Cationic Starch for Microalgae and Total Phosphorus Removal from Wastewater

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ABSTRACT: The city of Logan, Utah treats wastewater in 460 acres of facultative lagoons. Naturally growing algae in these lagoons uptake nutrients and remediate the wastewater, but require coagulation and flocculation for separation from wastewater. Cationic corn starch and cationic potato starch with a degree of substitution 1.34 and 0.82, respectively, were synthesized using 3-methacryloyl amino propyl trimethyl ammonium chloride and tested on *Scenedesmus obliquus* and lagoon wastewater. The zeta potential of the cationic starches tested over a pH range 5.0–10.0 showed an average of +16.0 and +15.1 mV for cationic corn starch and cationic potato starch, respectively. Total suspended solids removals of 90 and 85% were achieved with cationic corn starch and cationic potato starch, respectively, when tested with *S. obliquus*. Tests with wastewater showed total suspended solids removals of 80 and 60% for cationic corn starch and cationic potato starch, respectively. Total phosphorus removal values from wastewater were approximately 33, 29, and 42% for cationic corn starch, cationic potato starch, and alum, respectively, for a coagulant/algae ratio of 1.4 (wt/wt). These results indicate that cationic starch has the potential to replace alum for algae harvesting and wastewater treatment making it a suitable alternative to inorganic coagulants and flocculants. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 2572–2578, 2013

INTRODUCTION

Phosphorus and nitrogen in wastewater discharge may cause eutrophication in downstream reservoirs. The City of Logan, Utah biologically treats 15 million gallons per day of municipal wastewater in 460 acres of open ponds (facultative lagoons) that achieves primary and secondary treatments. In addition, naturally growing algae in this open pond system facilitates uptake of phosphorus and nitrogen, removing these nutrients from the wastewater and accomplishing tertiary treatment. With effective management of the system, algal biomass can be separated from the wastewater and used as animal feed and fertilizer, and can be further processed to generate biofuels such as biodiesel,[1] biosolvents,[2] and biogas.[3] However, the harvesting of algae from water is a technical and economic challenge.[4]

Harvesting microalgae, or separating microalgae from wastewater, presents a challenge in concentrating dilute microalgal suspensions mostly composed of two or more species exhibiting different size, shape, and surface charge that can influence the harvesting method chosen. The major techniques currently employed in microalgae harvesting and recovery include centrifugation, biofilm formation,[4] coagulation/flocculation, filtration and screening, gravity sedimentation, flotation, and electrophoresis techniques.[5] Of all these methods, coagulation/flocculation of microalgae is shown to be the most efficient method for harvesting biomass on a large scale.[6] Coagulation facilitates better interaction of the algae cells by charge neutralization, which otherwise forms a stable suspension due to electrostatic repulsion of inherent negative surface charges on algae. Coagulation is followed by flocculation, a mechanism by which the coagulated or neutralized particles are aggregated by bridging[7] and more easily separated from the wastewater.

Coagulation, or charge neutralization, of the algae colloid is conventionally achieved by the addition of electrolytes such as aluminum sulfate and ferric chloride. The positive charge on the multivalent Al^{3+} and Fe^{3+} ions facilitates charge neutralization of the colloidal particles.[7] After successful charge neutralization, flocculation is typically achieved by addition of a polymer, which is capable of forming a molecular "net" to trap the neutralized algae cells.[7] Polyelectrolytes such as Magnafloc have also been studied in the harvesting of *Chaetoceros calcitrans*.[8] Polyelectrolytes act to neutralize the particles and then form a bridge between the particles to facilitate flocculation.[9] Current methods of separation of algae using inorganic coagulants such as aluminum sulfate and ferric chloride are efficient, but require high dosages and contaminate the biomass with aluminum or iron, resulting in lower yields of the desired bioproducts.[10]

The drawbacks of inorganic coagulants and flocculants described above can be addressed by using organic coagulant/flocculants such as modified cationic starch. Vandamme et al.[10] showed that cationic starch is efficient in microalgae harvesting, focusing on specific fresh water and marine algal strains. Cationic starch was also used by Burr et al. (1975) for the flocculation of coal, clay, and sewage, among other studies using cationic starch as flocculants.[11,12] Conventionally, starch is modified by introducing quaternary ammonium functional groups to the starch backbone. This modification renders cationicity to the starch with an ability to neutralize the negative charge of colloids.[13] Furthermore, the inherent polymeric structure of the starch molecules provides flocculant properties to the cationic starch thus employing a dual mechanism of coagulation (charge neutralization) and flocculation (bridging) in algae harvesting. Starch can be cationized by various methods that include crosslinking,[14,15] oxidation,[16,17] etherification,[18] esterification,[19] and grafting.[20,21]

Although the science of cationic starch synthesis is well understood, there is a lack of available information addressing the charge acquired by the synthesized cationic starch and its interaction with the charge or zeta potential of the target colloid. Our research focused on the grafting method adopted by Gruber et al.[22] to cationize starch due to the simplicity of the process and minimal use of reagents to achieve cationization. Starch grafting is the most frequently used method to modify starch without altering its biological properties[21] and has also been shown to achieve higher cationic charge density than can be achieved using the other methods[23]. The objectives of this research were to: (1) synthesize cationic starch by graft polymerization and obtain a zeta potential titration curve, (2) use the cationic starch to remove algae and phosphorus from municipal wastewater (Logan lagoons), and to treat single strain suspensions of *Scenedesmus obliquus* isolated from the wastewater, and (3) compare cationic starch to aluminum sulfate (alum) with regard to algae and phosphorus removal.

MATERIALS AND METHODS

Potato starch was obtained from Fisher Scientific (Pittsburgh, PA). Food grade corn starch was obtained from a local grocery store. Ceric ammonium nitrate, 3-methacryloyl amino propyl trimethyl ammonium chloride (50% in water; MAPTAC), aluminum sulfate, and nitric acid (trace grade) were obtained from Sigma Aldrich (St. Louis, MO). All chemicals were used as received. *Scenedesmus obliquus* was isolated from the wastewater and grown in a Solar Simulated Reactor (SSR) to maintain unialgal conditions. *Scenedesmus obliquus* was chosen due to its high density in the lagoon wastewater.[2] Wastewater was collected from Logan lagoons for jar test experiments. Experiments were performed in a jar test apparatus (ECE DBT6). Total suspended solids (TSS) measurements were performed using Standard Methods 2450D.[24] Total phosphorus (TP) was measured using Lachat QuikChem 8500.

For cationic starch synthesis, 5.0 g of starch was dissolved in 100 mL water at 75–80°C. After complete dissolution, 1.0 g of ceric ammonium nitrate was added and heated at 75–80°C for 30 min. After free radical initiation, 15 mL of 3-methacryloylamino propyl trimethyl ammonium chloride was added slowly and

adjusted to pH 3 by the addition of nitric acid. The mixture was then heated at 80°C for 2 h, cooled, and pH neutralized with NaOH, and the starch was precipitated using ethanol as needed. The starch was then thoroughly rinsed with ethanol to remove any unreacted reagents. The washed starch was dried, pulverized, and stored until further use. This procedure was followed for the synthesis of both potato and corn cationic starches.

Total nitrogen in the starch was measured using Hach Test 'N Tube and the degree of substitution was calculated using *eq*. (1). Hach Test 'N Tube measures high range (0–150 mg/L) total nitrogen using persulfate digestion method and photospectrometric analysis (Hach Company, Colorado 2012).

$$\text{Degree of substitution, DS} = \frac{161 \times N\%}{[1400 - (220.74 \times N\%)]} \quad (1)$$

Where, 161 = Molecular weight of one anhydrous glucose unit, 220.74 = Molecular weight of MAPTAC, N % = % weight of nitrogen in starch. The degree of substitution (DS) is the average number of hydroxyl groups that have been substituted in one anhydrous glucose unit (AGU) of starch. The DS can range between 0–3. The higher the DS value the better is the reactivity of MAPTAC to starch.

To measure the extent of cationization of the modified starch, the zeta potential on the starch was measured using Brookhaven ZetaPlus zeta meter (Holtsville, NY). Zeta potential is a measure of the average charge of the colloid, measured in millivolts. The zeta potential on the cationic starches is a function of pH due to the adsorption of H^+ and OH^- charges on the surface of the starch particles. Besides this, the nitrogen attached to the starch molecule can be protonated and deprotonated depending on acidic or basic pH, respectively. The magnitude depends on the degree of substitution of the cationic starch. The effect of pH on the zeta potential was tested to identify the isoelectric point of the cationic starch and aluminum sulfate over the operating pH range of 5–10. The zeta potential of algae was also measured before and after jar tests to study the effect of starch and alum concentration on the surface charge neutralization of algae. ^{13}C-NMR was performed on the cationic starches using Jeol ECX-300 in D_2O at 298 K. The spectra from ^{13}C-NMR provided verification of attachment of MAPTAC to starch resulting in starch modification.

Three coagulants including: (1) cationic corn starch (CCS), (2) cationic potato starch (CPS), and (3) alum were tested for the jar test experiments on lagoon wastewater and *S. obliquus*.

Each set of experiments consisted of six jars with one control (no coagulant) and increasing concentrations of a particular coagulant chosen. Before adding coagulants to the jars, total suspended solids, total phosphorus, and initial zeta potential of the algal suspension was measured. After adding predetermined concentrations of the coagulants, the jars were flash mixed for two minutes at 200 rpm. The jars were then mixed at 25 rpm for 10 min after which, the mixing was stopped and the flocks were allowed to form and settle to the bottom for 1 h. Samples for TSS, total phosphorus and zeta potential were collected from the sampling ports on the jars. Jar tests were performed in triplicate for each combination of coagulant and colloid.
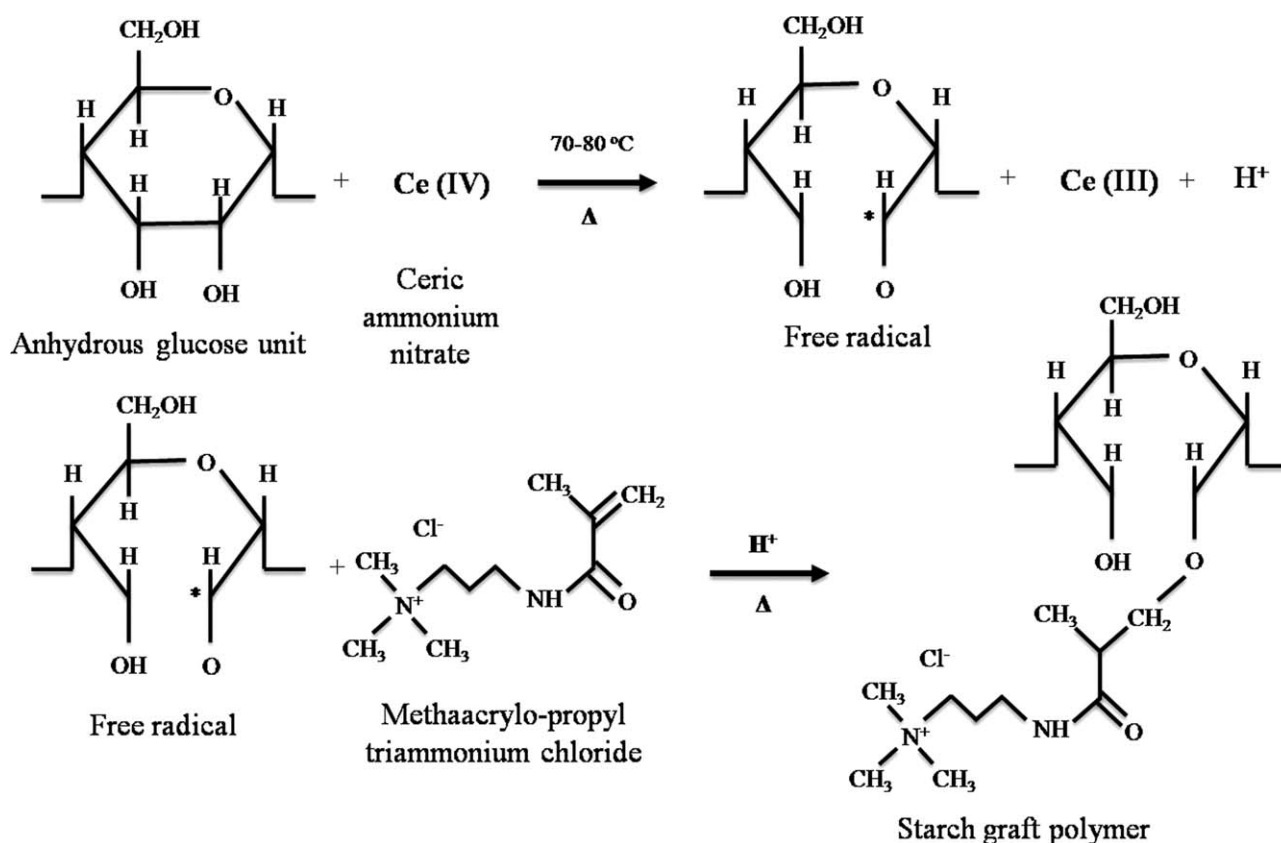
Figure 1. Mechanism of grafting 3-methacryloyl amino propyl trimethyl ammonium chloride on starch by radical initiation.

RESULTS AND DISCUSSION

Effect of Starch Type on the Degree of Substitution (DS)

For graft polymerization of starch, chemical initiation of radicals on the starch backbone is brought about by ceric ammonium nitrate. Although, the actual mechanism of the initiation is unclear, a very conclusive and accepted theory is that the radical is formed at the C2-atom of the anhydroglucose unit after breaking the C2–C3-bond[22] as shown in Figure 1. The monomers that can polymerize at the initiated radical are unsaturated and should have at least one double bond. The double bond of 3-methacryloyl amino propyl trimethyl ammonium chloride cleaves and is then grafted on to the C2 atom of the starch AGU.[25] The degree of substitution is a measure of the extent to which the hydroxyl groups on one anhydrous glucose unit of starch molecule is substituted by MAPTAC. Corn and potato cationic starches showed a DS of 1.34 and 0.82, respectively.

The relative reactivity of amylose is higher than amylopectin.[26] The number of amylose molecules per gram of starch ($x10^{20}$) is 30 and 130 for potato and corn starch, respectively.[27] Thus, it is postulated that due to the higher amount of amylose in corn starch than potato starch and due to the higher reactivity of amylose to substitution than amylopectin, the DS of cationic corn starch is higher than cationic potato starch. The effect of DS can be seen on the cationicity of the modified starches. High DS suggests higher nitrogen content in starch which leads to better dewatering performance.[23]

Effect of pH on the Zeta Potential of Cationic Starch

Zeta potential measured on a typical working pH range for corn and potato starches is presented in Figure 2. The results were compared with aluminum sulfate (alum) in order to evaluate the cationic potency of the modified starches with a standard coagulant.
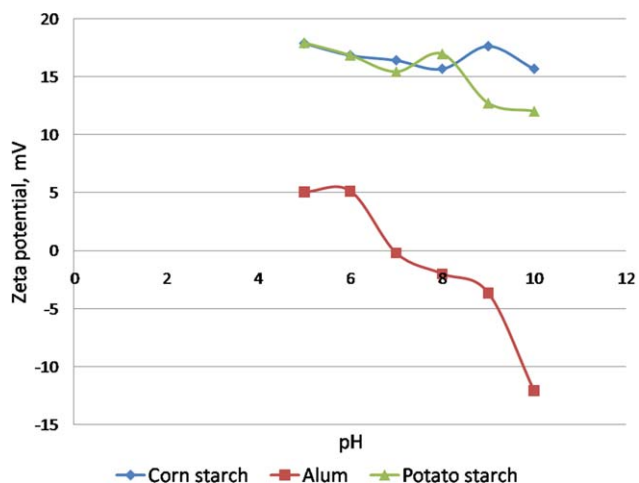

Figure 2. pH based zeta potential comparison of cationic corn starch, cationic potato starch, and alum. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
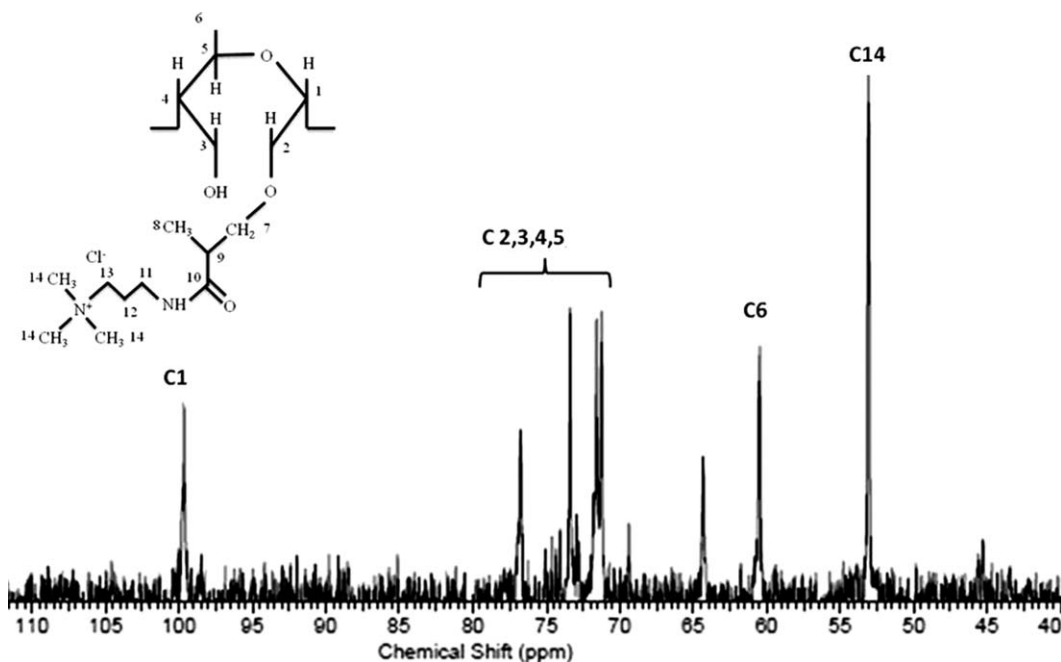
Figure 3. ^{13}C-NMR spectra of modified cationic corn starch (DS = 1.34) in D_2O at 298 K.

The cationic corn starch shows no or very little change in zeta potential with respect to the change in pH. This is due to the fact that all the bonding sites in quaternary ammonium are completely occupied leaving no room for protonation or deprotonation of nitrogen. The zeta potential of the cationic corn and potato starch averaged 16 and 15 mV, respectively. However, a slight drop in the zeta potential of potato starch is observed at basic pH values. This can be accounted for by the lower DS of cationic potato starch. The cationicity of the starches is relatively high as compared to alum which has a pH dependent zeta potential with an isoelectric point at pH 7.3 due to the fact that aluminum sulfate is a Lewis acid and acts by reducing the pH of the colloid.[28]

^{13}C-NMR Spectroscopy of Cationic Corn Starch
Standard ^{13}C-NMR spectra of the cationic corn starch is presented in Figure 3. The modified starch was dissolved in deuterium oxide (D_2O) and measured at 298 K. The peak for the C1 of the AGU appears at 99.62 ppm and peaks for C2–C6 of the AGU appear between 60 and 80 ppm. The introduction of 3-methacryloyl amino propyl trimethylammonium chloride results in the peak shown in Figure 3 as C14 at 53 ppm. The peak at 53 ppm is attributed to the carbons at 14th position ($CH_3(N+)$). The peak for C14 confirms MAPTAC attachment to the starch backbone and provides evidence of starch cationization The peak assignment was verified by Heinze et al.[25] using DEPT-135.

Effect of Cationic Starch on Total Suspended Solids Removal
Total suspended solids (TSS) removal experiments were performed in a Jar test apparatus with two different colloids; pure strain culture of microalgae *Scenedesmus obliquus* and wastewater from the Logan lagoons. Three coagulant/flocculants were tested for TSS removal efficiency: (1) cationic corn starch, (2) cationic potato starch, and (3) alum. Figure 4 presents the TSS experiment performed on *S. obliquus* at pH 7.

The initial concentration of *S. obliquus* was approximately 200–250 mg/L. Cationic corn starch treatment showed TSS removal of about 90% with a coagulant/algae weight ratio of 0.0053, while cationic potato starch showed 85% TSS removal and alum showed 15% TSS removal for the same ratio. The change in zeta potential of the colloids after addition of the coagulant is plotted on the secondary *x*-axis and suggests that reduction in zeta potential above −25 mV resulted in significant TSS removal efficiencies. It is postulated, by the values of zeta potential that the polymeric structure of the starch molecules resulted in flocculation after the initial charge neutralization (coagulation). The cationic starches showed high potency as coagulant/flocculant with high TSS removal efficiency when compared to alum. A coagulant/algae ratio of 1.0 was required for alum to effect 84% TSS removal not shown in Figure 4.

The initial algae concentration in lagoon wastewater was approximately 35–50 mg/L. Figure 5 shows TSS removal efficiency of the coagulants for wastewater from the lagoons. Cationic corn starch treatment showed TSS removal of approximately 80% with a coagulant/algae weight ratio of 1.4 : 1, while cationic potato starch achieved 60% TSS removal, and alum showed 30% TSS removal for the same ratio. Significant zeta potential reduction from approximately −16 mV to nearly 0 mV was observed with an increase in coagulant/algae ratio from 0.8 : 1 to 0.9 : 1 for both cationic corn starch and cationic potato starch, while alum only achieved approximately −16 mV to −13 mV reduction in zeta potential for the same change in ratio. High TSS removal with cationic starches without achieving complete charge neutralization is attributed to flocculation taking over coagulation as the predominant mechanism. The cationic starches showed high potency as coagulant/flocculent with high TSS removal efficiency when compared to alum. A significantly higher coagulant/algae ratio of 3.5 was required for alum to effect 63% TSS removal.
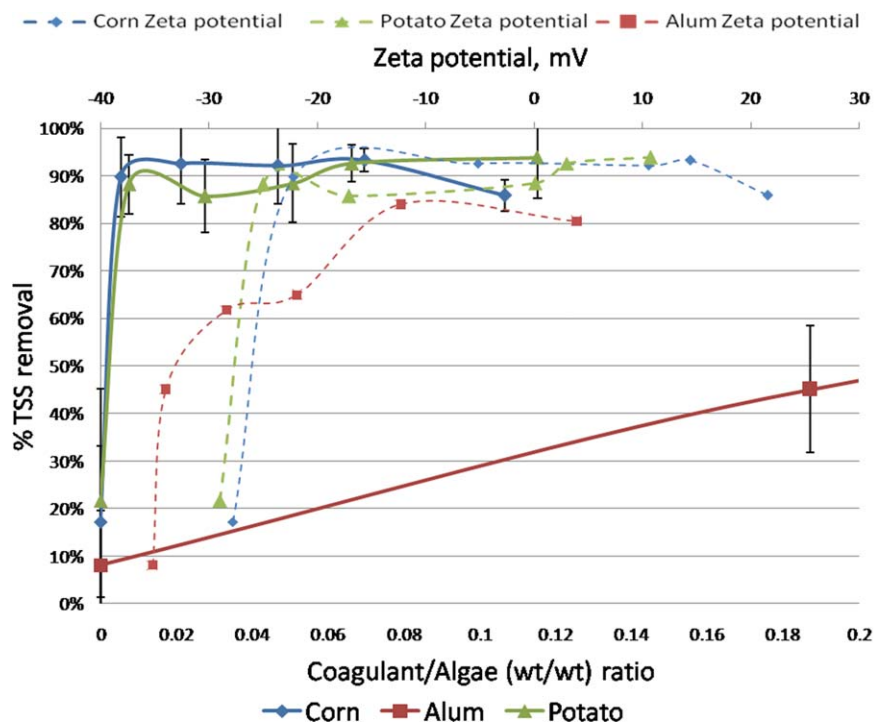
Figure 4. Comparison of TSS Removal from a Culture of *Scenedesmus obliquus* Using Cationic Corn Starch, Cationic Potato Sstarch, and Alum Treatments. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Effect of Cationic Starch on Total Phosphorus Removal

Total phosphorus (TP) removal using coagulants was evaluated for the wastewater. Initial concentrations of TP in the Logan lagoons wastewater ranged from 3.0 to 4.0 mg/L. Total phosphorus comprises both soluble and insoluble phosphorus. The insoluble phosphorus is primarily algae, or TSS, and is taken out of solution with the TSS. Soluble phosphorus is primarily in the form of orthophosphate. Figure 6 shows the total phosphorus removal efficiency of cationic corn starch, cationic potato starch, and alum treatment tested on the wastewater
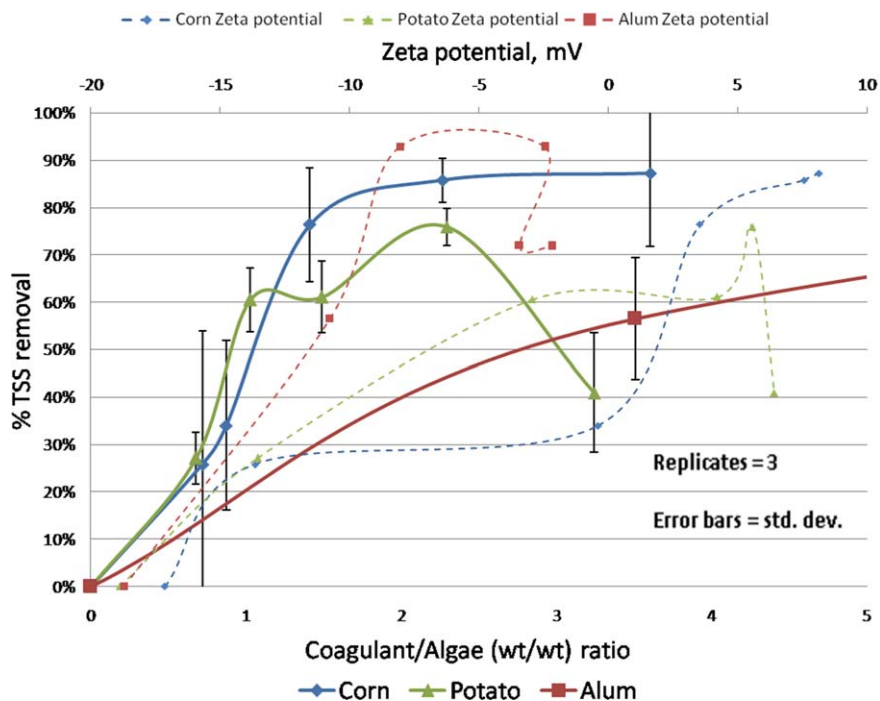

Figure 5. Comparison of TSS Removal from Logan Lagoon Wastewater Using Cationic Corn Starch, Cationic Potato Starch, and Alum Treatments. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 6. Comparison of Total Phosphorus Removal from Logan Lagoon Wastewater Using Cationic Corn Starch, Cationic Potato Starch, and Alum Treatments. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
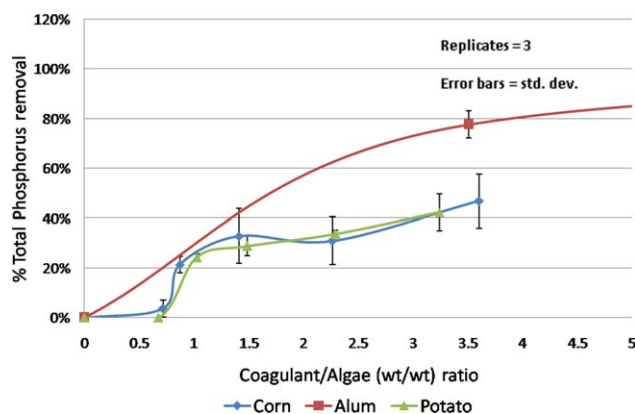
from the Logan lagoons. Cationic corn starch showed approximately 33% TP removal and cationic potato starch showed approximately 29% TP removal, while alum showed approximately 42% TP removal for the coagulant/algae ratio of 1.4 : 1. When compared to TSS removal (Figure 5) and TP removal (Figure 6), alum showed a simultaneous TP removal by first forming of aluminum phosphate by the dissociated aluminum and hydrogen and/or dihydrogen phosphate ions (HPO_4^{2-} or $H_2PO_4^{-}$) in the wastewater and achieving TP removal, and TSS removal by charge neutralization by the formation of aluminum hydroxide. However, the cationic starches showed high % TSS removal from wastewater (Figure 5) but low % TP removal compared to alum. This characteristic of the cationic starches suggests an initial TSS removal and then total phosphorus removal when compared to alum, which accomplishes simultaneous removal of TSS and TP.

CONCLUSIONS AND DISCUSSION

Cationic starch was successfully prepared by grafting 3-methacryloyl amino propyl trimethyl ammonium chloride on the backbone of corn and potato starch and the cationicity of the modified starch was measured as zeta potential at different pHs. The zeta potential titration procedure developed is a quick and easy technique for assessing the efficacy of the different cationization methods or testing different parameters of the cationization method chosen to achieve the desired charge on the starch particle. Zeta potential tests performed utilizing conventional jar tests helped optimize coagulant dosages and understand underlying mechanism of particle interaction. Cationic starch showed high total suspended solids removal with low dosage as compared to alum for both *Scenedesmus obliquus* and wastewater algae. Cationic starch showed higher preferential removal of algae cells measured as TSS, with lower dosages and demonstrated removal of total phosphorus in wastewater with an upward trend of TP removal with higher dosages.

Although the unit cost of alum chemical ($250/ton) is lower than the cost of cationic starch ($1000/ton),[10] additional costs of

using alum that include higher dosage requirement, greater volume of biomass handling, non-biodegradability, and disposal of flocculated solids must be included. On the other hand, cationic starches serve as substrates in anaerobic digestion or fermentation processes using the harvested biomass as feedstock and such biomass can be safely used as animal feed or fertilizer. The cost of cationic starch based algae separation can be further offset if the starch can be sourced from waste streams of corn or potato processing industries, and the flocculated algae biomass is used as feedstock for value bioproducts that include biosolvents, biodiesel, and biogas, in addition to animal feed and fertilizer.

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