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Microalgae flocculation: Impact of flocculant type, algae species and cell concentration

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ABSTRACT

Flocculation is an effective means of de-watering microalgae. This study was conducted to evaluate how cell type and concentration impact flocculation efficiency. Aluminum sulfate ($Al_2(SO_4)_3$) and two cationic starches with degree of substitutions of 0.5 and 0.2 (DS05 and DS02) were used to flocculate cells of *Scenedesmus* spp., *Chlamydomonas reinhardtii*, and *Schizochytrium limacinum* at three cell concentrations. The amount of cells flocculated per mg of flocculant used was 4–28 times greater with the modified starches than with $Al_2(SO_4)_3$. The maximum amount of cells flocculated per mg of flocculant was the greatest for *S. limacinum* (414 mg cells/mg DS05 and 25.6 mg cells/mg $Al_2(SO_4)_3$), which had a surface zeta potential of −9.97 mV. The flocs produced by the starches were more concentrated in cells and less prone to disruption than those produced with $Al_2(SO_4)_3$. In general, at high cell concentrations the mass of cells flocculated per unit mass of DS05 and $Al_2(SO_4)_3$ increased for all algae species. Cationic starches, especially those with high degree of substitution, provide an efficient and ecologically friendly way to harvest microalgae for biofuel production. This study achieved the goal of evaluating important factors and conditions that are unique for a particular algae production system in order to most efficiently harvest microalgae by flocculation.

1. Introduction

Microalgae rich in oil have attracted much attention because of their high efficiency to produce lipids as a feedstock for the production of biofuels, reducing CO_2 in the environment, and producing various value added products. Photosynthetic microalgae species have been proposed as a means to capture CO_2 [1]. Heterotrophic microalgae species could be grown on relatively inexpensive organic carbon sources such as biodiesel derived waste glycerol [2–4], for the production of lipids for food and non-food applications. For example, the heterotrophic strain *Schizochytrium limacinum* can accumulate up to 50% of its biomass as lipids and because of its high content of polyunsaturated fatty acids, it may be used as a feedstock for producing food supplements or biofuel [5]. The genetically modified *Chlamydomonas reinhardtii* 21st strain is a fresh water mutant that may accumulate lipids up to 35% of its biomass from our own lipid extraction testing (unpublished data). Microalgae have also been proposed as an alternative source of proteins [6].

Two important technological barriers to commercial microalgae biofuel production are biomass harvesting and dewatering. In general, microalgae cell cultures are grown at low dry cell concentrations (0.05–18 g/L) [2,7]. In addition, their small size and a density similar to that of water make their harvest very difficult. Centrifugation can harvest algal cells at a high cell density, but it is energy intensive. To harvest the algal cells from the liquid, it is necessary to flocculate the single cells into large cell aggregates. Flocculation is a chemically based separation process that requires less energy than centrifugation and ultrafiltration, and thus, is regarded as the most promising means for algae dewatering [8]. Flocculation is the result of the particle collision and charge interaction between charges of the flocculants and cell surface in a liquid medium. When particles cluster together as the result of the flocculation process the settling rate increases [9]. The apparent surface charge of the cells is represented by its zeta potential, which may affect flocculation efficiency [10].

Various flocculants have been studied in wastewater treatment processes, including both inorganic and organic types of flocculants. Inorganic flocculants include salts of polyvalent cations such as $Al_2(SO_4)_3$, $Fe_2(SO_4)_3$, and $FeCl_3$. Common organic flocculants include polyacrylamides [11]. Although more efficient than the inorganic salts, they are not preferred because of their low biodegradability in general [12]. Cationic starches as biodegradable flocculants [12] have been tested for harvesting photosynthetic microalgae [7]. These starches are made from natural sources (i.e. corn, wheat, or potato starch) and they can

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be partially hydrolyzed to their sugar monomers by α-amylase and glucoamylase [13]. The degree of substitution (DS) represents the number of substituting groups (i.e. cationic groups) per glucopyranosyl unit in the starch. The typical positively charged groups in cationic starches are quaternary amines. Then, greater DS means greater number of charges per glucopyranosyl unit in the starch. However, only starches with low DS (0.11 and 0.15) have been studied for harvesting photosynthetic algae [7]. In general, cationic starches with DS < 0.95 are considered less toxic than the polyacrylamide-based synthetic products, especially the cationic starches with DS < 0.6 [12]. In a kaolin clay particle model system, low concentrations of cationic starches with increasing DS were necessary to maximize the flocculation efficiency [14]. Therefore, it is necessary to explore the use of cationic starches with DS greater than 0.15 as flocculants for microalgae dewatering.

The objective of this study was to evaluate and compare the efficiency of an inorganic flocculant ($Al_2(SO_4)_3$) and two cationic starches with DS that are greater than 0.15 (DS of 0.2 and 0.5) on flocculation of high-oil microalgae including two photosynthetic and one heterotrophic species.

2. Materials and methods

2.1. Microalgae cells and sample preparation

S. limacinum SR-21 (ATCC MYA-1381) cells were grown in a medium containing 15 g/L glucose, 1.0 g/L yeast extract, and 1.0 g/L peptone in artificial seawater. Each liter of artificial seawater contained 18.0 g NaCl, 2.4 g $MgSO_4 \cdot 7H_2O$, 0.6 g KCl, 1.0 g $NaNO_3$, 0.3 g $CaCl_2 \cdot 2H_2O$, 0.05 g KH_2PO_4, 1.0 g Trizma base (Sigma Co.), 0.027 g NH_4Cl, 1.35×10^{-9} g vitamin B_{12}, 1 mL chelated iron solution to obtain 26 mM $Na_2EDTA \cdot 2H_2O$ and 3 mM $FeCl_3 \cdot 6H_2O$ in the final seawater, and 10 mL of a solution of trace elements containing boron, manganese, zinc, cobalt, and iron [15]. The medium was adjusted to pH 7–8 and autoclaved at 121 °C for 15 min. The cultures were grown in six 100-mL Erlenmeyer flasks and transferred into a 5-L BioFlo 310 New Brunswick fermentor/bioreactor (Edison, NJ) holding 4 L of a medium containing 5 g/L corn steep solids and 70 g/L glucose. The fermentor conditions were set at 25 °C, pH 7, 60% dissolved oxygen, and air flow 0.2 vvm (gas volume per liquid volume per minute). The cells at stationary phase were harvested with a cell concentration of 9.29 g dry weight/L.

C. reinhardtii 21st, with high lipid content (up to 35%) obtained from professor Martin Spalding (Iowa State University), were grown in 250 mL Erlenmeyer flasks with TAP medium [16,17] containing 2.42 g/L Trizma base (Sigma Co.), 0.375 g/L NH_4Cl, 0.100 g/L $MgSO_4 \cdot 7H_2O$, 0.050 g/L $CaCl_2 \cdot 2H_2O$, 0.179 g/L K_2HPO_4, 0.054 g/L KH_2PO_4, 1 mL/L glacial acetic acid, 1 mL/L of Hutner's trace elements solution, and pH 7.4 [18]. After 3 days, the cultures were transferred to two 1 L-bubble column containing the same medium. The material from this column was used as inoculum for a culture in a 16 L-flat panel photobioreactor. Both the column and the biophotobioreactor were aerated at a rate of 0.32 vvm and continuously illuminated with fluorescent light at 100 μmol m^{-2} s^{-1}. The cells in the photobioreactor were harvested at 72 h (stationary phase) with a final cell concentration of 1.06 g dry weight/L.

Scenedesmus spp. were grown in 15–20 cm depth and 30.5 m raceway open ponds in Roanoke, LA using the natural field nutrients (fertilizers) and local well water. The pH of the cultures was controlled between 8.5 and 9.5 with CO_2 bubbling. Four hundred and thirty five gallons of algae culture (0.11 g solids/L) were centrifuged upon arrival in the pilot plant of the Center for Crops Utilization Research at Iowa State University, using an Alfa Laval BTPX-205TGD-14/34 CDP-60 centrifuge at 10,000 rpm. The process was done at ambient temperature and the concentrated material (17.1 g solids/kg) was stored overnight at 5 °C before being re-suspended in the culture medium (collected as supernatant after centrifugation) for the flocculation experiments.

The stock cell suspensions were diluted with their respective medium to the desired cell concentration levels. For each algae species, flocculation tests were done at three different algae concentrations. For *S. limacinum*, the cell concentrations used were: 0.09, 0.93, and 4.65 g/L; for *C. reinhardtii*, they were 0.03, 0.31, and 1.06 g/L; and for *Scenedesmus* spp., 0.05, 0.20, and 1.00 g/L.

2.2. Flocculants used

Three different flocculants were studied: $Al_2(SO_4)_3$ (Fisher Scientific, Pittsburgh, PA) and two cationic starches with different DS. The positively charged groups are quaternary amines attached to the polymeric glucose units, and the DS05 and DS02 starches are with DS of 0.5 and 0.2, respectively. These starch samples were provided by a vendor who requested to be anonymous.

2.3. Flocculation experiment

The effect of the flocculant type and concentration on flocculation efficiency was determined using a jar test [7,19]. Briefly, the algae suspension (100 mL) was stirred at 250 rpm in a 100 mL beaker. After the flocculant was added, the stirring continued for 2 min. Then, the stirring stopped and the suspension was allowed to set for 20 min when an aliquot of the supernatant was taken 2 cm from the surface of the liquid and its absorbance at 550 nm was measured in a 10-mm path length plastic cuvette using a DU720 spectrophotometer (Beckman Coulter Inc., Brea, CA). A calibration curve was prepared with dilutions of the microalgae suspension (100, 50, 25, 12.5, 6.3, 3.1, and 1.6%) for each species at each cell concentration. Eq. (1) was used to obtain the concentration of microalgae in the supernatant suspension. The efficiency values reported are the percentage microalgae concentration reduction from the starting microalgae concentration used in the jar test (Eq. (2)).

$$A = a \times C_{algae} + b \tag{1}$$

$$Efficiency(\%) = 100 \times \left(1 - \frac{C_{algae\ in\ the\ supernatant}}{C_{algae_o}}\right) \tag{2}$$

where A is the absorbance, a is the slope and b is the Y-intercept, and C_{algae} is the concentration of algae in suspension at which the absorbance was measured, $C_{algae\ in\ the\ supernatant}$ is the concentration of algae still in the supernatant, and C_{algae_o} is the concentration of algae before the addition of the flocculant.

The relative efficiency (mg of cells flocculated per mg of flocculant used when the flocculation efficiency was maximum) for each cell concentration and flocculant (Eq. (3)) was calculated as:

$$Relative\ efficiency = Maximum\ efficiency_{(\%)} \times \frac{C_{algae}}{C_{flocculant}} \tag{3}$$

where C_{algae} is the initial algae cell concentration and $C_{flocculant}$ is the concentration of flocculant when the efficiency is maximum, and $Maximum\ efficiency_{(\%)}$ is the maximum value obtained for each algae concentration and flocculant type as calculated in Eq. (2).

After decanting the supernatant, the concentrated algae suspension was transferred into a graduated tube with tapered bottom, and the settled volume of the cell concentrate was read directly on the tube after 10 min.

2.4. Zeta potential determination

The zeta potential of the different algae species was determined with a Malvern ZetaSizer Nano ZS90 (Malvern Instruments Ltd.) using 1 mL cell dispersions in their respective medium at concentrations of

0.18 g/L (*S. limacinum*) and 0.05 g/L (*C. reinhardtii* and *Scenedesmus*) without pH and osmotic adjustment [20].

2.5. Statistical analysis

All the flocculation treatments were run in duplicate and the means of the efficiencies and floc volumes were analyzed using one-way ANOVA and contrasts for the comparison of the means with the Proc GLM from SAS 9.1 (SAS Institute Inc., Cary, NC). The significance level was established at $P = 0.05$ unless otherwise noted.

3. Results and discussion

3.1. S. limacinum flocculation

S. limacinum is a marine heterotrophic species, which can grow, by fermentation, to a greater cell concentration than autotrophic species. As shown in Fig. 1, the use of $Al_2(SO_4)_3$ as a flocculant resulted in flocculation efficiencies greater than 90% at cell concentrations of 0.93 g/L and 4.65 g/L. However, the maximum flocculation efficiency was reduced to 68% when at a lower cell concentration (0.09 g/L). At this low cell concentration the flocculation efficiency also decreased with increasing flocculant concentration. The reason can be that too many charges from $Al_2(SO_4)_3$ interacted with the surface of the small number of algae cells. The counteraction of extra charges in the suspension diminished the flocculating effect of $Al_2(SO_4)_3$ and stabilized the suspension. In addition, the effect of the $Al_2(SO_4)_3$ (an acidic salt) on the pH of the medium may result in a change of charge density on the surface of the cells with a consequent change in the flocculation efficiency [21]. The negative values observed in this and other treatments are a result of not forcing the calibration curves through zero in order to maximize the fitting of the curves. At increasing cell concentration, the relative efficiency of $Al_2(SO_4)_3$ increased slightly and then it plateaued (Fig. 2).

For the cationic starches, DS02 was not effective at any of the cell concentrations tested. The low density of cationic groups may not have been sufficient to induce cell aggregation. DS05, a cationic starch with a greater degree of substitution had maximum efficiencies greater than 80% for the two greater cell concentrations. For the 0.09 g/L cell concentration treatments, after reaching a maximum 37%, the efficiency of the flocculation started to rapidly decrease. The same decreasing trend was observed for the 0.93 g/L cells. This decrease was also the result of an excess of positive charges, which contributed to the stabilization of the particles in suspension by repelling each other, as well as steric hindrance when polymers were used [7]. Several studies proposed that some cationic polymers are not effective in flocculating marine algae species because of the high concentration of NaCl, with the ratio of starch to algae needing to be close to one [7,11]. In the study published by Vandamme et al. [7], the DS of the cationic starches tested was 0.15 and 0.11, lower than the DS values used in this work (DS 0.2 and 0.5). Their starches showed different effectiveness toward 4 microalgae with three being different from the ones tested in this study. Their cationic starch was effective for freshwater microalgae (*Parachlorella* and *Scenedesmus*) but not for marine microalgae (*Phaeodactylum* and *Nannochloropsis*). We have shown that marine alga *S. limacinum* can be flocculated with our cationic starch with DS of 0.5, and the cells could also be flocculated much more efficiently compared to other algae (Fig. 2) as discussed later.

The results obtained with DS05 demonstrate that efficient flocculation is possible at high NaCl concentration (18 g/L) with a cationic starch having greater DS. Another factor to consider is the zeta potential of *S. limacinum* (Table 1), which is closer to zero than the other two algae, making it easier to flocculate. In a previous study, flocculation of four algae species was shown to be highly dependent on their zeta potential, with maximum flocculation efficiencies at zeta potentials between −8 and +2 mV after flocculant addition [10]. The zeta potential of *S. limacinum* before flocculant addition was very close to this range (−9.97 mV).

The relative efficiency of DS05, at 0.93 and 4.65 g/L cell concentrations was greater than for $Al_2(SO_4)_3$ (Fig. 2). DS05 was much more efficient at greater cell concentrations than at lower cell concentrations, probably because of a more efficient interaction of the cells with the charges carried by the starch. At greater cell concentrations, the probability of the cationic groups in a chain of starch interacting with more cells is greater. Wyatt et al. [21] also reported a possible flocculation mechanism change at higher cell concentration from the "bridging" to "sweep" flocculation when ferric chloride was used as a flocculant. This can be used to explain the observations in the present study as well. Both flocculants, DS05 and $Al_2(SO_4)_3$, were much more efficient when flocculating *S. limacinum* than when flocculating either one of the other species in terms of amount of cells flocculated per mg of flocculant used (Fig. 2). The initial zeta potential of the system, a consequence of the cell–medium interaction, was much lower than for the other microalgae species, and as discussed above, it was closer to the optimum range for flocculation (−8 mV to +2 mV) proposed by Henderson et al. [10]. Therefore, any modification of the zeta potential of algae particles surface, such as genetic modification to change the chemical composition of the cell wall thus its charge type and density, or more practically modifying the cell growth cycle and medium conditions thus altering algae cell surface characteristics, may have an important impact on the economics of the process by minimizing the amount of flocculant necessary to maximize flocculation yield.

3.2. C. reinhardtii flocculation

The strain of *C. reinhardtii* used is a high oil producing strain, and it may accumulate up to 35% oil as energy reserve. In the case of this fresh water photosynthetic algae species, $Al_2(SO_4)_3$ was able to flocculate more than 90% of the cells in suspension at the three cell concentration levels studied (0.03, 0.31, and 1.06 g/L) (Fig. 3). The decreasing efficiency observed on the 0.03 g/L treatment at high $Al_2(SO_4)_3$

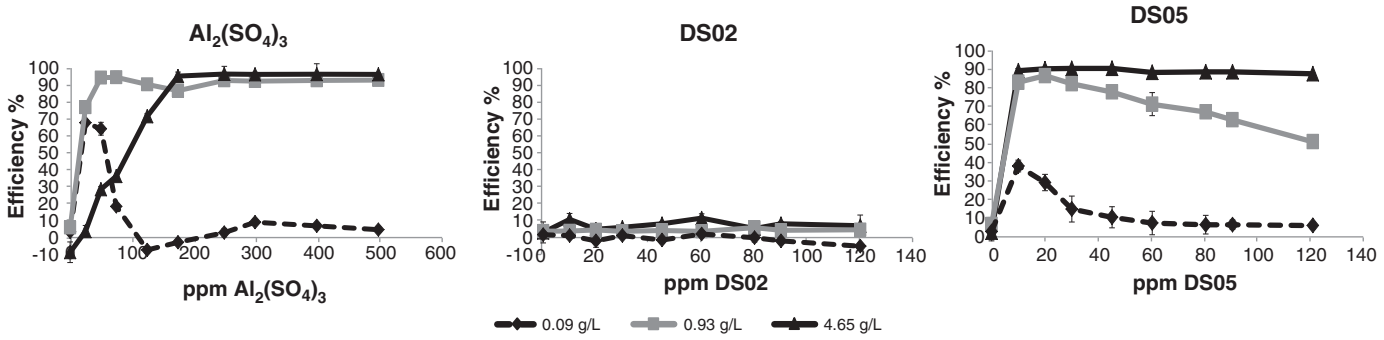

Fig. 1. Flocculation efficiencies of $Al_2(SO_4)_3$ and cationic starches DS02 and DS05 at three cell concentrations of *S. limacinum*. Error bars denote standard deviations of the means.

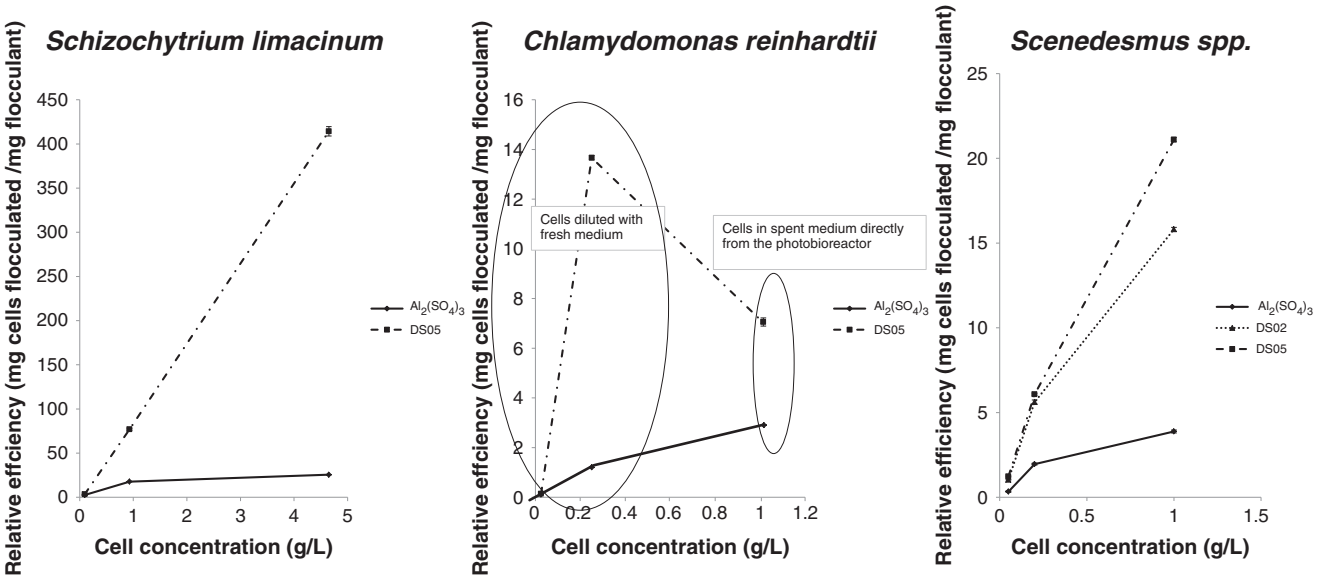

Fig. 2. Relative efficiencies (mg cells flocculated per mg of flocculant) of the different flocculants at selected cell concentrations of *Schizochytrium limacinum*, *Chlamydomonas reinhardtii*, and *Scenedesmus* spp. Note that the scales for the different species are different. Error bars denote standard deviations of the means.

concentrations may be the effect of flocculant on the pH of the medium, thus decreasing the number of negatively charged groups on the surface of the cells and the reduced flocculation efficiency [20]. Another plausible explanation could be that the amount of flocculant that exceeded the optimum concentration could contribute to an excess of positive charges, thus stabilizing the cell particles in suspension by charge repelling, as well as by steric hindrance [7]. Greater cell concentrations increased the cell recovered per mg of $Al_2(SO_4)_3$ used, i.e., increased relative flocculation efficiency (Fig. 2).

DS02 was only effective when treating the intermediate cell concentration (0.31 g/L). At 0.03 g/L, neither DS02 nor DS05 flocculated *C. reinhardtii*, probably because the concentrations of the flocculants used were relatively too high for such a low cell concentration. On the other hand, at 1.06 g/L cell concentration, even the maximum DS02 concentration may not have been sufficient to induce the flocculation of *C. reinhardtii* as a consequence of the low DS. DS05 had efficiencies greater than 85% for the two greater cell concentrations. At 0.31 g/L, after reaching a maximum at 45 ppm DS05 (Fig. 3), the efficiency slowly decreased with increasing DS05 concentrations. This effect was not observed for the 1.06 g/L treatment, because the greatest DS05 concentration tested (120 ppm) was not enough to produce the particle stabilization effect observed at the high cell concentration. The relative efficiency of DS05 increased with a 10-fold magnitude in the cell concentration from 0.031 to 0.31 g/L; however, at the highest cell concentration, it decreased (Fig. 2). In this case, the cells had not been diluted to the desired concentration with fresh medium, but rather the test was done directly on the material as it came out of the photobioreactor. The medium had changed in composition and pH had increased from 7.4 to 8.4 as a result of the algae growth and the consumption of the nutrients; thus this may have resulted in different flocculation performances.

Table 1
Zeta potential of the selected microalgae species at the time of harvest.

Microalgae species	Zeta potential (mV)
Shizochytrium limacinum	−9.97[b]
Chlamydomonas reinhardtii	−19.95[a]
Scenedesmus spp.	−20.60[a]

[a–b]Different superscripts denote significant differences at $p < 0.05$.

3.3. Scenedesmus spp. flocculation

The $Al_2(SO_4)_3$ had efficiencies greater than 90% for all three cell concentrations (0.05, 0.20, and 1.00 g/L) of *Scenedesmus* spp., another fresh water photosynthetic algae species (Fig. 4). At the lowest cell concentration the effect of the excessive $Al_2(SO_4)_3$ resulted in a slight decrease of flocculation efficiency. For the other two greater concentrations, the amounts of flocculant used were not high enough to produce this negative effect.

DS02 and DS05 had comparable effects on the flocculation of *Scenedesmus* spp. (Fig. 4). For the 0.05 g/L cell concentration, both starches reached maximum efficiencies greater than 60% and for 0.20 and 1.00 g/L treatments, both surpassed 90% efficiencies. DS02 and DS05 had increasing relative efficiencies with increasing cell concentrations. However, at a concentration of 1.00 g/L it is evident that the greater DS starch increased the amount of cells flocculated per mg of DS05 when compared to DS02 (Figs. 4 and 2). The reason why DS02 was effective at all cell concentration levels may be because of the pH of *Scenedesmus* spp. suspension being quite alkaline (pH > 8). This alkaline condition favored the presence of negative charges on the cells, thus favoring their interaction with the quaternary amine groups of the starch [7].

These flocculation experiments illustrate that each type of alga responded very differently to flocculation treatments. Different flocculants also had a very different effect on flocculation efficiency. Therefore, as a commercial operation growing a particular alga, flocculants need to be tested and optimized in great detail to ensure the identification of the best conditions for the most effective flocculation and the highest flocculant efficiency.

3.4. Volume and solid content of the precipitated algae concentrate

In general, $Al_2(SO_4)_3$ produced fluffier, less concentrated flocs than the cationic starches (Table 2). This is probably because the cells accumulated along the starch chains in a more ordered structure [22] than when $Al_2(SO_4)_3$ was used. Cell concentration in the various concentrates was calculated. For example, the initial volume of the flocculation experiment was 0.1 L, initial *Chlamydomonas* concentration was 0.31 g/L, the efficiency was 87% and the concentrate (floc) volume was 3.75 mL for the DS05 treatment replicate 1, then, the algae concentration in the concentrate = (0.1 L * 0.31 g/L * 0.87 * 1000 mL/L) / (3.75 mL) = 7.2 g/L. These calculated concentration values also indicate

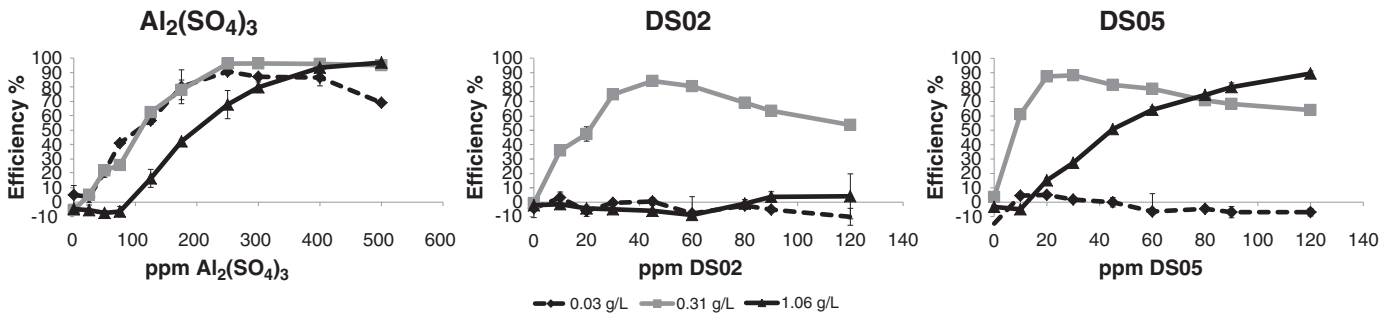

Fig. 3. Flocculation efficiencies of $Al_2(SO_4)_3$ and cationic starches DS02 and DS05 at three cell concentrations of *C. reinhardtii*. Error bars denote standard deviations of the means.

that the cationic starch-produced cell concentrates had higher mass content than the $Al_2(SO_4)_3$-produced concentrates. It again shows that *S. limacinum* can be compacted much more than the other two algae.

Cationic starches are a biodegradable alternative to other synthetic polymers. As previously discussed, toxicological studies demonstrated that toxicity of cationic starches was lower than that of polyacrylamide-based synthetic products, especially for cationic starches with DS < 0.6 [12]. Cationic starches are used as paper sizing agents, and DS02 is approved for use in paper that may be in contact with food products. Because most of the cationic starch flocculant will remain with the flocculated biomass, this can translate to an addition of up to ~10% material to *C. reinhardtii*. Since cationic starches may be hydrolyzed to sugars [13], part of the cost of using cationic starch may be recovered as part of the fermentable biomass after defatting.

3.5. Effect of medium composition on flocculation efficiency

To evaluate the effect of medium on flocculation efficiency, *Scenedesmus* spp. cells concentrated by centrifugation and stored frozen at −22 °C were re-suspended in deionized water (DI water) or growth medium at 0.1 g solids/L. This system was treated with $Al_2(SO_4)_3$ at a concentration of 125 ppm. At this flocculant concentration, cells flocculated at all concentrations when the experiment was done in the fresh growth medium. In the DI water test, however, no flocculation was observed. When the cells re-suspended in DI water were adjusted to pH in the range 3 to 11, flocculation was only observed when the initial pH was 11. After the addition of the $Al_2(SO_4)_3$ the pH of the DI water system decreased substantially, to values of 4 and below. However, when the cells were re-suspended in the growing medium at the same concentration (0.1 g solids/L), flocculation was observed and the system pH decreased from 8.14 to 6.40. This pH reduction was much smaller than when DI water adjusted to the same pH was used to re-suspend the cells, probably because of the buffering capacity of the growing medium. The effect of the pH induced by inorganic salts ($FeCl_3$) on the flocculation efficiency of *Chlorella zofingiensis* was explained by Wyatt et al. [21]. Briefly, pH determines the charges present on the surface of the cells and then the effect is dependent on the type and number of charged groups (amines, carboxylic acids, phosphates) on the cell surface, which would interact with the flocculant. Therefore, the pH of the medium will impact the interaction of the flocculant with these charged groups, thus influencing its flocculation efficiency.

The effect of the suspension medium on the efficiency of the cationic starches was also studied. Maximum flocculation efficiency occurred at lower DS02 and DS05 concentrations when the cells were dispersed in DI water than when they were dispersed in the growing medium (Fig. 5). As the result of the dissolved salts, the growing medium has more charges in solution (pH 8.14) than DI water does. These charges may interact with the cationic groups from the starches, requiring a greater amount of cationic starch to maximize flocculation efficiency. Therefore, the suspension medium plays a major role on the efficiency and the concentration of the flocculant necessary for the flocculation of microalgae. It is important to point out that the *Scenedesmus* spp. cells used for this preliminary study had been frozen for storage purpose, and therefore the results should not be directly compared to those presented in Fig. 4.

During the flocculation of *C. reinhardtii*, the 0.03 and 0.31 g/L treatments were obtained as dilutions. The 1.06 g/L treatment was from fresh media and the actual cell concentration in the photobioreactor at harvesting. The decrease in the relative efficiency at the greatest cell concentration of 1.06 g solids/L (Fig. 2) was probably the result of the differences in the medium composition between the fresh medium and the nutrient depleted medium. Medium pH increased from 7.4 to 8.4 at the time of harvest. Therefore, the dilution with fresh medium would not only have decreased the pH (affecting the zeta potential) but also contributed to a diluted extracellular organic matter that could influence flocculation efficiency [23]. Therefore, not only cell type and concentration, but also the medium conditions and compositions will affect flocculation efficiency. It is thus recommended that the optimum flocculation conditions should be determined for each algae operation and under a set of unique conditions.

The mechanism of particle flocculation is in itself a scientific discipline. Various mathematical modelings of flocculation have been discussed [24]. Flocculation process mainly involves two discrete steps that are transport and attachment. The transport step leads to the

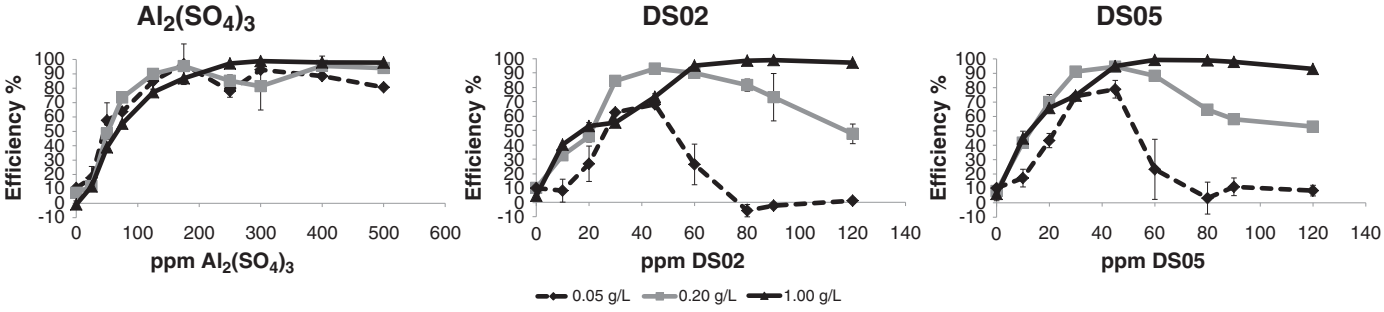

Fig. 4. Flocculation efficiencies of $Al_2(SO_4)_3$ and cationic starches DS02 and DS05 at three cell concentrations of *Scenedesmus* spp. Error bars denote standard deviations of the means.

Table 2
Volume of concentrate (mL) produced by $Al_2(SO_4)_3$, DS02, and DS05 at maximum efficiency conditions for selected concentrations of *Schizochytrium limacinum*, *Chlamydomonas reinhardtii*, and *Scenedesmus* spp.

Microalgae species	Cell concentration	Concentrate volume (mL)			Cell concentration in the concentrate (g/L)		
	(g/L)	$Al_2(SO_4)_3$	DS02	DS05	$Al_2(SO_4)_3$	DS02	DS05
Schizochytrium limacinum	0.09	1.0	–	–	6.1	–	–
	0.93	3.5^{x}	–	1.25^{y}	25.9^{x}	–	64.1^{y}
	4.65	21.3^{a}	–	11.8^{a}	21.8^{x}	–	35.4^{y}
Chlamydomonas reinhardtii	0.03	9.3	–	–	0.3	–	–
	0.31	12.3^{a}	$2.8^{b\ x}$	$3.9^{b\ y}$	2.4^{b}	$9.6^{a\ x}$	$7.0^{a\ y}$
	1.06	22.5^{a}	–	17.5^{a}	4.6	–	5.4
Scenedesmus spp.	0.05	12.5^{a}	2.5^{b}	2.0^{b}	0.3^{c}	1.2^{b}	1.9^{a}
	0.20	10.0^{a}	1.5^{b}	1.4^{c}	1.5^{c}	11.3^{b}	13.0^{a}
	1.00	20.5^{a}	7.8^{b}	7.8^{b}	4.9^{y}	12.6^{x}	12.6^{x}

$^{a–c}$Different superscripts within the same row denote significant differences at $p < 0.05$.
$^{x–y}$Different superscripts within the same row denote significant differences at $p < 0.07$.

collision of two particles which is achieved by particle velocities through the random Brownian motion of the particles, mechanical mixing, and differences in settling velocities of individual particles. Attachment is then induced by van der Waals and electrostatic attractions which are largely pertaining to the nature of the surfaces themselves. These interparticle forces among microorganisms in aqueous dispersion and their aggregation behavior can be quantitatively explained by the DVLO theory. Flocculation kinetics and particle collision and flocculation frequencies are influenced by agitation speed, medium pH, ionic strength, particle size, and particle concentration [25]. In addition, during the algae cell flocculation process, the size and shape of particles or clusters will change from the conventional spherical assumption; thus fractal mathematics can be used to model particle coagulation and aggregation [26]. Many of the observational studies of microalgae flocculation may be modeled using the extensive knowledge developed in colloidal and water treatment sciences.

4. Concluding remarks

The efficiency of the flocculants tested was highly dependent upon the type of cells, their concentration and the medium conditions. In general, the amount of cells flocculated per mg of flocculant increased with increasing cell concentrations. Cationic starches, especially those with high DS, are an effective and ecologically friendly alternative for the processing of algae for biofuel production purposes. However, more detailed analyses including their impact on other processing aspects such as lipid and protein extraction may be necessary. In addition, a more thorough quantification of how a flocculant changes pH and surface zeta potential under various conditions should be conducted in future work; hence, more insights on mechanism of flocculation can be obtained. This study serves as an example of studying a few factors that can greatly affect algae flocculation. It is necessary to examine more levels of treatments and at conditions that are unique for a particular algae production system in order to achieve the most efficient flocculation.

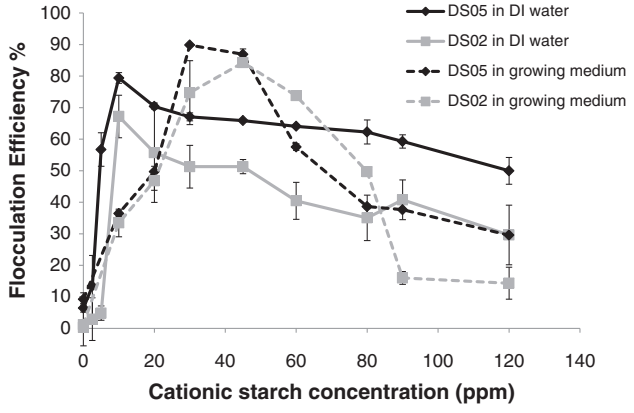

Fig. 5. Flocculation efficiency of cationic starches DS02 and DS05 in 0.10 g/L *Scenedesmus* spp. cells, which were concentrated by centrifugation, stored frozen at −22 °C, and resuspended in either growing medium or deionized water (DI water). Error bars denote standard deviations of the means.

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