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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 CO_2 to increase their biomass through photosynthesis and are important for the value added product. The presence of COOH and NH_2 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.

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 disperse air floatation (Xu et al., 2010) are well studied. Use of magnetic (Fe^{3+}) 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-

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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 tora* 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. N2324′51″; E8526′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 GeneBank 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 initially 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 cationizaion 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 Ubbelodhe viscometer (CS/S: 0.003899) at 25 °C. The viscosities were measured in 1 M $NaNO_3$ 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 (t_0, for distilled water), relative viscosity ($\eta_{rel} = t/t_0$) was obtained. Specific viscosity was calculated from the relation $\eta_{sp} = \eta_{rel} - 1$. Reduced viscosity ($\eta_{sp}$/C) and the inherent viscosity ($\ln \eta_{rel}$/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. η_{sp}/C *vs* C and ln η_{rel}/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 hydrodyanimic 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).