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Study of algal biomass harvesting using cationic guar gum from the natural plant source as flocculant

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ABSTRACT

Microalgae are small in size with negatively charged surface. They are usually stable in suspension culture and hard to flocculate. The present work emphasizes on the synthesis of cationic guar gum (CGG) by the introduction of quaternary amine groups onto the backbone of guar gum (GG) from N-(3-chloro-2-hydroxypropyl) trimethyl ammonium chloride (CHPTAC). The optimal dosage of the synthesized cationic guar gum is used to flocculate two different green algae viz. *Chlorella* sp. CB4 and *Chlamydomonas* sp. CRP7.

1. Introduction

Microalgal biomass is being proposed as next generation biofuel. To fulfill the current energy needs algae might be the best option. Microalgal species have been widely used for carbon dioxide sequestration as the global temperature is increasing day by day due to industrialization and fossil fuel burning. Microalgae are also getting priority in the varied research areas like aquaculture feed, food supplements and natural pigments. Another most important aspect is in the field of biofuel. The biodiesel yield is about 20 times higher than other oil crops. The energy efficient pathway for the conversion of biomass to biofuel which can be applied at commercial level. The recent developments regarding biofuel are well described (Banerjee, Bandopadhyay, & Shukla, 2011; Boer, Moheimani, Borowitzka, & Bahri, 2012; Chisti, 2007; Duong, Li, Nowak, & Schenk, 2012). For all the above purposes, we need microalgae which have desirable traits for biodiesel production and highly valued byproducts. Many microalgae species are reported and cultivated but the major problem is related with the biomass harvesting. Flocculation is one of the best techniques reported so far for algal biomass harvesting (Banerjee et al., 2012b).

Chemically, guar gum is a polysaccharide composed of the sugars galactose and mannose. The backbone is a linear chain of β-1,4-linked mannose residues to which galactose residues are 1,6-linked at every second mannose, forming short side-branches. Guar gum is a biopolymer obtained primarily from the endosperm of two different annual leguminous species of *Cyamopsis tetragonalobus* and *Cyamopsis psoraloides* (Ma & Pawlik, 2007). Guar gum has been the subject of study in both native and modified form for a variety of applications. Grafted agar (Mishra, Sen, Rani, & Sinha, 2011) and grafted guar gum (Mishra & Sen, 2011) has been successfully studied for the flocculation purpose as well as matrix for controlled drug release (Sen, Mishra, Jha, & Pal, 2010).

Flocculation involves solid–liquid separation by an aggregation process of colloidal particles (Barkert & Hartmann, 1988). Synthesis of cationic tamarind kernel polysaccharide (Pal, Ghosh, Sen, Jha, & Singh, 2009), cationic glycogen (Pal, Sen, Karmakar, Mal, & Singh, 2008) and amphoteric amylopectin (Singh, Pal, Rana, & Ghorai, 2012) has been carried out and used to flocculate textile industry waste water, coal suspension, kaolin and iron ore respectively. Flocculation of different algae like *Spirulina*, *Oscillatoria*, *Chlorella* and *Synechocystis* has been studied using chitosan (Divakaran & Pillai, 2002) at varying pH. Greenfloc 120 cationic starch was also used to flocculate *Parachlorella* and *Scenedesmus* (Vandamme, Foubert, Meesschaert, & Muylaert, 2010).

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The sulfate and carboxyl group are responsible for imparting the negative charge and thus stabilizing the algae in suspension form. So, a low-cost technique of harvesting and dewatering of algae (Gudin & Thepenier, 1986) is to be developed for fulfilling the current energy demands.

Apart from flocculation, there are other means of harvesting freshwater microalgae which include centrifugation (Molina Grima, Belarbi, Acien Fernandez, Robles Medina, & Chisti, 2003) and filtration (Danquah, Ang, Uduman, Moheimani, & Forde, 2009). Flocculation of *Chlorella minutissima* by inorganic salts such as Zn^{2+}, Al^{3+}, Fe^{3+} has been studied broadly (Papazi, Makridis, & Divanach, 2010). Recently, electro coagulation flocculation (Vandamme et al., 2011) and electro flocculation with disperse air floatation (Xu et al., 2010) has also been studied elaborately with respect to the flocculation purpose. Use of Fe^{3+} nanoparticle for flocculation of microalgae with higher efficiency rate and less time was also studied (Xu, Guo, Wang, Zheng, & Liu, 2011).

Combined use of chemical coagulants such as $CaCl_2$ and $FeCl_3$ and bioflocculant from *P. polymyxa* AM49 for harvesting a high density culture of *Scenedesmus* sp. (Kim, La, Ahn, Park, & Oh, 2011) have been reported. The medium was further used for the next phase of culture.

In the present study, the cationic guar gum (CGG) is used as a natural bioflocculant for microalgae harvesting. The advantage of CGG as flocculant is the low dosage requirement for quick dewatering of algal biomass.

2. Materials and methods

2.1. Synthesis of cationic guar gum

The synthesis of CGG was carried out by the insertion of cationic moiety (CHPTAC) onto the polysaccharide backbone. The required amount of GG powder was dispersed at room temperature in 150 ml of 70% isopropanol (IPA) solution with constant stirring for about 30 min. This was followed by adding intended amount of caustic solution (15 ml) with continuous stirring for 10 min. Next, 0.062 mol of cationic reagent was added slowly to that solution.

The flask was immersed in a thermostatic water bath between 50 °C and 55 °C and the reaction was allowed to proceed for 180 min. The mechanism has been elaborated in Scheme 1. Dilute hydrochloric acid was added for lowering the pH below seven to stop the cationization process (Larsson & Wall, 1998). Thereafter the solution was cooled to the room temperature. The polymer was precipitated by addition of excess isopropanol (1l) and consecutively was washed twice with an aqueous IPA solution (IPA:Water = 80:20; 100 ml). Initially the product was dried at room temperature and then in hot air oven at 40 °C for 6 h.

2.2. Isolation and culturing

Chlorella sp. CB4 and *Chlamydomonas* sp. CRP7 were isolated from the nature (N 23°24′51″; E 85°26′24″). The above two samples were isolated by phototaxis method (Banerjee, Bandopadhyay, & Shukla, 2012a; Droop, 1954) followed by plating in TRIS-Acetate-Phosphate (TAP) medium. As the samples were collected directly from the natural environment, so an optimum dosage of 500 μg/ml of cefotaxime was added to remove the bacterial contamination. The isolated algae belonged to the genus *Chlorella* and *Chlamydomonas* and it was further confirmed by Inter Transcribed Spacer (ITS1, 5.8S, and ITS2 regions of the ribosome) amplification and sequence characterization (Gene Bank ID: JQ710683 for *Chlorella* sp. CB4; Gen Bank ID: JQ408690 for *Chlamydomonas* sp. CRP7). The isolated algae was cultured in TAP medium (Gorman & Levine, 1965) and incubated at 25 °C under light (10,000 lx) with 16:8 h light/dark photoperiods. The culture was harvested at its stationary phase.

2.3. Characterization

2.3.1. Zeta potential measurement and microscopic examination of algal floc

Zeta potential of algal cells (*Chlorella* sp. CB4 and *Chlamydomonas* sp. CRP7) before and after the flocculation was measured by electrophoresis method (Zeta NS, Malvern Institute, UK). Morphological examination of algal cells after flocculation was observed by taking out the floc from the bottom of the beaker by the microscopic (Leica FW4000, Germany) study at low magnification (10×).

2.3.2. Intrinsic viscosity measurement

Viscosity measurements of the polymer solutions were carried out with an Ubbelodhe viscometer at 25 °C. The viscosities were measured in 1 M $NaNO_3$ solution. 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 $NaNO_3$ solution), relative viscosity ($\eta_{rel} = t/t_0$) was obtained. Specific viscosity (η_{sp}), reduced viscosity (η_{red}) and inherent viscosity (η_{inh}) was mathematically calculated as:

$$\eta_{sp} = \eta_{rel} - 1$$

$$\eta_{red} = \frac{\eta_{sp}}{C}$$

and

$$\eta_{inh} = \frac{\ln \eta_{rel}}{C}$$

[where C is polymer concentration in g/dL]

The intrinsic viscosity was calculated by plotting η_{sp} versus C and η_{inh} versus C and then extrapolating to common intercept at $C = 0$ of the best fitted straight lines through the two sets of points as described earlier (Nayak & Singh, 2001; Rani, Mishra, & Sen, 2012; Rani, Sen, Mishra, & Jha, 2012).

2.3.3. Elemental analysis

The elemental analysis of GG and CGG was analyzed using an elemental analyzer (Vario EL III, Elementar, Germany). The estimation of three elements i.e., carbon, hydrogen, nitrogen was undertaken.

The degree of substitution (DS) was also calculated (Huang, Yu, & Xiao, 2007) by the following equation and summarized in Table 3.

$$DS = \frac{162.2 \times N(\%)}{1401 - 151.6 \times N(\%)}$$

2.3.4. FTIR spectroscopy

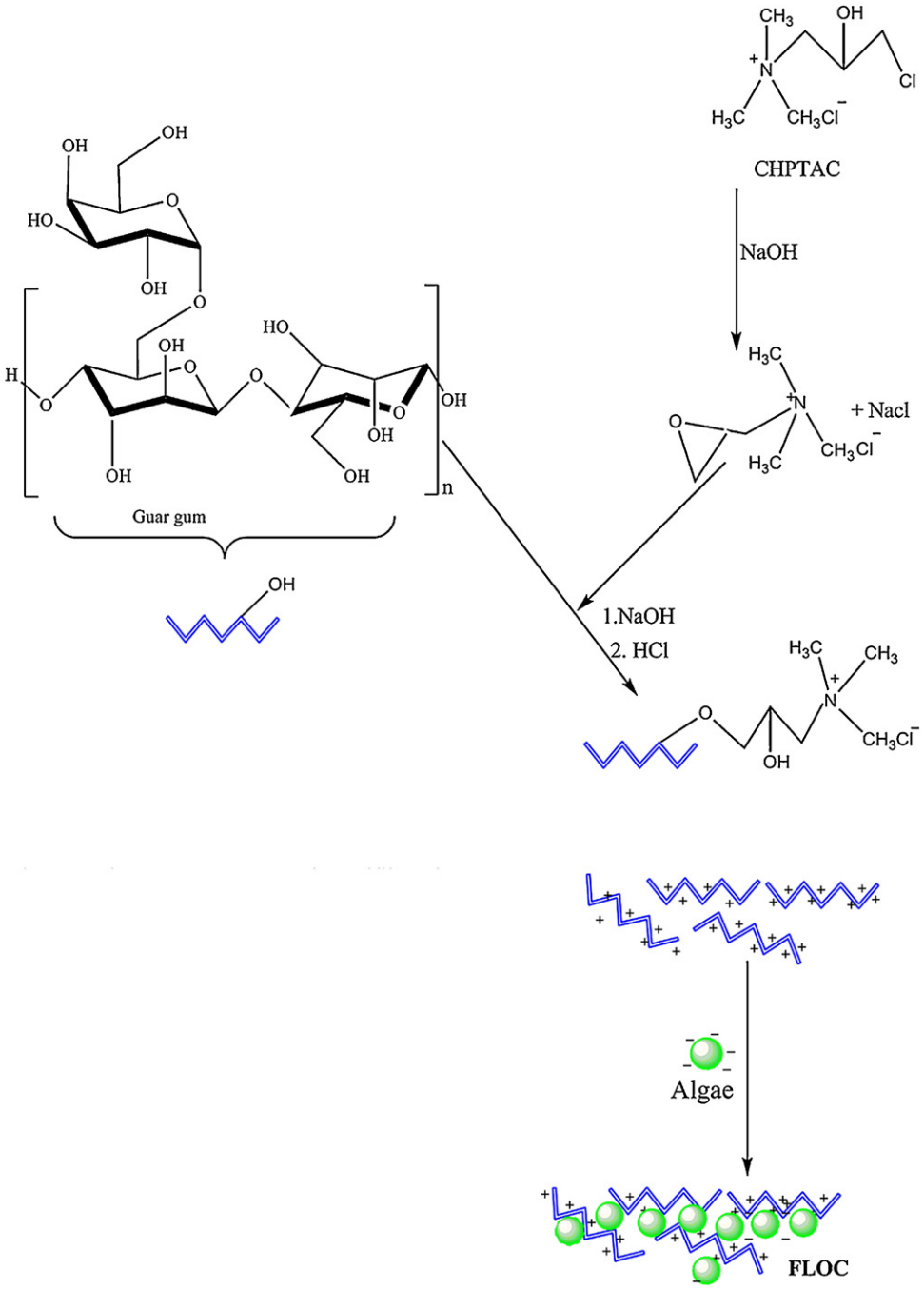
The FTIR spectrums of GG, CHPTAC and CGG were recorded in the solid state by a KBr pallet method using an FTIR spectrophotometer (IR-Prestige 21, Shimadzu Corporation, Japan) between 400 and 4000 cm^{-1}.

2.3.5. Scanning electron microscopy (SEM)

Both GG and CGG were made conductive by gold sputtering and surface morphology was analyzed in scanning electron microscopy (SEM) in powdered form (JSM-6390LV, Jeol, Japan).

2.4. Study of algal flocculation efficacy of cationic guar gum and dosage optimization

The algal flocculation efficacy of the synthesized CGG was studied with fresh water microalgae *Chlorella* sp. CB4 and *Chlamydomonas* sp. CRP7 through standard "Jar test" procedure.



Scheme 1. Schematic representation of cationic guar gum synthesis and floc formation.

The study involved taking in 200 ml of the algal culture in 250 ml identical beakers. The flocculant under study (GG and CGG) was added in calculated amount to result dosage in the beakers that varied from 0.0 ppm (control) to 100.0 ppm. The content of these beakers was stirred identically (in "Jar test" Apparatus, Simeco, India) at 75 rpm for 2 min, followed by 25 rpm for 5 min and then kept for proper settling. Consequently, the supernatant was collected from each beaker and optical density at 750 nm was measured to plot flocculation curves. The flocculation efficacy, which is an indication of the viability of CGG towards algal harvesting, is studied by comparing the flocculation curves of GG and CGG.

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 *Chlorella* sp. CB4 and *Chlamydomonas* sp. CRP7.

The percentage removal of algae *Chlorella* sp. CB4 and *Chlamydomonas* sp. CRP7 from the culture has been evaluated as in an earlier study (Salim, Bosma, Vermue, & Wijffels, 2011) by the relation:

$$\text{Recovery}\quad (\%) = \frac{\text{OD}_{750}(t_0) - \text{OD}_{750}(t)}{\text{OD}_{750}(t_0)} \times 100$$

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

Table 1
Details of synthesis and intrinsic viscosity of cationic guar gum.

Material	Amount of guar gum (mol)	Volume of NaOH (mol)	Amount of CHPTAC (mol)	Temp. (°C)	Time (h)	Intrinsic viscosity (dL/g)
CGG	0.31	0.12	0.062	50–55	3	13.8
GG	–	–	–	–	–	8

Table 2
Flocculation characteristic of *Chlorella* sp. CB4 and *Chlamydomonas* sp. CRP7.

Material used	Zeta potential (mV)		Algae used	Percentage recovery	Biomass used for flocculation (gm/L)	Optimized dosage (ppm)	pH	Time (min)
	Before flocculation	After flocculation						
CGG	−19	−2.17	*Chlorella* sp. CB4	94.5	0.78	40	7.52	30
CGG	−23.8	−4.57	*Chlamydomonas* sp. CRP7	92.15	0.89	100	7.34	15

3. Results and discussion

3.1. Synthesis of cationic guar gum

Though there are different procedures to incorporate cationic moiety, but N-(3-chloro-2-hydroxyl propyl) trimethyl ammonium chloride has been used recently for cationization (Huang et al., 2007; Pal et al., 2008, 2009; Singh, Pal, & Mal, 2006). The synthesis details were summarized in Table 1. The substitution reaction is responsible for the expansion of CGG from GG, which has been described in Scheme 1.

3.2. Characterization

3.2.1. Measurement of zeta potential

Initially the zeta value for *Chlamydomonas* sp. CRP7 and *Chlorella* sp. CB4 in culture medium was found to be −19 mV and −23.8 mV but after flocculation with CGG the value increases to −2.17 mV and −4.57 mV respectively. The high negative zeta value of algal culture necessitates use of cationic flocculant for effective flocculation. The details were summarized in Table 2.

3.2.2. Estimation and interpretation of intrinsic viscosity

It is obvious that intrinsic viscosity of CGG will be greater than that of GG. 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 two reasons:

1. Added volume of cationic moiety.
2. Repulsion between the added cationic moiety–stretching/uncoiling the backbone of guar gum.

3.2.3. Elemental analysis

The results of elemental analysis for both GG and CGG are given in the 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 (0.15%) in GG reflects the presence of trace amount of protein in commercial grade.

Table 3
Elemental analysis of guar gum, cationic guar gum and N-(3-chloro-2-hydroxypropyl) trimethyl ammonium chloride.

Material used	%C	%H	%N	Degree of substitution (DS)
GG	38.42	7.346	0.15	
CGG	41.31	7.467	1.321	0.1784
CHPTAC	36.38	7.58	7.12	

3.2.4. FTIR spectroscopy

In case of GG (Fig. 1A), the broad band at 3421 cm^{-1} is due to stretching mode of the O—H groups. The band 2915 cm^{-1} is assigned to the C—H stretching vibration. Two strong bands at 1025 and 975 cm^{-1} are attributed to C—O—C stretching vibrations.

In case of CHPTAC (Fig. 1B), the broad band at 3348 cm^{-1} is for O—H stretching vibration. The bands at 2820 and 1355 cm^{-1} are assigned to the C—H and C—N stretching vibration respectively. A strong band at 675 cm^{-1} is due to the C—Cl absorption band.

Fig. 1C shows the FTIR spectrum of CGG. The broad peak at 3384 cm^{-1} is for O—H stretching vibration. A strong band at 2905 cm^{-1} is due to C—H stretching vibration. Two strong bands at 1055 and 975 cm^{-1} are for C—O—C stretching vibration. The presence of an additional band at 1490 cm^{-1} can be assigned to C—N stretching vibration, which is absent in GG. These additional bands in CGG are clear proof of incorporation of the cationic

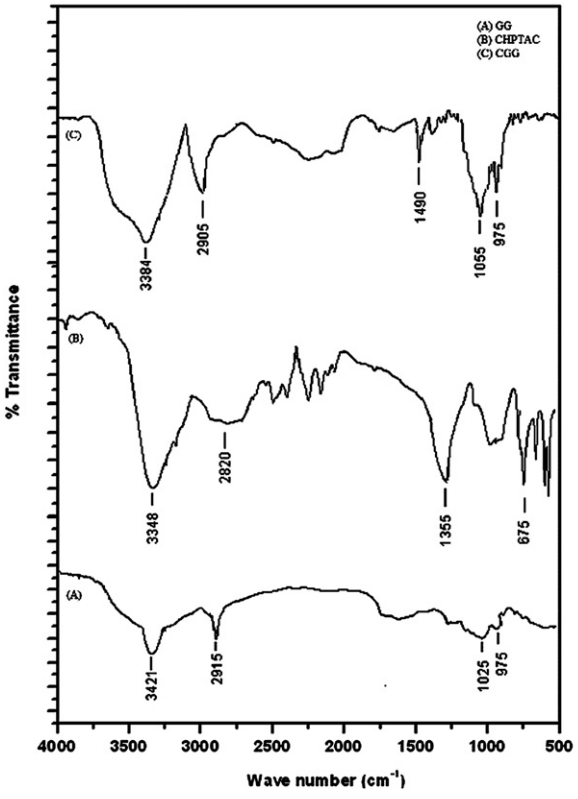


Fig. 1. FTIR spectra of (A) GG, (B) CHPTAC and (C) CGG.

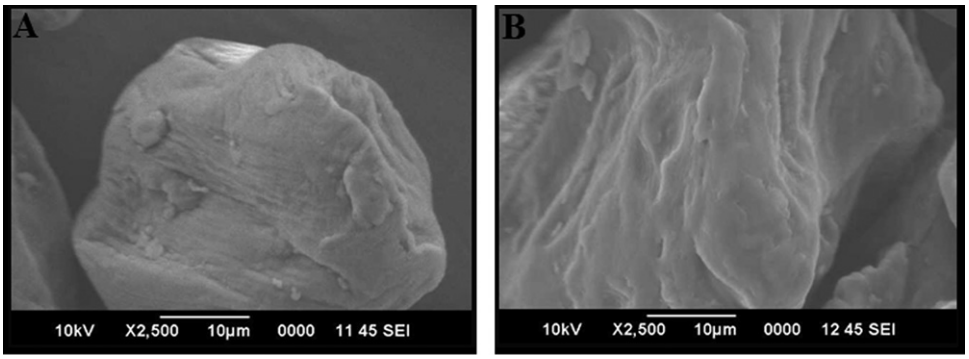


Fig. 2. SEM micrographs of (A) GG and (B) CGG.

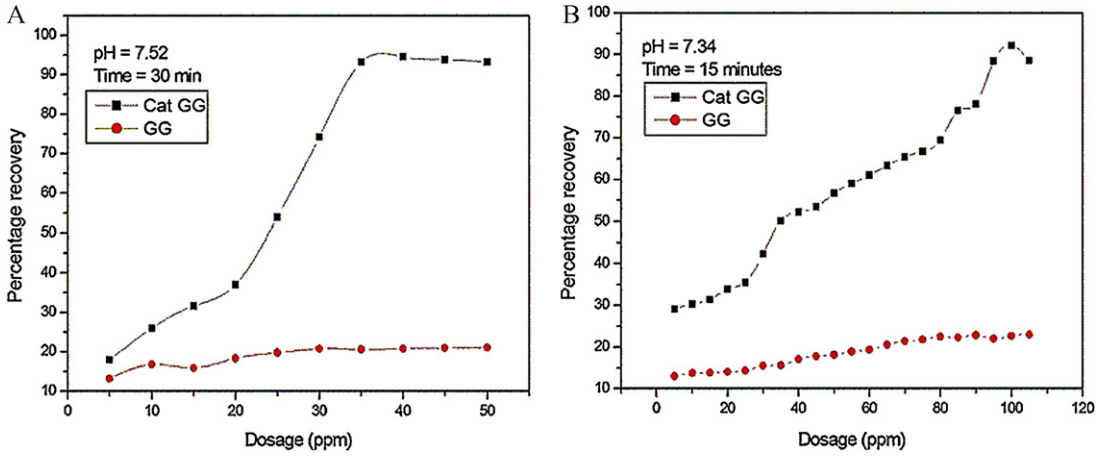


Fig. 3. Flocculation characteristic of (A) *Chlorella* sp. CB4 and (B) *Chlamydomonas* sp. CRP7.

moiety onto the polysaccharide backbone. Furthermore, the band corresponding to C—Cl at 675 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 GG (Fig. 2A) and CGG (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 CHPTAC onto the backbone of GG.

3.3. Application of the cationic guar gum as flocculant for algal harvesting

The flocculation efficacy of CGG has been investigated through standard "Jar test" procedure in algal culture solution. The flocculation efficacy has been determined in terms of decrease in optical density (at 750 nm) of the supernatant collected after completion of the "jar test" procedure.

Although the floc are visible at 40 ppm of CGG for both *Chlorella* sp. CB4 and *Chlamydomonas* sp. CRP7. The CGG was found to dewater the algae from the culture medium, compared to GG,

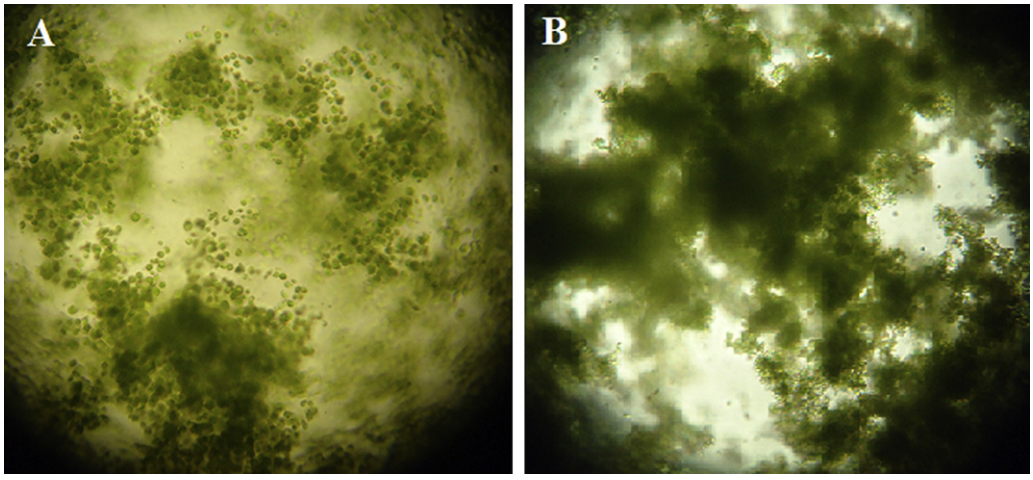


Fig. 4. Microscopic images for floc formation for (A) *Chlorella* sp. CB4 and (B) *Chlamydomonas* sp. CRP7.

as evidenced by analysis of supernatants drawn from the "jar test" procedure at an optimized flocculant dosage of 40 ppm and 100 ppm for *Chlorella* sp. CB4 (Fig. 3A) and *Chlamydomonas* sp. CRP7 (Fig. 3B) respectively. 94.5% and 92.15% recovery occur for *Chlorella* sp. CB4 and *Chlamydomonas* sp. CRP7 at above said dosage concentration within 30 and 15 min respectively. The higher dosage in case of *Chlamydomonas* sp. CRP7 may be due to higher biomass and zeta potential value than compared to *Chlorella* sp. CB4. The lowering of zeta potential value also conferred the effectiveness of CGG towards algae flocculation. The details were summarized in Table 2.

CGG has been found to be higher flocculation efficacy then GG as a consequence of its higher hydrodynamic volume (intrinsic viscosity) as expected by Brostow, Singh and Pal's model of flocculation (Brostow, Pal, & Singh, 2007).

In the present study, lower dosage (0.04 g L^{-1} for *Chlorella* sp. CB4 and 0.1 g L^{-1} for *Chlamydomonas* sp. CRP7) as well as lower time interval (30 min for *Chlorella* sp. CB4 and 15 min for *Chlamydomonas* sp. CRP7) was the added advantage. On the other hand, other flocculation studies (Papazi et al., 2010) had reported 60% *Chlorella minutissima* dewatered by the addition of 1 g L^{-1} of $Al_2(SO_4)_3$ and $ZnCl_2$ in 1.5 and 6 h respectively.

The algal flocculation can be explained by bridging mechanism (Scheme 1) in which the positive charged moiety in CGG is going to adsorb or binds partly or fully the negative charged algal cells resulting in the formation of flocs and thus settles down. The microscopic images of floc formation for *Chlorella* sp. CB4 (Fig. 4A) and *Chlamydomonas* sp. CRP7 (Fig. 4B) clearly indicate the bridging mechanism (Ruehrwein & Ward, 1952)

4. Conclusion

From the preliminary studies, it is resolved that by incorporating a cationic moiety (CHPTAC) on the backbone of GG, an effective flocculating agent for harvesting algal biomass can be developed. The algal flocculation efficacies of the synthesized CGG were studied through standard "jar test" procedure. The higher the degree of substitution, the higher is its intrinsic viscosity and thus higher the algal flocculation efficacy. This synergistic relationship between intrinsic viscosity and flocculation efficacy is in good agreement with the contemporary models of flocculation (Brostow, Singh & Pal's model of flocculation). CGG neutralize negetive charge of the algae cells resulting in neutralization to form floc. The algal flocculation efficacy of CGG can be well explored for commercial algal harvesting. The dosage (e.g. 40 ppm for *Chlorella* sp. CB4 and 100 ppm for *Chlamydomonas* sp. CRP7) of the flocculant required is an added advantage as it is not expected to interfere with the product quality of the harvested algal biomass. The harvested algal biomass can be used for industrial applications (e.g. biodiesel production) or for food security (e.g. animal feed or as human food supplements).

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