Study of algal biomass harvesting through cationic cassia gum, a natural plant based biopolymer

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HIGHLIGHTS

• Cationic cassia was synthesized and characterized.
• Flocculation dosage was optimized for two different green algae.
• Flocculation mechanism was hypothesized.
• Cationic cassia was proved to be a better flocculant than cassia.

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ABSTRACT

Green unicellular microalgae have a capacity to entrap CO₂ to increase their biomass through photosynthesis and are important for the value added product. The presence of COOH and NH₂ groups are responsible for imparting negative zeta value. The present work emphasizes on the synthesis of cationic cassia (CCAS) by the insertion of quaternary amine groups onto the backbone of cassia (CAS) from N-3-Chloro-2-hydroxypropyl trimethyl ammonium chloride (CHPTAC) which was further characterized via FTIR, SEM, elemental analysis and intrinsic viscosity. The optimal dosage of the synthesized cationic cassia is used to flocculate two different green fresh water algae viz. Chlamydomonas sp. CRP7 and Chlorella sp. CB4 were evaluated. 80 and 35 mg L⁻¹ was optimized dose for dewatering of above algae, respectively.

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1. Introduction

Depleting non renewable fuels pave the way to switch towards ecofriendly renewable energy source. Microalgae have the capacity to sequester greenhouse gas and transform into value added products. The biodiesel yield is 20 times higher than other oil crops, the energy efficient pathway for the conversion of biomass to biofuel to be applied in commercial level and recent development regarding unifying framework towards policy making for biofuel are well described (Chisti, 2007; Gasparatos et al., 2013). Microalgae as the name suggests are of smaller size (3–30 μm). Negative charge and low concentration are the key factors for stability in a suspended form. This stability makes the harvesting process more challenging (Gudin and Thepenier, 1986; Chen et al., 2013).

Harvesting techniques like centrifugation (Molina Grima et al., 2003), filtration (Danquah et al., 2009), electrocoagulation flocculation (Vandamme et al., 2011), electroflocculation with dispense air floatation (Xu et al., 2010) are well studied. Use of magnetic (Fe³⁺) nanoparticle for flocculation with higher efficiency rate and less time was also studied (Xu et al., 2011).

Beside this, inorganic flocculants such as aluminum sulfate and iron chloride (Uduman et al., 2010), aluminum nitrate sulfate (Rwehumbiza et al., 2012) also used in flocculation. Extracellular biopolymers from the self-flocculating microalga Scenedesmus obliquus was used to dewater S. obliquus and C. vulgaris (Guo et al., 2013).

Although a lot of techniques of harvesting microalgae have been studied by various workers. Centrifugal based separation technique is found to be energy intensive and high cost. Filtration based separation technique is of high cost and the operation gets interrupted frequently due to membrane fouling. Flocculation based technique seems to be the ultimate promise for commercial harvesting of microalgae. However inorganic flocculants (e.g. Alu-
minum as flocculants) pose the problem of contamination of the ultimate products which limits its usability as animal feed. So the ultimate approach is the use of organic flocculants based on biopolymeric material and modified biopolymeric material whose presence in the ultimate product might not lead to serious complication.

Chemically, cassia is a polysaccharide composed linear chain of 1,4-β-D-mannopyranose units with 1,6 linked α-D-galactopyranose units and are obtained primarily from the endosperm of two different leguminous plant species *Cassia torea* and *Cassia obtusifolia*.

Chitosan contains a positive charge due to positive amino groups. Under acidic condition these chitosan molecules have high positive charge and thus are active in flocculation by binding with microorganism having negatively charged cell surface. Because of its cationic nature, biodegradability and low-toxicity, chitosan is also used in wastewater treatment (Lertsittichai et al., 2007; Wang et al., 2008).

Biopolymers such as guar gum which has been the subject of study in both native and modified form for a variety of applications. Grafted guar gum (Mishra and Sen, 2011), grafted tamarind kernel polysaccharide (Ghosh et al., 2010), hydrolyzed polyacrylamide grafted tamarind kernel polysaccharide (Ghosh et al., 2011), Cationic guar gum (Singh et al., 2006; Banerjee et al., 2013) has been elaborately studied for the flocculation purpose. Starch grafted polyacrylamide was also studied in relation to algal flocculation (Banerjee et al., 2012b).

Similarly chemical modification of tamarind kernel polysaccharide (Pal et al., 2009), glycogen (Pal et al., 2008) and amylopectin (Singh et al., 2012) has been carried out and used to flocculate textile industry waste water, coal suspension and kaolin, iron ore respectively.

Recently different approaches in flocculation process, challenges and possible solutions in algal flocculation area are well addressed (Vandamme et al., 2013). The commercial viability towards the high value product from algae needs a high biomass and its downstream processing, which includes the harvesting step.

The present study involves synthesis of CCAS and its application in harvesting of microalgae (*Chlamydomonas* sp. CRP7 and *Chlorella* sp. CB4). No such study has been reported to the best of our knowledge. The zeta potential value and percentage recovery was thoroughly investigated. The harvested biomass was also assessed by light microscopy to check their cell integrity. The advantage of cationized cassia (CCAS) over normal cassia (CAS) as flocculant is the lowest dosage requirement for quick dewatering of algae.

2. Methods
2.1. Isolation and culturing

*Chlamydomonas* sp. CRP7 and *Chlorella* sp. CB4 were isolated from the specific area of Global Positioning System coordinates viz. N.2324.51’S; E.8526.24’. They were isolated by phototaxis method. DNA amplification and characterization of ITS region was performed according to Banerjee et al. (2012). They were submitted to GenBank under accession No. JQ408690 for *Chlamydomonas* sp. CRP7 and JQ710683 for *Chlorella* sp. CB4. The isolated algae was cultured in TAP medium (Gorman and Levine, 1965) and incubated at 25 °C under light (107 μmol m⁻² s⁻¹) with 16:8 h light/dark photoperiods. The stationary phase culture was used for flocculation.

2.2. Synthesis of cationic cassia

The synthesis of cationic cassia was carried out by insertion of cationic moiety, N-3-Chloro-2-hydroxypropyl trimethyl ammonium chloride (CHPTAC) onto the polysaccharide backbone. Cassia powder was dispersed at room temperature in 150 ml of 70% Isopropanol solution (IPA) by constant stirring for about 30 min. Required amount of caustic solution (15 ml) was added with continuous stirring effect for 20 min. This was followed by adding intended amount of cationic reagent. The flask was immersed in a thermostatic water bath keeping the temperature (55–60 °C) at which the reaction was allowed to proceed for the desired duration. Dilute hydrochloric acid was added for lowering the pH below 7.0 to stop the cationization process (Larsson and Wall, 1998). The mechanism has been illustrated in Scheme 1.

The solution was thereafter cooled to room temperature and was precipitated in excess Isopropanol followed by washing with 200 ml of aqueous 80% IPA solution (Banerjee et al., 2013). The product was finally dried at room temperature and then in hot air oven at 50 °C for 6 h.

2.3. Characterization

2.3.1. Zeta potential value measurement and microscopic examination of algal floc

Zeta potential value of algal cells (*Chlamydomonas* sp. CRP7 and *Chlorella* sp. CB4) before and after the flocculation was measured using electrophoresis method (Zeta NS, Malvern Institute, UK). Zeta potential value indicates the stability of colloidal suspension. High positive and high negative value indicate more stability. Any effective coagulant/flocculant is supposed to drastically reduce the zeta potential value (tends to zero) so that the particle can approach each other and aggregate.

Morphological examination of algal cells after flocculation was observed by taking out the floc from the bottom of the beaker by the microscopic system (Leica FW4000, Germany) study at low magnification (10×) to check the integrity of algal cells.

2.3.2. Elemental analysis

The elemental analysis of cassia (CAS) and cationic cassia (CCAS) was analyzed using an elemental analyzer (Vario El III, Elementar, Germany). The estimation of three elements i.e., carbon, hydrogen, nitrogen was undertaken. Degree of cationization (DC) was also calculated by the following equation:

\[ DC = \frac{\%N \text{ in C-CAS} - \%N \text{ in CAS}}{\%N \text{ in CHPTAC}} \]

Degree of cationization is the ultimate parameter quantifying the extent of substitution by quaternary ammonium group.

2.3.3. Intrinsic viscosity measurement

Viscosity measurements of the polymer solutions were carried out with an Ubbelohde viscometer (CS/S: 0.003899) at 25 °C. The viscosities were measured in 1 M NaNO₃ solution. The pH of the solution was neutral. The time of flow for solutions was measured at four different concentrations. From the time of flow of polymer solutions (t) and that of the solvent (t₀, for distilled water), relative viscosity (ν = t/t₀) was obtained. Specific viscosity was calculated from the relation ν = ν₀ − 1. Reduced viscosity (νr) and the inherent viscosity (νi/C) were calculated, where C is the polymer concentration in g/dL. The intrinsic viscosity was obtained from the point of intersection after extrapolation of two plots, i.e. νr/C vs C and ln νi/C vs C, to zero concentration.

As investigated in earlier studies (Brostow, Singh and Pal’s model of flocculation) the efficacy of a flocculant is proportionally to the surface area and consequently the hydrodynamic volume of the molecule i.e. intrinsic viscosity. Thus it is expected that a positive correlation exist between this two aspects (Intrinsic viscosity and flocculation efficacy).
2.3.4. FTIR spectroscopy
The FTIR spectrums of CAS, CHPTAC and CCAS were recorded in the solid state by KBr pellet method, using FTIR spectrophotometer (IR-Prestige 21, Shimadzu Corporation, Japan) between 400 and 4000 cm$^{-1}$.
Appropriate peaks in FTIR spectrum essentially confirms the intended chemical modification on the targeted biopolymer.

2.3.5. SEM study
Both CAS and CCAS were made conductive by gold sputtering and surface morphology was analyzed in scanning electron microscopy (SEM) in powdered form (JSM-6390LV, Jeol, Japan).
The change in surface morphology due to cationization of the biopolymer is of research interest.

2.4. Study of algal flocculation efficacy of cationic cassia and dosage optimization
Standard “Jar test” procedure was used to study algal flocculation efficacy of the synthesized CCAS, with fresh water microalgae *Chlamydomonas* sp. CRP7 and *Chlorella* sp. CB4. 200 ml of the algal culture (*Chlamydomonas* sp. CRP7 and *Chlorella* sp. CB4) in 250 ml identical beakers. Calculated amount of the flocculants in this study (CAS and CCAS) were added. The dosage was varied from 0.0 mg L$^{-1}$ (control) to 100.0 mg L$^{-1}$. The content of these beakers were stirred identically in “Jar test” Apparatus (Simeco, India) at 75 rpm for 2 min, followed by 25 rpm for 5 min and then kept undisturbed for proper settling. Consequently, the supernatant was collected from each beaker and optical density at 750 nm was measured to plot flocculation curves (Percentage recovery vs Dosage). The flocculation efficacy, which is an indication of the viability of CCAS towards algal harvesting, is studied by comparing the flocculation curves of CAS and CCAS.

Further in each case, the beakers were continued to be kept undisturbed and the supernatant was collected after specified time. The percentage recovery was plotted against different flocculant dosage for *Chlamydomonas* sp. CRP7 and *Chlorella* sp. CB4. The percentage recovery of algae *Chlamydomonas* sp. CRP7 and *Chlorella* sp. CB4 from the culture has been evaluated as in an earlier study (Salim et al., 2011) by the relation:

\[
\text{Recovery} \% = \left( \frac{OD_{750}(t) - OD_{750}(t_0)}{OD_{750}(t_0)} \right) \times 100
\]

where $t_0$ is the initial reading (at 0 h) and $t$ is the final reading (at time $t$).

The flocculation experiment was done at constant pH. The pH for *Chlamydomonas* sp. CRP7 and *Chlorella* sp. CB4 was 7.8 and 7.6, respectively.

3. Result and discussion

3.1. Synthesis of cationic cassia
CHPTAC based cationization was used for cationization of different biopolymers (Pal et al., 2006). The synthesis details has been tabulated in Table 1. Substitution reaction was accountable for the expansion of CCAS from CAS, which is represented in Scheme 1.

3.2. Characterization
3.2.1. Zeta potential value measurement
Initially the zeta potential value for *Chlamydomonas* sp. CRP7 and *Chlorella* sp. CB4 in culture medium was found to be $-23.5$ mV and $-19.2$ mV but after flocculation with CCAS the value increases to $-9.62$ mV and $-5.77$ mV respectively. The details are
summarized in Table 2. The zeta potential value was initially negative and its magnitude decreased with increase in flocculant dose. The ability of flocculant to decrease the magnitude of zeta potential warrants its high efficacy.

3.2.2. Estimation and interpretation of intrinsic viscosity

It is obvious that intrinsic viscosity of CCAS will be greater than that of CAS. This can be explained by an increase in hydrodynamic volume due to incorporation of cationic moiety onto the backbone of the parent polymer as shown in Table 1.

The increase in hydrodynamic volume is due to following reasons:

- Repulsion between the added cationic moiety–stretching/uncoiling the backbone of cassia.
- Added volume of cationic moiety.

The higher intrinsic viscosity of the final product than the raw material ensure superior flocculation properties as intended.

3.2.3. Elemental analysis

The results of elemental analysis for both CAS and CCAS are given in Table 3. The presence of the high percentage of nitrogen in the products confirms that the CHPTAC has been incorporated into the polymer backbone. The slight amount of nitrogen (0.25%) in CAS reflects the presence of trace amount of protein in commercial grade.

The incorporated quaternary ammonium group enhance the hydrodynamic volume of the moiety effecting higher intrinsic viscosity as evidence in earlier section and consequently higher flocculation efficacy.

3.2.4. FTIR spectroscopy

The FTIR spectra of cassia, N-3-chloro-hydroxypropyl trimethyl ammonium chloride (CHPTAC) and cationic cassia gum are shown in Supplementary respectively.

In case of CAS (Supplementary Fig. 1a), the broad band at 3380 cm\(^{-1}\) is due to stretching mode of the O–H groups. The band at 2908 cm\(^{-1}\) is assigned for C–N stretching vibration, which is absent in cassia. It further confirms that chlorine is liberated during the reaction.

In case of CHPTAC (Supplementary Fig. 1b), the band at 3345 cm\(^{-1}\) is for O–H stretching vibration. The bands at 2815 and 1360 cm\(^{-1}\) are assigned to the C–H and C–N stretching vibration respectively. A strong band at 680 cm\(^{-1}\) is due to the C–Cl absorption band.

Supplementary Fig. 1c shows the FTIR spectrum of CCAS. The broad peak at 3390 cm\(^{-1}\) is for O–H stretching vibration. A strong band at 2908 cm\(^{-1}\) is because of C–H stretching vibration. Two strong bands at 1050 and 977 cm\(^{-1}\) are for C–O–C stretching vibration. The presence of an additional band at 1450 cm\(^{-1}\) can be assigned for C–N stretching vibration, which is absent in cassia. This is a clear proof of incorporation of Cationic moiety onto the polysaccharide backbone. Furthermore, the band corresponding to C–Cl at 680 cm\(^{-1}\) is absent in the cationic product. It further confirms that chlorine is liberated during the reaction.

3.2.5. Scanning electron microscopy

It is evident from the SEM micrographs of CAS (Supplementary Fig. 2A) and CCAS (Supplementary Fig. 2B) that profound morphological changes, in the form of transition from granular smooth surface to fibrillar structure have taken place because of incorporation of cationic moiety onto the backbone of CAS.

3.3. Cationic cassia as algal flocculant

The result shows that cationic cassia was an effective flocculant against indigenous isolated two green algae viz. Chlamydomonas sp. CRP7 and Chlorella sp. CB4. The flocculation efficacy of CCAS has been investigated through standard “jar test” procedure in two different algal culture solution viz., Chlamydomonas sp. CRP7 and Chlorella sp. CB4. The flocculation efficacy has been determined in terms of decrease in optical density (at 750 nm) of the supernatant collected at the end of the “jar test” procedure. Although the flocc are visible at 30 mg L\(^{-1}\) of CAS for both Chlamydomonas sp. CRP7 and Chlorella sp. CB4.

Optimized flocculant dosage of 80 mg L\(^{-1}\) and 35 mg L\(^{-1}\) for Chlamydomonas sp. CRP7 (Fig. 1A) and Chlorella sp. CB4 (Fig. 1B) respectively is sufficient for maximum dewatering of algae. The recovery rate was 93% and 92% for Chlamydomonas sp. CRP7 and Chlorella sp. CB4 at optimized dosage of CCAS within 15 and 30 min respectively. The pH of culture suspension was not altered during the entire study. CCAS has been found to be of higher flocculation efficacy then CAS as a consequence of its higher

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**Table 1**

Synthesis detail and intrinsic viscosity for CCAS and CAS.

<table>
<thead>
<tr>
<th>Material used</th>
<th>Amount of cassia (mol)</th>
<th>Volume of NaOH (mol)</th>
<th>Amount of CHPTAC (mol)</th>
<th>Temp. (°C)</th>
<th>Time (h)</th>
<th>Intrinsic viscosity (dL g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCAS</td>
<td>0.41</td>
<td>0.13</td>
<td>0.062</td>
<td>50–55</td>
<td>2</td>
<td>8.9</td>
</tr>
<tr>
<td>CAS</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**Table 2**

Flocculation characteristics.

<table>
<thead>
<tr>
<th>Material used</th>
<th>Zeta potential (mV)</th>
<th>Algae used</th>
<th>Percentage recovery</th>
<th>Biomass used for flocculation (gm L(^{-1}))</th>
<th>Optimized dosage (mg L(^{-1}))</th>
<th>pH</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre flocculation</td>
<td>Post flocculation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCAS</td>
<td>–19.2</td>
<td>–9.62</td>
<td>Chlorella sp. CB4</td>
<td>93</td>
<td>0.75</td>
<td>7.6</td>
<td>30</td>
</tr>
<tr>
<td>CCAS</td>
<td>–23.5</td>
<td>–5.77</td>
<td>Chlamydomonas sp. CRP7</td>
<td>92</td>
<td>0.85</td>
<td>7.8</td>
<td>15</td>
</tr>
</tbody>
</table>

**Table 3**

Elemental analysis.

<table>
<thead>
<tr>
<th>Material used</th>
<th>%C</th>
<th>%H</th>
<th>%N</th>
<th>Degree of cationization (DC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS</td>
<td>41.70</td>
<td>7.420</td>
<td>0.185</td>
<td></td>
</tr>
<tr>
<td>CCAS</td>
<td>40.99</td>
<td>7.972</td>
<td>1.452</td>
<td>0.1779</td>
</tr>
<tr>
<td>CHPTAC</td>
<td>36.38</td>
<td>7.58</td>
<td>7.12</td>
<td></td>
</tr>
</tbody>
</table>
hydrodynamic volume (intrinsic viscosity) as expected by Brostow, Singh and Pal’s model of flocculation (Brostow et al., 2007).

The low dosage of flocculant required (80 mg L\(^{-1}\) for Chlamydomonas sp. CRP7 and 35 mg L\(^{-1}\) for Chlorella sp. CB4) combined with absence of utility of high technology equipments ensures higher economic viability. Further nontoxic nature and plant based origin of the flocculant ensures higher applicability of end product. This investigation is of bench scale level (200 ml batches). Due to absence of complicated procedures the process is expected to be easily scalable to higher level.

Flocculation using inorganic salts involves hidden cost in removing salt contamination from harvested biomass. Ultrasound based technique, centrifugation and magnetic nanoparticle based separation techniques are of prohibitive cost. Biopolymer based flocculation is the promising candidates to be scaled for commercial level. The cost of harvesting algae by the synthesized CCAS seems to be the lowest among all the existing technologies for algal harvesting from cultured biomass. The current cost of CCAS is $1.47/kg which is less than other cationic polymers available as for example cationic guar gum cost approximately $ 4.88/kg. This is lowest cost of cationic polymer ever reported to the best of our knowledge.

The process has been applied for freshwater green microalgae. Further work is required to evaluate the application of such a method to the marine microalgae.

The lower dosage (80 mg L\(^{-1}\) for Chlamydomonas sp. CRP7 and 35 mg L\(^{-1}\) for Chlorella sp. CB4) as well as low time interval (15 min for Chlamydomonas sp. CRP7 and 30 min for Chlorella sp. CB4) was the added advantage for CCAS as flocculant.

On the other hand, chitosan based flocculation require 120 mg L\(^{-1}\) for C. vulgaris (Rashid et al., 2013).

The magnitude of negative value of the zeta potential decreased with the increase in flocculant dose and on further increasing the dose it will touch the positive plateau. The algal flocculation can be explained by bridging/patch mechanism (Scheme 1) in which the positive charged moiety in CCAS is going to adsorb or binds partly or fully the negative charged algal cells resulting in the formation of flocs and thus settles down. Charged polymers can also neutralize or even reverse the surface charge of particles to cause flocculation through charge neutralization or electrostatic patch aggregation (Vandamme et al., 2013).

The microscopic images of floc formation for Chlamydomonas sp. CRP7 (Supplementary Fig. 3A) and Chlorella sp. CB4 (Supplementary Fig. 3B) clearly indicate the above said mechanism and the cells after flocculation are well intact which further approve this process for algal harvesting.

The exoskeleton of microalgae has sugars namely, galacturonic acid, ribose, arabinose, xylose, glucose, galactose and rhamnose (Bafana, 2013). The COO\(^{-}\) residues of galacturonic acid imparts a overall negative charge on algal surface. Electrostatic attraction between these COO\(^{-}\) groups and positive charge of this carboxylated cassia is responsible for the bridging bond between the two. The polymer chain simultaneously bonds (bridges) with many such algal surface thus establishing a structural network in form of heavy floc. The floc thus formed settles down and thus get separated from bulk liquid. This concept of interaction between the algal surface and the polymeric flocculant has been hypothesized in Fig. 2.

4. Conclusion

Incorporation of cationic moiety (CHPTAC) onto the backbone of cassia, resulted in a novel effective flocculating agent for harvesting algae. The low dosage (e.g. 80 mg L\(^{-1}\) for Chlamydomonas sp. CRP7 and 35 mg L\(^{-1}\) for Chlorella sp. CB4) of the flocculant required is an added advantage as it is not expected to interfere with the product quality of the harvested algal biomass (Krentz et al. 2006; Vandamme et al., 2010). Physiological characterization further confirms the insertion of cationic moiety does take place. The harvested biomass is intended to be utilized as biofuel.

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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.2013.10.035.

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