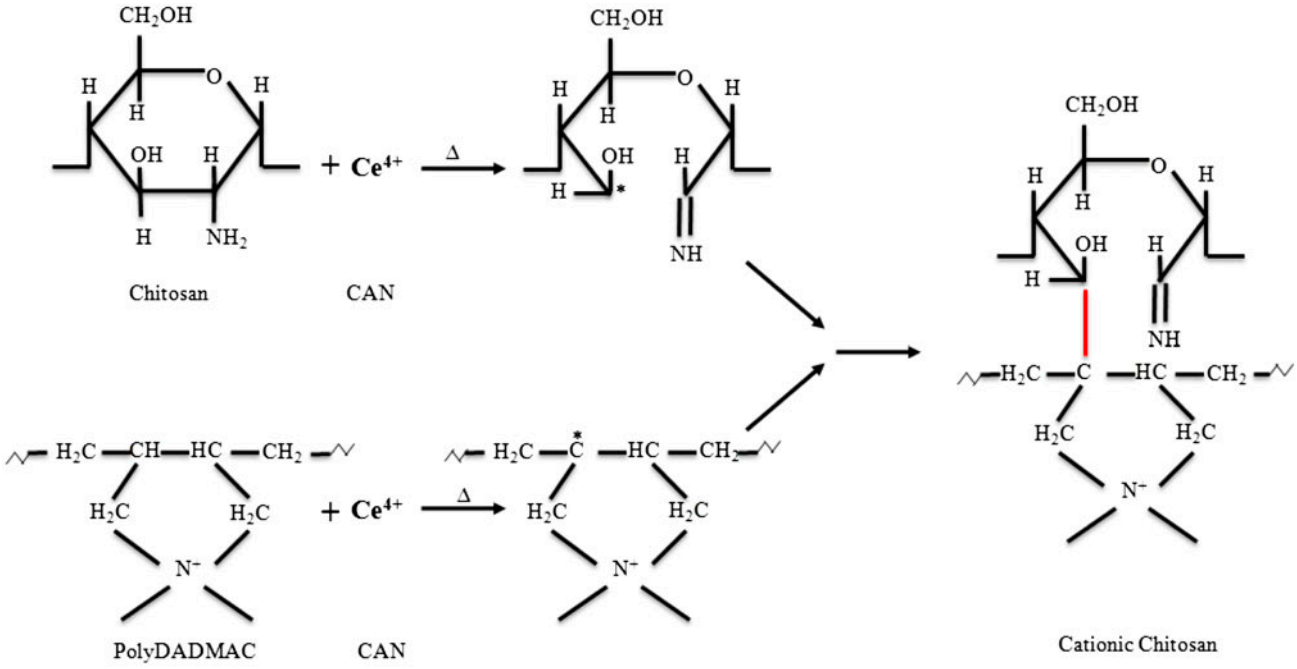

Fig. 1. General reaction scheme of grafting polyDADMAC onto chitosan.

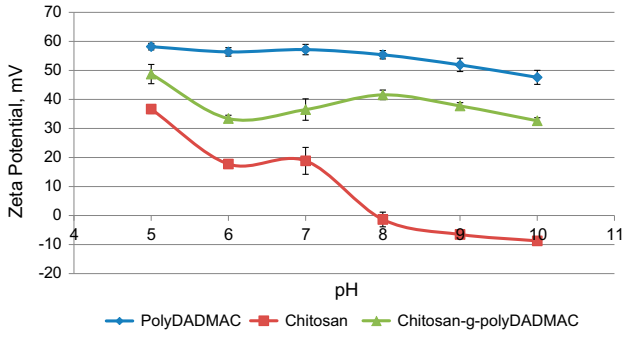

Fig. 2. Zeta potential titration curve for polyDADMAC, chitosan, and chitosan-g-polyDADMAC.

where 165.2 = molecular wt. of one repeating unit of chitosan, 161.5 = molecular wt. of one repeating unit of polyDADMAC, and N % = (wt. of nitrogen in chitosan-g-polyDADMAC − wt. of nitrogen in chitosan)/total wt. of cationic chitosan. Zeta potential titration curves for chitosan, polyDADMAC, and chitosan-g-polyDADMAC were prepared for pH 5–10 using Brookhaven Zeta plus zeta meter (Holtsville, NY). ^{13}C-NMR was performed for chitosan-g-polyDADMAC using Jeol ECX-300 in D_2O at 298 K. The NMR spectra provided confirmation of successful grafting of polyDADMAC onto the chitosan molecule.

Wastewater was collected from Logan city WWTP (facultative lagoons). The Logan city WWTP consists of five consecutive open ponds or cells designed to achieve primary, secondary, and tertiary treatment. Tertiary treatment which is the removal of dissolved nitrogen and phosphorus from the wastewater is accomplished in the fourth and the fifth cells of the WWTP by naturally growing algae. The wastewater for this study was collected from the outlet of the fifth cell, and the suspended solids is considered to be composed entirely of a mixed culture of algae as shown by Griffiths [19]. Flocculation experiments were performed in a jar test apparatus (ECE DBT6) at pH 7.0.

TSS were measured using Shimadzu UV-1800 spectrophotometer (550 nm) using a previously established correlation between the concentration of microalgae in wastewater and absorbance. TP removal was measured using Lachat QuikChem 8500 which employs the 4500-P E Standard Methods [18]. Jar tests were performed for the wastewater with chitosan, polyDADMAC, and chitosan-g-polyDADMAC in triplicate. Each run consisted of six jars with no flocculant in the first jar (control), and predetermined increasing concentrations in the following five jars. After addition of the coagulants, the jars were flash mixed for 10 s and then mixed at 30 rpm for 10 min. The mixing was completely stopped after 10 min and the microalgae aggregates were allowed to form and settle to the bottom for an hour. Samples for TSS, TP, and zeta potential were collected before addition of the coagulants–flocculants and after completion of the jar test.

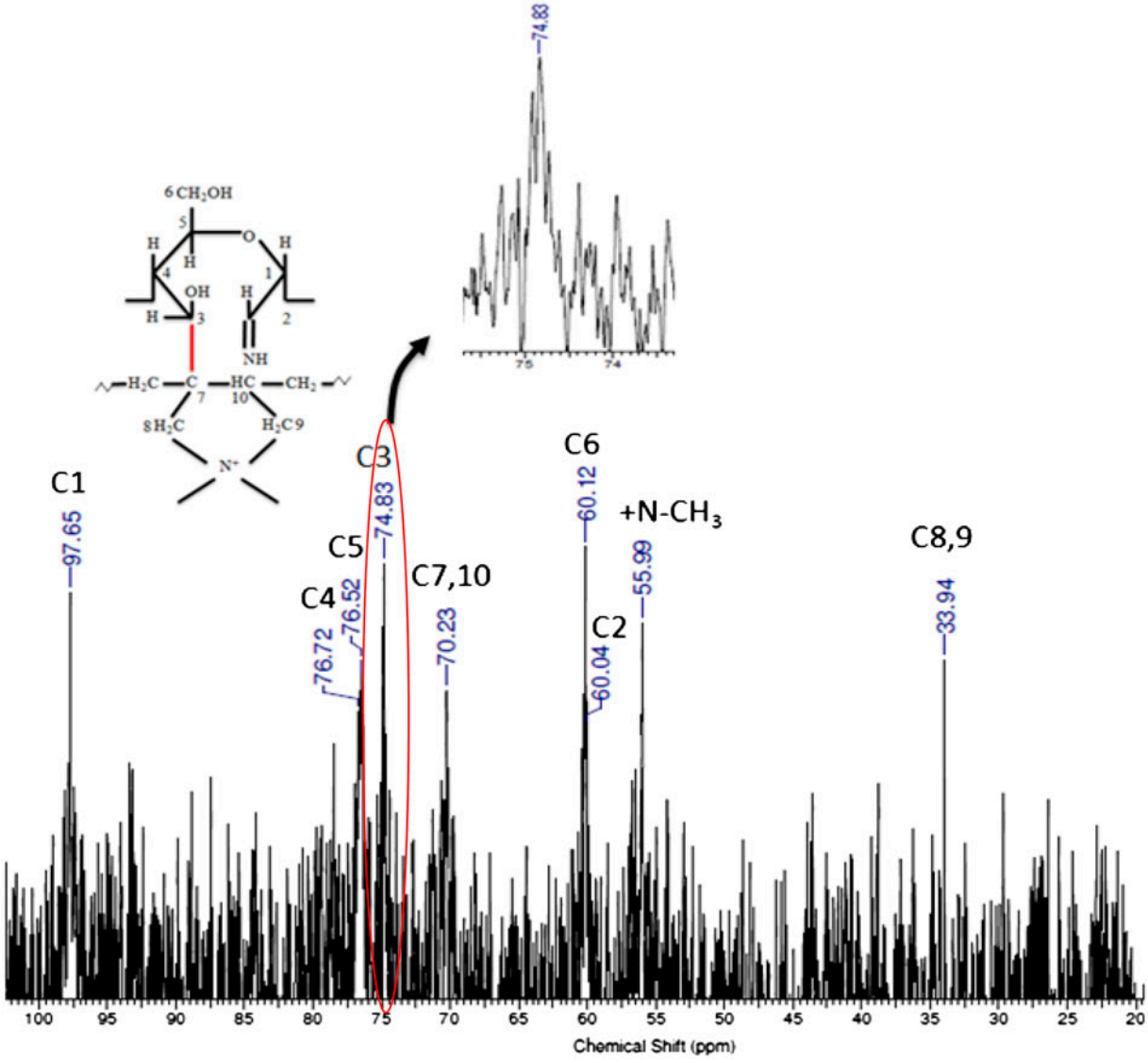

Fig. 3. ^{13}C-NMR spectra of chitosan-g-polyDADMAC measured in D_2O (1% HCl) at 298 K.

3. Results and discussion

3.1. Cationic chitosan synthesis

The general reaction scheme for chitosan grafting is presented in Fig. 1. It is postulated that the addition of CAN to chitosan initiates radicals at C3 of the chitosan molecule by breaking the C2–C3 bond [20]. Similarly, reaction of CAN with polyDADMAC results in free radicals at the tertiary carbons as shown in Fig. 1 [21]. The two molecules with free radicals namely chitosan and polyDADMAC, then combine with each other at the radicals by termination reaction [22]. The degree of substitution achieved for the reaction was 0.016.

3.2. Zeta potential titration curve

Zeta potential titration was performed in order to determine the charge acquired by the flocculants at various pH values, and to establish an efficient operating pH range with positive zeta potential for chitosan-g-polyDADMAC. The titration curve for polyDADMAC was nearly constant and independent of pH as seen in Fig. 2 with high positive zeta potential (+50–+60 mV). Constant titration curve was obtained due to the presence of the quaternary ammonium cation on the polyDADMAC molecule. Chitosan showed a pH dependent zeta potential with pH > 8 resulting in negative values of zeta potential. The change in zeta

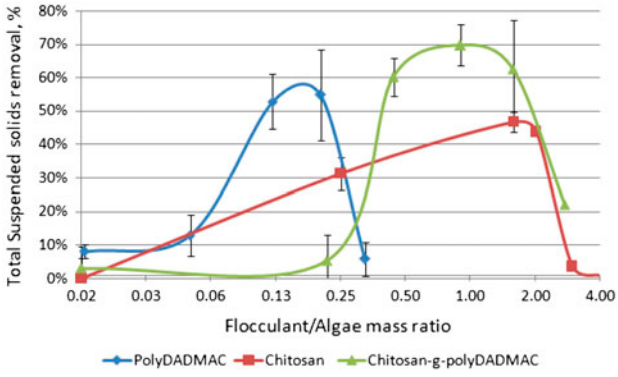

Fig. 4. Comparison of % TSS removal from Logan lagoon wastewater using polyDADMAC, chitosan, and chitosan-g-polyDADMAC.

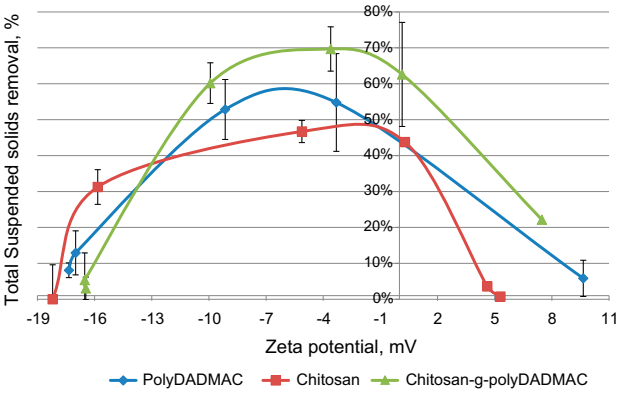

Fig. 5. Correlation between zeta potential and % TSS removal from Logan lagoon wastewater using polyDADMAC, chitosan, and chitosan-g-polyDADMAC.

potential is attributed to the protonation and deprotonation of the nitrogen atom on the chitosan at acidic and basic pH, respectively. Chitosan-g-polyDADMAC, however, showed improved flocculation properties than chitosan exhibiting higher average positive zeta potential, and nearly insignificant change of zeta potential values with pH. The modification of chitosan with polyDADMAC improved the cationic potency of chitosan and completely eliminated the isoelectric point, characteristic of unmodified chitosan.

3.3. ^{13}C-NMR of Chitosan-g-polyDADMAC

^{13}C-NMR spectra for chitosan-g-polyDADMAC measured in D_2O (1% HCl) at 298 K is presented in Fig. 3. The peak at 97.65 ppm is attributed to carbon C1 of the chitosan as shown in Fig. 3. Peaks for carbons C3–C5 appear between 70–80 ppm. Peaks for C2 and C6 appear close to 60 ppm. The peak assignment for chitosan was provided by de Abreu and Campana-Filho [23]. The peaks for C7 and C10 of the grafted polyDADMAC appear at 70.23 ppm. Peaks for C8 and C9 appear at 33.94 ppm. The peak at 55.99 is attributed to the CH_3-N^+-CH_3 carbons. The peak assignment for polyDADMAC was verified by John [24]. Splitting of the C3 peak at 74.83 ppm as shown in the inset of Fig. 3 confirmed successful grafting as predicted.

3.4. TSS removal from wastewater

Initial concentration of microalgae in the wastewater ranged from 40 to 67 mg/L for the jar tests. The dosage of polyDADMAC required to achieve 50–60% TSS removal was about 10–20% of the initial concentration of algae (Fig. 4). An increase in polyDADMAC dosage beyond 20% of initial concentration of algae caused a reversal of average colloidal charge from negative to positive resulting in colloid stability and low TSS removal rates. The dosage of chitosan was nearly twice or 200% of the initial concentration of algae to achieve about 50% TSS removal. This can be attributed to low zeta potential (±20 mV) of chitosan at pH 7 as compared with polyDADMAC as shown in Fig. 2. Further addition of chitosan resulted in charge reversal and low TSS removal. On the contrary, chitosan-g-polyDADMAC showed higher average TSS removal than polyDADMAC and chitosan. The dosage of chitosan-g-polyDADMAC was about 100% of the initial concentration of algae to achieve 70% TSS removal. This result suggested that significant improvement in flocculating properties of chitosan by grafting resulting in lower dosages, higher TSS removal, and in turn lower costs of harvesting.

Zeta potential measurements of microalgal suspensions during jar tests helped to understand the mechanism of charge neutralization and inter-particle bridging taking place after addition of the coagulant–flocculants. Initial zeta potential values of the wastewater ranged from −15.0 to −18.0 mV. Complete destabilization of a colloid is achieved when its average zeta potential has been reduced to 0 mV. However, as shown in Fig. 5, polyDADMAC, chitosan, and chitosan-g-polyDADMAC achieved maximum % TSS removal as the zeta potential approached 0 mV. This is due to the polymeric nature of the flocculants resulting in inter-particle bridging after initial reduction of negative zeta potential of the microalgal suspension. Higher % TSS removal was observed for chitosan-g-polyDADMAC than chitosan and polyDADMAC at the same zeta potential values. This could be due to the longer polymer chains of

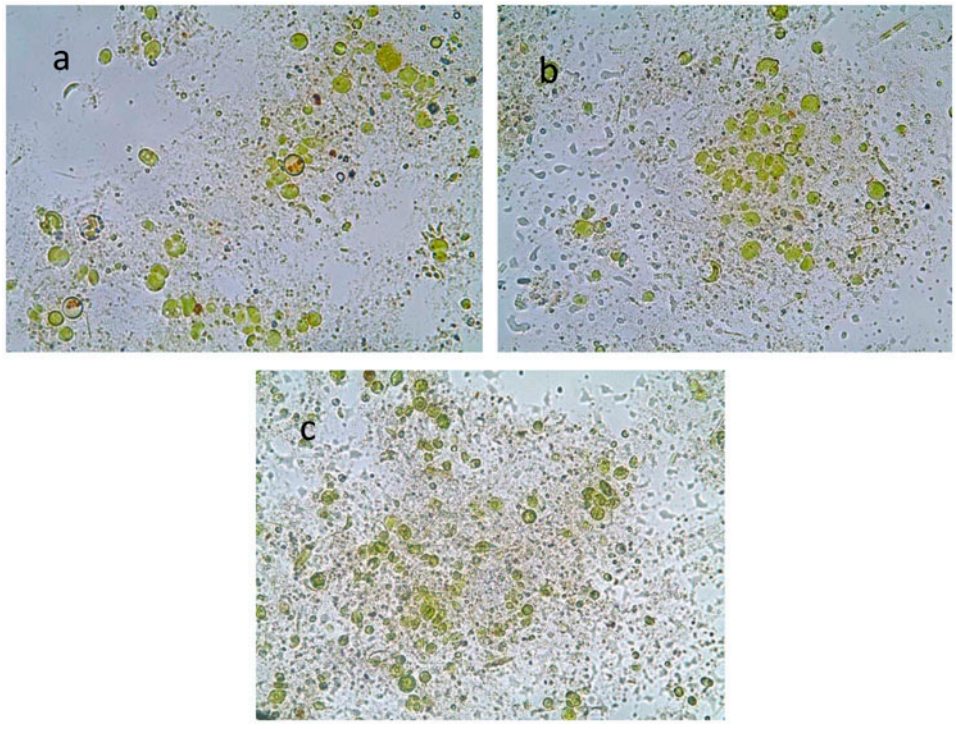

Fig. 6. Microscopic images (40×) of aggregates formed during the jar test for (a) polyDADMAC, (b) chitosan, and (c) chitosan-g-polyDADMAC.

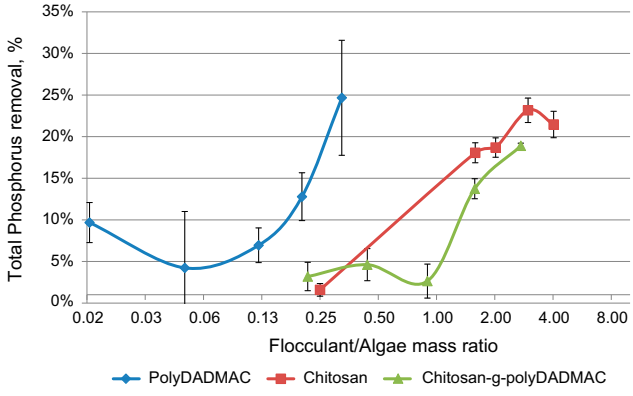

Fig. 7. Comparison of TP removal from Logan lagoon wastewater using polyDADMAC, chitosan, and chitosan-g-polyDADMAC.

chitosan-g-polyDADMAC that more effectively flocculate microalgae after initial charge reduction. Fig. 6 makes a comparison of the aggregates of the three flocculants formed during the jar test. A visual qualitative assessment can be made using Fig. 6 of the larger size of the aggregates of chitosan-g-polyDADMAC compared with polyDADMAC and chitosan.

3.5. TP removal from wastewater

The initial concentration of TP in the wastewater ranged from 2.0 to 3.0 mg/L. At pH 7.0, TP consisted of insoluble phosphorus (microalgae) and soluble phosphorus in the form of hydrogen and/or dihydrogen phosphate ions (HPO_4^{2-} or $H_2PO_4^-$). PolyDADMAC showed about 25% TP removal with a flocculant to algae ratio of 0.4. Chitosan also showed nearly 25% TP removal however, the flocculant to algae ratio being 3.5 for chitosan was much higher as compared with polyDADMAC. The removal mechanism of negatively charged phosphate ions follows the principle of charge neutralization similar to solids removal. As seen from Fig. 7, chitosan-g-polyDADMAC followed the same trend as chitosan for TP removal, however the % TP removal was lower compared with chitosan. Further tests for TP removal are required to optimize flocculant dosage to obtain higher phosphorus removal efficiencies.

4. Conclusions

Chitosan-g-polyDADMAC was successfully synthesized using a simple procedure and exhibited superior coagulant and flocculant properties derived from polyDADMAC. Chitosan-g-polyDADMAC

showed higher positive zeta potential values than chitosan at all the values of pH tested. The zeta potential values for chitosan-g-polyDADMAC were not affected by pH allowing it to be used as an effective coagulant for colloids exhibiting a wide range of pH values. Chitosan-g-polyDADMAC achieved higher % TSS removal than chitosan at lower dosage, which could help reduce material costs of harvesting by nearly 50%. Due to the high positive zeta potential of chitosan-g-polyDADMAC, its application can be extended to harvest marine algae. Future work will focus on replacing the synthetic polyDADMAC with biogenic amines from waste source to improve the biodegradability, sustainability, and its potential use as a carbon substrate in fermentation or digestion processes that use the harvested biomass as feedstock.

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