Chapter 4

Removal of Algae by Flocculation

Contents

4.1 Introduction
   4.1.1 Algal Removal
   4.1.2 Flocculation of suspended algae

4.2 Materials and methods
   4.2.1 Development of algal cultures
   4.2.2 Flocculation of algae
   4.2.3 Comparison of flocculants
   4.2.4 Sludge volume index of algal cultures flocculated by chitosan and alum
   4.2.5 Standardisation of measurement parameter of algal flocculation
   4.2.6 Effect of pH on flocculation by chitosan
   4.2.7 Effect of turbidity and algal species
   4.2.8 Biochemical composition of flocculated and centrifuged algal biomass
   4.2.9 Chitosan-algal assay

4.3 Results
   4.3.1 Comparison of flocculants
   4.3.2 Sludge volume index (SVI) of algal biomass flocculated by chitosan and alum
   4.3.3 Comparison of measurement parameters of algal flocculation
   4.3.4 Effect of pH on flocculation by chitosan
   4.3.5 Effect of turbidity and algal species
   4.3.7 Chitosan-algal assay

4.4 Discussion

4.5 References
4.1 Introduction

4.1.1 Algal Removal

Eutrophication related algal problems are gaining world wide attention in water treatment. The major algae-related problems in conventional water treatment are unpleasant tastes, odours, and filter clogging. Increased disinfection by-product (DBP) concentration, and microbial re-growth in distribution systems are other algae related problems. The production of toxins by some algae is also of concern in the drinking water industry (Gray, 2005).

Controlling or harvesting suspended algae is always a major bottleneck in water treatment and biomass recovery. Algal removal operations as function of treatment technique include micro straining, centrifugation, flocculation in combination with sedimentation or flotation, rapid/slow sand filtration, oxidation and direct filtration (Petrusevski, 1996). Over the years, these methods have been followed to remove algae with varying degrees of success. Significant shortcomings are still there for all existing methods. An associated problem with any type of filtration is fast filter clogging. There is chance of oxidation by-products which may harm living beings in case of oxidation process. Process optimization of operation is the hurdle in case of coagulation and related processes.

Based on the size, fresh water phytoplanktons are divided into four major groups (Lee, 2008). They are picoplankton (0.2 to 2 µm), nanoplankton (2 to 20 µm), microplankton (20 µm to 200 µm), and macroplankton (>200 µm). The smaller size of phytoplankton causes difficulty in water treatment as it passes through the filters and when present in large concentrations it rapidly clogs filters. Algal properties having influence on water treatment include size, shape, mobility, cell surface characteristics, ability to produce extracellular organic matter and low density associated with vacuole formation. Even some species of algae may show a significant different behaviour as a function of growth stage or difference in the composition of its
Removal of Algae by Flocculation

Removal of Algae by Flocculation

suspension medium (Petrusevski et al., 1993). The characteristics of dominant species in the water body affect the efficiency of treatment mainly.

Mobility allows phytoplankton to be in a suitable environment by responding to external stimuli like gravitational, chemical and thermal gradients. Mobility may be of active (mucilage extrusion, cilia and flagella) and inactive (buoyancy) type (Sigee, 2005). Several types of algal motility may interfere with water treatment processes. Motile organisms have the ability to free themselves and move through the coagulated suspension.

Extracellular organic matter from algae may act as coagulant aid or may hinder coagulation depending on the species of algae and extracellular concentrations (Bernhardt & Calsen, 1991). Extracellular organic matter can be a threat to public health as a trihalomethane precursor (Petrusevski et al., 1996).

Prior research has shown that algae are negatively charged (Bernhardt & Calsen, 1991). Negative algal surface charge hinders algal agglomeration and in case of filtration it inhibits algal adherence onto the filtering material (Petrusevski et al., 1996). The zetapotential and charge density at a constant $pH$ varied from genus and even varied between different species of the same genus (Ives, 1959).

4.1.2 Flocculation of suspended algae

Flocculation is reported to be cheap, simple, and volume independent method for concentrating algal cells when compared to other existing methods. Algal removal by coagulation and sedimentation can be economical options for the mitigation of filter clogging problems in conventional water treatment plants as it requires little or no capital investment. Conventional flocculants are mineral additives (ferric chloride, aluminium chloride, aluminium sulphate, poly aluminium chloride), or synthetic polymers (such as polyacrylamide). Numerous studies have evaluated the effectiveness of trivalent metal salts, inorganic polymers and organic polymers for algal
Removal of Algae by Flocculation

coagulation, (Ives, 1959; Tenney et al., 1969; Tilton et al., 1972; Lubián, 1989; Jun et al., 2001; Strand et al., 2003; Yan & Jameson, 2004; Knuckey et al., 2006).

Multivalent metal salts are effective flocculants or coagulants. The commonly used salts include ferric chloride (FeCl₃), aluminum sulfate (Al₂(SO₄)₃) and ferric sulphate (Fe₂(SO₄)₃). Coagulation efficiency of metal ions increases with increasing ionic charge. Multivalent metal salts, such as alum, have been widely used to flocculate algal biomass in wastewater treatment processes (McGarry, 1970; Moraine et al., 1980; Lincoln, 1985). Alum is an effective flocculant for S. quadricauda and Chlorella (Golueke & Oswald, 1965). However, flocculation by metal salts may be unacceptable if biomass is to be used in aquaculture and other related applications. Polyferric sulfate (PFS) is observed to be a better flocculant compared to the more traditional non-polymerized metal salt flocculants (Jiang et al., 1993). Flocculation with pre-polymerized metal salts is said to produce flocs that are easily dewatered and effective over a wider pH range than non-polymerized salts. An alternative to using metal salts is the use of cationic polymers (Tenney et al., 1969).

Most of the conventional flocculants have several environmental consequences. An increase in aluminium concentration has been cited as a possible cause of Alzheimer’s disease. Aluminium (Al) is clearly a powerful neurotoxicant. Flaten (2001) had reviewed on the epidemiological evidence linking aluminium and Alzheimer’s disease and observed that nine out of thirteen published cases have statistically significant positive relations. Production of large volumes of sludge is a disposal problem in the case of alum as reported by Pan et al. (1999). Dispersion of acrylamide oligomers may cause health hazards (Roussy et al., 2004). Page et al. (2003) depicts the presence of non coagulable organic matter especially polysaccharide compounds with a higher hydrophilic character after alum treatment, indicating that these components are refractory to alum treatment.
Cationic polymers doses between 1 mg/L and 10 mg/L can induce flocculation of freshwater algae; however, high salinity of the marine environment can inhibit flocculation by polyelectrolytes (Bilanovic et al., 1988). Auto flocculation produced by modifying the culture medium, has been investigated for algal cultures in both fresh and seawater systems (Suh et al., 1997; Lee et al., 1998). The bacterium *Paenibacillus* sp. AM49 is known to produce a bioflocculant that has proved effective for harvesting *Chlorella vulgaris* (Hee-Mock et al., 2001). Aqueous extract of dry seeds of *Moringa oleifera* act as effective coagulants or as coagulant aid for water and wastewater treatment (Ndabigengesere & Narasiah, 1998; Okuda et al., 2001; Katayon et al., 2006; Bhatia et al., 2007; Kwaambwa & Maikokera, 2008). Other natural coagulants include extracts of *Prosopis juliflora* and *Cactus latifaria* (Diaz et al., 1999) and Okra and Nirmali seeds (Samawi & Shokralla, 1996).

Therefore, alternative flocculants have been considered for environmental application. Comparison among studies are difficult to make because of differences in model or natural water composition, algae type, strength of coagulant stock solution and the choice of coagulant pH. An ideal flocculant should be inexpensive, nontoxic, and effective in low concentration. In addition, the flocculant should be selected so that further downstream processing is not adversely affected by its use. Bio-macromolecules may be of great interest since they are natural products characterized by their environment friendly behaviour. Among these biopolymers, chitosan may be considered as an emblematic material to be used for coagulation-flocculation.

Chitosan is a partially deacetylated polymer of acetyl glucosamine. Chitosan is prepared from chitin by alkaline de-N-acetylation. Chitin is found in a wide range of natural sources such as crustaceans, fungi, insects, annelids, molluscs, coelenterata etc. However, chitosan is manufactured only from crustaceans, primarily because large amount of crustacean exoskeleton is available as by-product from food processing industry.
The most important and significant developments in chitin and chitosan technology are in the medical and environmental applications. Chitosan is well known as a complexing agent for many metal ions (Guibal, 2004, Gerente et al., 2007), phenolic compounds (Popa et al., 2000), natural and synthetic polyanions (Peniche & Arguelles-Monal, 2001; Guibal, 2004) and pesticides (Yoshizuka et al., 2000). It was previously reported that chitosan is a non-toxic biodegradable polymer with moderate bacteriostatic effect (Koide, 1998). All these properties make chitosan very attractive for applications in water treatment. Another benefit of chitosan application is a possibility to use chitosan containing sludge formed during water treatment in production of fertilizers or additives to animal feeding mixtures (Bratskaya et al., 2004).

The largest single use of chitosan is the clarification of waste and effluent water (Onsoyen & Skaugrud, 1990). Chitosan has been studied for use as a coagulant or flocculant for a wide variety of suspensions including the following: fish processing (Guerrero et al., 1998), food industry (Savant & Torres, 2000), silt in river water, (Divakaran & nd Pillai, 2002a), latex particles (Ashmore & Hearn, 2000), microorganisms (Strand et al., 2002, Barany & Szepesszentgyfrgyi, 2004; Zou et al., 2006) and mineral colloids (Roussy et al., 2004).

Chitosan is reported as algal flocculant by a few authors (Lavoie & Noue, 1983; Lubían, 1989; Divakaran & Pillai, 2002b; Grima et al., 2003). Chitosan is required in low dosages in freshwater but its flocculating power is reduced in salt water. Optimum flocculation dose of chitosan varies greatly,
optimum flocculation of *Tetraselmis chui*, *Thalassiosira pseudonana* and *Isochrysis* sp. has been observed at a chitosan dosage of 40 mgL⁻¹ and in contrast 150 mgL⁻¹ was required for optimum flocculation of *Chaetoceros muellari*. Heasman *et al.* (2000) did not observe any consistent correlation between the taxonomic group of the algae and the quantity of chitosan needed for optimal flocculation. In view of the variable results reported it is attempted to verify the use of chitosan as flocculant in water treatment to meet water quality standards.

**Objectives of the study**

- To check the effectiveness of chitosan as an algal flocculant and compare it with conventional flocculant alum.
- To check the effect of algal pH, turbidity and species on flocculation by chitosan
- To compare biochemical composition of algae after centrifugation and flocculation as a concentration technique.
- To check whether chitosan has algistatic or algicidal property

**4.2 Materials and methods**

**4.2.1 Development of algal cultures**

- **Development of algal stock cultures**

  Pure cultures of *C. pyrenoidosa*, *S. elongatus* and *S. quadricauda* were obtained from the culture collection of the School of Environmental Studies, CUSAT. The algae were grown in one litre Borosil culture flasks aseptically. *C. pyrenoidosa* and *S. quadricauda* were cultured in Ward and Parish medium (Ward & Parrish, 1982) and *S. elongatus* was cultured in BG 11 medium (Stainer *et al.*, 1971). Cultures were illuminated by a bank of day light fluorescent lamps to stimulate photosynthesis. This light source imparted an intensity of 1500 lux to the culture surface on a 12 h light and 12 h dark cycle.
Development of test cultures

The stock cultures of the three species were inoculated into filtered tap water enriched with respective algal medium in 10 L glass tanks and illuminated as before. The inoculum size was controlled to yield cell densities in the range $10^3$ to $10^6$ cell/mL in a growth period of 10 days. These cultures were used for flocculation studies. A natural bloom of fresh water algae was induced by inoculating pond water sample to filtered water sample dosed with garden nutrient mix.

4.2.2 Flocculation of algae

Preparation of chitosan solution

Chitosan used in this study was obtained from M/s South India Sea Foods, Kochi, Kerala, India. It was extracted from crustacean exoskeletons, had an average molecular weight of 180 kDa, and was 80% deacetylated. Different concentrations of chitosan were prepared by dissolving in 10 mL (0.1 N) HCL and making up to 100 mL with distilled water.

Jar test

The jar test method was used for the study of coagulation- flocculation. The well mixed algal cultures were taken in one litre beaker. The pH of the algal suspension was measured and adjusted to a fixed value (depending upon the experiments) using dilute hydrochloric acid solution (0.1 N). Then it was dosed with flocculant; flash mixed for 1 minute, flocculated at 40 rpm for 30 minutes, and settled for 40 minutes. All jar tests were performed at an ambient temperature of $30 \pm 2^\circ$ C. The performance of the flocculants was visually evaluated in situ for dose-response characteristics in respect to size of the floc, and by turbidity. The pH of the supernatant was determined after flocculation. Turbidity of algal suspension was measured using Nephelometer calibrated as per standard procedure (Standard methods, 1999). pH values of the suspensions were measured using digital pH meter. Flocculation was carried out on a six spindle multiple stirrer unit with stainless steel paddles.
All experiments were performed with three replicates and mean value was taken for quantification.

4.2.3 Comparison of flocculants

This experiment was designed to evaluate and compare flocculation efficiency of a conventional flocculant alum, and chitosan which has a GRAS (generally regarded as safe) status. Flocculation efficiency of both flocculants was tested in *S. elongatus* culture having turbidity range 20-25 NTU. The initial pH of the culture was 10.5. The ten-day old culture of *S. elongatus* was transferred to one litre beaker for jar test. The pH was adjusted to 7.0 by adding hydrochloric acid. One hundred mg chitosan powder was accurately weighed and dissolved in 100 mL distilled water to obtain a solution containing 1.0 mg/mL of solution. Alum was prepared by adding 1.0 g alum to 1000 mL distilled water. Alum was added to the cultures in the beaker at concentrations 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L and 100 mg/L to determine the range of dose limits for effective flocculation. The flocculation efficiency was computed based on turbidity estimation of the supernatant. Turbidity was measured in nephelometer and expressed in NTU.

In a second set of experiment, chitosan was added to *S. elongatus* cultures at concentrations 5 mg/L, 10 mg/L, 20 mg/L, 40 mg/L, 60 mg/L, and 80 mg/L. Flocculation efficiency was determined as before. The results of the two sets of experiments were compared based on the flocculation efficiency and resultant pH.

4.2.4 Sludge volume index of algal cultures flocculated by chitosan and alum

The sludge volume index is the volume in milliliters occupied by 1 g of suspension after 30 minutes settling. *S. elongatus* with a turbidity of 20-25 NTU was dosed with chitosan at a concentration of 5 mg/L and flash mixed for 1 minute, flocculated at 40 rpm for 30 minutes. *S. elongatus*-floc suspension was gently stirred and it was then transferred to 1 L graduated
measuring cylinder and allowed to settle for 30 minutes. The volume occupied by the flocculated algal mass was measured. Suspended solids of the S. elongatus-floc suspension were determined by gravimetric method. A well-mixed suspension was filtered through a weighed glass-fiber filter and the residue retained on the filter was dried to a constant weight at 105°C. The increase in weight of the filter was expressed as the total suspended solids. The same experiment was repeated with alum as flocculant at dose of 20 mg/L. The sludge volume index was calculated as:

$$SVI = \frac{\text{settled sludge volume (mL/L)} \times 1000}{\text{Suspended solids (mg/L)}}$$

Same experiment was repeated with C.pyrenoidosa and natural bloom of same turbidity and at a chitosan concentration of 10 mg/L and an alum dosage of 20 mg/L.

4.2.5 Standardisation of measurement parameter of algal flocculation

In order to find out better flocculation efficiency parameter S. elongatus was flocculated with chitosan at 5 mg/L. C. pyrenoidosa culture and Natural bloom were flocculated with chitosan at concentration of 10 mg/L by jar test experiment 4.2.2. The turbidity of algal cultures was 20-25 NTU. The algal turbidity and chlorophyll a of supernatant of the C.pyrenoidosa, S.elongatus and natural bloom were measured before and after flocculation and compared. Chlorophyll a was extracted from the algal concentrate into 90 % aqueous acetone solution and the absorbance of the extract was determined with a spectrophotometer. The concentration of chlorophyll a was computed using the trichromatic equation (APHA, 1999)

$$\text{Chlorophyll a} = 11.85(\text{OD664}) - 1.54(\text{OD647}) - 0.08(\text{OD630})$$

4.2.6 Effect of pH on flocculation by chitosan

The test cultures of C. pyrenoidosa and S. elongatus having turbidity range 20 – 25 NTU and with pH 9.8 were transferred to jar test beakers.
pH was adjusted at three levels i.e. 7, 8 and 9. Chitosan was added at 5 mg/L, 10 mg/L, 20 mg/L, 40 mg/L, 60 mg/L and 80 mg/L at each pH level. The flocculation efficiency was estimated based on turbidity measurements.

The experiment was repeated under post pH correction i.e. the test cultures were inoculated with chitosan at 5 mg/L, 10 mg/L, 20 mg/L, 40 mg/L, 60 mg/L and 80 mg/L, well mixed and the pH was adjusted to 7, 8 and 9. The effect of pH adjustment prior to, and after addition of chitosan was analysed. The results were analysed by two way analysis of variance to elucidate the interference of pH with chitosan flocculation efficiency.

4.2.7 Effect of turbidity and algal species

Test cultures of S. elongatus and C. pyrenoidosa and natural bloom at turbidity levels of 20 - 25 NTU, and 30 - 35 NTU were taken in beakers. The pH was adjusted to 7. Chitosan was added to each at concentrations 5 and 10 mg/L. The jar test was performed and flocculation efficiency was determined as before.

4.2.8 Biochemical composition of flocculated and centrifuged algal biomass

Algal cultures of C. pyrenoidosa, S. elongatus and S. quadracauda in exponential growth phase were harvested by flocculation with chitosan and by centrifugation. Harvested biomass was then dried at 60°C in hot air oven. Dried sample was then powdered in glass mortar and kept in desiccator over silica gel.

- **Determination of total carbohydrate by anthrone method**

Fifty mg of the dried algal biomass was weighed out into a clean glass tube and hydrolyzed by keeping it in a boiling water bath for 3 hours with 5 mL of 2.5 N HCl and cooled to room temperature. It was neutralized with solid Na₂CO₃ until the effervescence ceased. The supernatant was collected by centrifuging and solution was made up to 10 mL. Three different samples were treated in the same manner. Four mL of anthrone reagent were added to 1 mL
of the supernatant, then heated for 8 minutes in a boiling water bath, cooled rapidly and read the green colour at 630 nm in spectrophotometer (Hedge and Hofreiter, 1962). The amount of total carbohydrate present in the samples was calculated and expressed as mg/100 mg of the sample.

- **Determination of total protein by organic nitrogen estimation**

Total organic nitrogen of dried algal biomass was determined in CHNS analyser. The determination in CHNS was based on Isotope Ratio Mass spectroscopy (IRMS). Quantitative combustion was carried out by oxygen jet injection directly at the sample. Exactly 5 mg of the algal sample were fed to the digestion chamber of the instrument. The gases were pre-separated in the elemental analyzer and injected into mass spectrometer by continuous flow procedure. Digestion temperature was kept at 950°C. Injection of reference gases was also performed automatically. Total protein content of the algal sample was calculated by multiplying total organic nitrogen by a factor 6.25 (Jones, 1931).

- **Estimation of lipid by sulphophosphovanillin method**

A sample of five hundred mg of dried algal biomass was taken in a homogeniser and added 10 mL of Chloroform- Methanol mixture (2:1) and mixed well. The homogenate was filtered through Whatman No: 1 filter paper, added 2mL of 0.9% Nacl solution and shaken well. Each of this was transferred to a small separating funnel and allowed to stand overnight at 4°C. The lower phase of the biphasic layer, which contains all the lipids, was removed and the volume was adjusted to 10 mL by the addition of chloroform. A 0.5 mL sample of the above extract was measured into clean test tube and allowed to dry in a vacuum desiccator over silica gel. Then added 0.5 mL of concentrated H2SO4 and mixed well. After plugging with non absorbent cotton it was heated in a boiling water bath for 10 minutes, and then cooled to room temperature. A 0.2 mL sample of the acid digest was taken in a separate clean glass tube and added 5 mL of Vanillin reagent (0.1 gm vanillin dissolved
in 50 mL 80% Orthophosphoric acid). The mixture was mixed well in a cyclomixer and allowed to stand for 30 minutes. The absorbance was measured at 520 nm (Barnes and Blackstock, 1973). Concentration of total lipid is expressed as mg/100 mg of the sample.

4.2.9 Chitosan-algal assay

- **Plate assay**

  Algal lawn of *C.pyrenoidosa*, *S.elongatus* and *S.quadricauda* was prepared by double layer method. Sterile Petri plates were laid with a lower layer of algal medium with 2% agar. An upper layer algal medium with 1.2% agar incorporated with desired algae was prepared by adding concentrated algal biomass into molten algal medium. Preparations were incubated at room temperature, under the light intensity of 1500 lux with 12h light: 12h dark photo-cycle for 2 days. Different concentrations of chitosan were prepared by dissolving in 10 mL 0.1 N HCl and making up to 100 mL with distilled water. After mixing properly, a 20 µL sample of the Chitosan solution was impregnated on sterile filter paper disc and allowed to dry for 30 minutes. The process was repeated to get 40 µL extract impregnated per disc and dried in vacuum desiccator for 24 hours. Control discs were kept with distilled water alone. The impregnated discs were placed on the algal lawn. The algal plates were examined daily for 5 days, and any yellowing, or clearing zone in the treated lawns compared to control.

- **Liquid culture assay**

  The algal cultures of *C.pyrenoidosa*, *S.elongatus* and *S.quadricauda* were inoculated at a cell density of $10^4$ cells to respective algal medium in borosilicate culture flasks, in triplicate and amended with chitosan solution at 2.5 mg/L, 5 mg/L, 10 mg/L, 20 mg/L and 40 mg/L concentrations. Cultures were illuminated by a bank of day light fluorescent lamps of an intensity of 1500 lux to the culture surface on a 12 h light and 12 h dark cycle. At the end of 96 hour incubation (12/12 light cycle) total algal cell count was enumerated.
in a haemocytometer. Duplicate control cultures in respective algal medium were also kept.

4.3 Results

4.3.1 Comparison of flocculants

Alum effected 83 % to 100 % flocculation of *S. elongatus* in the range 20 mg/L -100 mg/L. The highest settling of algae occurred at 100 mg/L giving a clear supernatant. The pH of the supernatant was 5.8. The flocculation efficiency is likely to decline at < 20 mg/L as per the trend observed (Fig. 4.2). In the range 20 to 100 mg/L, alum treatment reduced the pH of the supernatant medium to 5.5-6.0, which is not advisable in water quality management. Chitosan effected 99 % flocculation at 5 mg/L which successively decreased as the concentration increased (fig 4.2). The pH remained nearly unchanged at ~7 following treatment.

![Flocculation Efficiency Graph](image)

**Fig.4.2** Flocculation efficiency of *S.elongatus* (algal turbidity 20-25NTU) by Alum and chitosan treatment.
4.3.2 Sludge volume index (SVI) of algal biomass flocculated by chitosan and alum

Table 4.1 comparison of sludge volume index between chitosan and alum in three different algae

<table>
<thead>
<tr>
<th>Algal Species</th>
<th>Sludge Volume Index (mL/L)</th>
<th>Chitosan</th>
<th>Alum</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. elongatus</td>
<td>60.38 ± 0.17</td>
<td>132.5 ± 2.95</td>
<td></td>
</tr>
<tr>
<td>C. pyrenoidosa</td>
<td>72.33 ± 0.35</td>
<td>124.03 ± 1.40</td>
<td></td>
</tr>
<tr>
<td>S. quadricauda</td>
<td>86.46 ± 0.45</td>
<td>155.73 ± 0.90</td>
<td></td>
</tr>
</tbody>
</table>

The results of SVI are expressed in the Table 4.1. SVI for algal species flocculated with chitosan falling in the range 60 mL/L -87 mL/L. The range was high with alum-algae sludge (124 mL/L - 156 mL/L).

4.3.3 Comparison of measurement parameters of algal flocculation

Flocculation efficiency of chitosan as flocculant was checked by two parameters-turbidity and chlorophyll \( a \) of the supernatant. The results are expressed in Table 4.2. The flocculation efficiency based on turbidity and chlorophyll \( a \) of both, the pure cultures \( S. elongatus \) and \( C. pyrenoidosa \) and natural bloom, were nearly same. Therefore, turbidity was taken as measurement parameter for further experiments.

Table 4.2 Comparison of filtrate turbidity and Chlorophyll \( a \) following chitosan flocculation

<table>
<thead>
<tr>
<th>Algal Species</th>
<th>Filtrate Turbidity</th>
<th>Chlorophyll ( a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( S. elongatus )</td>
<td>99.2 ± 0.10</td>
<td>99.1 ± 0.05</td>
</tr>
<tr>
<td>( C. pyrenoidosa )</td>
<td>98.1 ± 0.06</td>
<td>98.1 ± 0.08</td>
</tr>
<tr>
<td>Natural bloom</td>
<td>96.47 ± 0.15</td>
<td>96.2 ± 0.05</td>
</tr>
</tbody>
</table>
4.3.4 Effect of pH on flocculation by chitosan

Flocculation efficiency of chitosan in *C. pyrenoidosa* and *S. elongatus* was pH dependent. In case of *C. pyrenoidosa* it can be observed that the most effective flocculation with minimum chitosan requirement i.e. 10 mg/L was obtained in neutral pH (Fig. 4.3) At pH 8 and 9 maximum efficiency was obtained with 20 mg/L. At all pH levels flocculation efficiency decreased as the concentration increased beyond 20 mg/L.

![Flocculation efficiency of Chitosan at 7, 8 & 9 pH](image)

**Fig.4.3** Flocculation efficiency of Chitosan at 7, 8 & 9 pH (pre-treatment pH correction) in *C. pyrenoidosa* (Algal Turbidity 20-25 NTU)
Effect of pH on flocculation efficiency in *C. pyrenoidosa*, was found to be significant as $p < 0.005$ ($p = 1.36 \times 10^{-23}$). Similarly, effect of chitosan concentration on flocculation efficiency was also statistically different ($p = 3.32 \times 10^{-44}$) Combined effect of pH and chitosan concentration on flocculation efficiency also have a p value of $1.95 \times 10^{-26}$, thus rejecting the hypothesis - all groups are equal (Table 4.3).

**Table 4.3** ANOVA on data of flocculation Efficiency (20-25 algal turbidity) at different chitosan concentration and pH in *C. pyrenoidosa*

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>F crit</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>1489.371</td>
<td>2</td>
<td>744.6857</td>
<td>317.4128</td>
<td>3.259446</td>
<td>1.36E-23</td>
</tr>
<tr>
<td>Chitosan</td>
<td>27598.07</td>
<td>5</td>
<td>5519.614</td>
<td>2352.665</td>
<td>2.477169</td>
<td>3.32E-44</td>
</tr>
<tr>
<td>Interaction</td>
<td>3609.881</td>
<td>10</td>
<td>360.9881</td>
<td>153.8666</td>
<td>2.106054</td>
<td>1.95E-26</td>
</tr>
</tbody>
</table>
Post pH correction (i.e., pH correction after chitosan addition) could effect flocculation up to 67 % only in all pH levels tested (Fig 4.4). The flocculation efficiency trend was similar for three pH levels tested. Flocculation efficiency increased up to a concentration of 10 mg/L, then the efficiency remained unchanged up to the highest concentration tested i.e. 80 mg/L. Statistical interaction between pH variation and concentration variation was provided by statistical analysis (Table 4.4). ANOVA gave $p$ value of $1.54 \times 10^{-6}$ indicating a significant difference between pH and concentration interaction. The significant difference among pH ($p < 0.005$) was evident as given in table 4.4.

**Table 4.4** ANOVA on data of flocculation Efficiency (20-25 algal turbidity) at different chitosan concentration and pH in *C.pyrenoidosa* with post pH correction

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>F crit</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>14.79788</td>
<td>2</td>
<td>7.398941</td>
<td>5.782213</td>
<td>3.259446</td>
<td>0.006643</td>
</tr>
<tr>
<td>Chitosan</td>
<td>7092.87</td>
<td>5</td>
<td>1418.574</td>
<td>1108.604</td>
<td>2.477169</td>
<td>2.37E-38</td>
</tr>
<tr>
<td>Interaction</td>
<td>100.8335</td>
<td>10</td>
<td>10.08335</td>
<td>7.880057</td>
<td>2.106054</td>
<td>1.54E-06</td>
</tr>
</tbody>
</table>

*S.elongatus* required only 5 mg/L chitosan for 99 % flocculation efficiency at pH 7. Chitosan concentration of 10 mg/L was required at pH 8 and at pH 9, 20 mg/L were needed for maximum flocculation efficiency (Fig 4.5). A decreasing trend of similar fashion with increasing concentration was observed in *S.elongatus* as in *C.pyrenoidosa*.
Fig 4.5 Flocculation efficiency (%) of chitosan at different pH (pre-treatment pH correction) in S. elongatus culture (Algal turbidity 20-25 NTU)

Fig 4.6 Flocculation efficiency (%) of chitosan with post pH correction in S. elongatus bloom (Algal turbidity 20-25 NTU)
The significant interaction between Chitosan concentration and pH in flocculation efficiency was provided by statistical analysis - Two factor ANOVA, i.e. a probability value $3.7 \times 10^{-28}$, rejecting the null hypothesis that there is no interaction between pH and concentrations (Table 4.5).

Table 4.5 ANOVA on data of flocculation Efficiency (20-25 algal turbidity) at different chitosan concentration and pH in *S.elongatus*

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>F crit</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>509.3472</td>
<td>2</td>
<td>254.6736</td>
<td>90.58622</td>
<td>3.259446</td>
<td>8.93E-15</td>
</tr>
<tr>
<td>Concentration</td>
<td>40626.34</td>
<td>5</td>
<td>8125.269</td>
<td>2890.121</td>
<td>2.477169</td>
<td>8.27E-46</td>
</tr>
<tr>
<td>Interaction</td>
<td>5422.531</td>
<td>10</td>
<td>542.2531</td>
<td>192.8769</td>
<td>2.106054</td>
<td>3.7E-28</td>
</tr>
</tbody>
</table>

In ‘post pH correction’ experiments maximum flocculation efficiency obtained was up to 66 % (Fig 4.6). ANOVA on data of flocculation efficiency at different Chitosan concentration and pH showed significant interaction with a probability value of $4.58 \times 10^{-11}$ (Table 4.6).

Table 4.6 ANOVA on data of flocculation Efficiency (20-25 algal turbidity) at different chitosan concentration and pH in *S.elongatus* with post pH correction

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>F crit</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>123.9982</td>
<td>2</td>
<td>61.99912</td>
<td>40.60004</td>
<td>3.259446</td>
<td>5.93E-10</td>
</tr>
<tr>
<td>Concentration</td>
<td>5525.511</td>
<td>5</td>
<td>1105.102</td>
<td>723.6746</td>
<td>2.477169</td>
<td>4.78E-35</td>
</tr>
<tr>
<td>Interaction</td>
<td>270.4374</td>
<td>10</td>
<td>27.04374</td>
<td>17.70956</td>
<td>2.106054</td>
<td>4.58E-11</td>
</tr>
</tbody>
</table>

4.3.5 Effect of turbidity and algal species

The effect of algal turbidity on flocculation was compared at 5 mg/L and 10 mg/L chitosan concentration in three algal species. The results were compared using t-test. At low turbidity (20-25 NTU) the culture of *C.pyrenoidosa* and natural bloom behaved similar i.e. the flocculation
increased with concentration of chitosan. The flocculation efficiency of *S. elongatus* was similar at 5 and 10 mg/L chitosan. At high cell density (turbidity 30-35 NTU) overall flocculation efficiency decreased at respective chitosan concentration (Table 4.7, 4.8, 4.9).

**Table 4.7** Turbidity related flocculation efficiency (%) of chitosan in *C. pyrenoidosa*

<table>
<thead>
<tr>
<th>Chitosan (mg/L)</th>
<th>Turbidity</th>
<th>mean</th>
<th>t stat</th>
<th>t critical</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>20-25 NTU</td>
<td>75.33± 1.53</td>
<td>2.287</td>
<td>2.132</td>
<td>0.0421</td>
</tr>
<tr>
<td></td>
<td>30-35 NTU</td>
<td>72.73±1.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>20-25 NTU</td>
<td>98.33± 0.31</td>
<td>18.862</td>
<td>2.132</td>
<td>2.3266E-05</td>
</tr>
<tr>
<td></td>
<td>30-35 NTU</td>
<td>84.41±1.29</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 4.8** Turbidity related flocculation efficiency (%) of chitosan in *S. elongatus*

<table>
<thead>
<tr>
<th>Chitosan (mg/L)</th>
<th>Turbidity</th>
<th>mean</th>
<th>t stat</th>
<th>t critical</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>20-25 NTU</td>
<td>99.29±0.05</td>
<td>40.901</td>
<td>2.132</td>
<td>1.0677E-06</td>
</tr>
<tr>
<td></td>
<td>30-35 NTU</td>
<td>74.82±1.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>20-25 NTU</td>
<td>98.36±0.34</td>
<td>14.331</td>
<td>2.132</td>
<td>6.8879E-05</td>
</tr>
<tr>
<td></td>
<td>30-35 NTU</td>
<td>93.37±0.41</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 4.9** Turbidity related flocculation efficiency (%) of chitosan in Natural bloom

<table>
<thead>
<tr>
<th>Chitosan (mg/L)</th>
<th>Turbidity</th>
<th>mean</th>
<th>t stat</th>
<th>t critical</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>20-25 NTU</td>
<td>74.82±1.01</td>
<td>9.270</td>
<td>2.132</td>
<td>0.00038</td>
</tr>
<tr>
<td></td>
<td>30-35 NTU</td>
<td>66.24± 1.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>20-25 NTU</td>
<td>93.44±0.41</td>
<td>17.612</td>
<td>2.132</td>
<td>2.3266E-05</td>
</tr>
<tr>
<td></td>
<td>30-35 NTU</td>
<td>85.76± 0.70</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 4.10** Differential response of the species to flocculation efficiency ( turbidity 20 – 25)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>F crit</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>162.613</td>
<td>2</td>
<td>81.307</td>
<td>116.919</td>
<td>5.143</td>
<td>1.57E-05</td>
</tr>
<tr>
<td>Within Groups</td>
<td>4.172</td>
<td>6</td>
<td>0.695</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>166.786</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.11 Differential response of the species to flocculation efficiency (turbidity 30 – 35)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>F crit</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>46.822</td>
<td>2</td>
<td>23.411</td>
<td>95.485</td>
<td>5.143</td>
<td>2.83 E-05</td>
</tr>
<tr>
<td>Within Groups</td>
<td>1.471</td>
<td>6</td>
<td>0.245</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>48.293</td>
<td>8</td>
<td>0.245</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.3.6 Biochemical composition of flocculated and centrifuged algal biomass

Fig 4.7 Biochemical composition of flocculated and centrifuged algal biomass
The biochemical compositions (protein, carbohydrate, lipids and ash) of three species of freshwater algae were characterized. Interspecies comparisons showed that the carbohydrate content of *S. quadricauda* was greater than that of the other two species. *S. quadricauda* had the same value, 34.66 ± 0.26, for both flocculated as well as centrifuged mass. Probability value from the t-test gave a value higher than 0.05 and hence there is no significant difference between carbohydrate levels of both flocculated and centrifuged biomass (Table 4.11).

**Table 4.11 Results of biochemical comparison – carbohydrate**

<table>
<thead>
<tr>
<th>Algae</th>
<th>Concentration method</th>
<th>Mean (mg %)</th>
<th>t stat</th>
<th>t critical</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. elongatus</em></td>
<td>Flocculated</td>
<td>14.57 ± 0.04</td>
<td>0.572</td>
<td>2.776</td>
<td>0.598</td>
</tr>
<tr>
<td></td>
<td>Centrifuged</td>
<td>14.55 ± 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. quadricauda</em></td>
<td>Flocculated</td>
<td>34.66 ± 0.26</td>
<td>0</td>
<td>2.776</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Centrifuged</td>
<td>34.66 ± 0.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. pyrenoidosa</em></td>
<td>Flocculated</td>
<td>28.08 ± 0.54</td>
<td>0.564</td>
<td>3.182</td>
<td>0.306</td>
</tr>
<tr>
<td></td>
<td>Centrifuged</td>
<td>27.87 ± 0.35</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*S. elongatus*, *S. quadricauda* and *C. pyrenoidosa* were checked for protein concentration in the flocculated and centrifuged mass. The results are shown in the Table 4.12. *S. elongatus* had a higher protein content among the three species but the t-test gave a probability > 0.05 accepting the hypothesis all groups are equal.

**Table 4.12 Results of biochemical comparison - protein**

<table>
<thead>
<tr>
<th>Algae</th>
<th>Concentration method</th>
<th>Mean (mg %)</th>
<th>t -stat</th>
<th>t critical</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. elongatus</em></td>
<td>Flocculated</td>
<td>54.800 ± 0.766</td>
<td>0.800</td>
<td>3.182</td>
<td>0.241</td>
</tr>
<tr>
<td></td>
<td>Centrifuged</td>
<td>55.490 ± 1.282</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. quadricauda</em></td>
<td>Flocculated</td>
<td>26.977 ± 1.036</td>
<td>1.094</td>
<td>2.776</td>
<td>0.168</td>
</tr>
<tr>
<td></td>
<td>Centrifuged</td>
<td>26.167 ± 0.757</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. pyrenoidosa</em></td>
<td>Flocculated</td>
<td>29.203 ± 0.525</td>
<td>1.999</td>
<td>3.182</td>
<td>0.070</td>
</tr>
<tr>
<td></td>
<td>Centrifuged</td>
<td>28.493 ± 0.320</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Lipid content of the three species used in the experiment was checked and results are given in the Table 4.13. *S.quadrivacuda* has lipid content higher than that of *S.elongatus*, the value being 21.73 ± 0.35 and 21.70 ± 0.24 in the flocculated and centrifuged mass. *C.pyrenoidosa* has the highest lipid content among the three species tested, flocculated mass has a value of 30.14 ± 0.37 and 29.88 ± 0.21 for the centrifuged mass. The t-test between flocculated and centrifuged mass gave probability > 0.05 accepting the hypothesis that there is no difference in the lipid content of both groups.

**Table 4.13** Results of biochemical comparison- lipid

<table>
<thead>
<tr>
<th>Algae</th>
<th>Concentration method</th>
<th>Mean (mg %)</th>
<th>t stat</th>
<th>t critical</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S.elongatus</em></td>
<td>Flocculated</td>
<td>13.93 ± 0.08</td>
<td>-0.346</td>
<td>2.776</td>
<td>0.373</td>
</tr>
<tr>
<td></td>
<td>centrifuged</td>
<td>13.95 ± 0.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S.quadrivacuda</em></td>
<td>Flocculated</td>
<td>21.73 ± 0.35</td>
<td>0.150</td>
<td>2.776</td>
<td>0.444</td>
</tr>
<tr>
<td></td>
<td>centrifuged</td>
<td>21.70 ± 0.24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C.pyrenoidosa</em></td>
<td>Flocculated</td>
<td>30.14 ± 0.37</td>
<td>1.059</td>
<td>3.182</td>
<td>0.184</td>
</tr>
<tr>
<td></td>
<td>centrifuged</td>
<td>29.88 ± 0.21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 4.3.7 Chitosan-algal assay

- **Plate assay**

  There was no plaque formation or yellowing in any of the chitosan impregnated algal lawns. This showed that chitosan did not inhibit algal growth.

- **Liquid culture assay**

  Algal growth was observed visually by the development of colour and turbidity in all the test cultures of *C.pyrenoidosa, S.elongatus* and *S.quadrivacuda*, but the algae didn’t form homogenous suspension as in the control cultures; instead clumps of algae were formed and settled at the
bottom. Upon microscopic examination, it was found that the cells were intact, but stuck together

![Microphotograph of algal floc after chitosan flocculation](image)

**Fig 4.8** Microphotograph of algal floc after chitosan flocculation
(a) *C.pyrenoidosa*  (b) *C.pyrenoidosa* floc  (c) *S.elongatus*  
(d) *S.elongatus* floc

In all cases except control, algae tend to remain the clumps even after gentle shaking it remain settled at the bottom of the culture flask. So cell count was not a reliable measurement. Visual and microscopic observation of algal flocs was taken into account to check whether chitosan has any effect on algal cells and its growth. The observation under the microscope showed that the algal cells were intact after flocculation, but stuck together in massive clumps at all chitosan concentrations.
4.4 Discussion

The results of the experiments conducted to evaluate the efficiency of alum and chitosan confirmed the better efficiency of chitosan at minimum concentration of 5 mg/L, when compared to a high concentration of 80 mg/L of alum. Earlier reports had suggested that chitosan can effectively flocculate algal species at 5 mg/L to 200 mg/L. Divakaran and Pillai (2002b) reported a 90% turbidity removal at 5 mg/L of chitosan in an algal suspension having a turbidity of 30 NTU. These results have a similarity with the trend observed in this study. A 96–98% reduction in the number of suspended cells (Euglena gracilis culture) was obtained in the cultures with 200 mg/L of chitosan at pH 7.5 in the study of Gualtieri et al. (1988). For a pH range of 6·0–9·0, a mixed culture of Chlorophyceae dominated by Chlorella sp. in a high-rate algal pond (HRAP) fed with dilute pig-waste obtained a flocculation efficiency of 95–100% at 20 mgL⁻¹ chitosan (Buelna et al., 1990).

A high coagulation demand in case of alum may be due the chelate complex formation between extracellular organic matter/cellular organic matter of alga and alum. Pivokonsky et al. (2006) and Auvray et al. (2006) gave an experimental evidence to the effect of algogenic organic matter in coagulation which in turn produce coagulation demand. Auvray et al. (2006) emphasized that both extracellular organic matter (EOM) and cellular organic matter (COM) disturbed the flocculation of suspended kaolin with PACl (polyaluminium chloride), and proteins in COM were identified as possible inhibitory substances for the coagulation with PACl. These proteins could consume PACl in the coagulation process due to the formation of chelate complexes between these inhibitory proteins and the coagulant. The consumption of alum by cyanobacterial proteins could be one of the important causes of the increase in coagulant demand.
The pH of the supernatant after flocculation in case of chitosan remains unchanged and that of alum treated get reduced to 5.89. This also favours the usage of chitosan as lower pH is not advisable in water quality management.

Flocculation efficiency of chitosan is reported as pH dependent. Most of the studies of this nature were done in mineral particles (Roussy et al., 2004). The effect of pH in flocculation of colloids of biological origin was investigated by very few authors (Strand et al., 2003 and Divakaran and Pillai, 2002 b). The pH dependency of chitosan flocculation was studied at different pH of 7, 8 and 9. Neutral and alkaline pH was selected for the study because the pH of algal blooms is always high. The flocculation efficiency at three pH was studied at six chitosan concentrations ranging from 5 mg/L – 80 mg/L. The result of pH dependency on flocculation showed that the best flocculant concentrations varied with the pH. At neutral pH the most effective flocculation occurred with a minimum chitosan concentration of 10 mg/L in *C.pyrenoidosa* and 5 mg/L in *S.elongatus*. At pH 8 and 9 maximum efficiency was observed with 20 mg/L in both species. These observations give a proof for pH dependency of chitosan flocculation and most effective flocculation with minimum chitosan requirement was observed at neutral pH. Another series of experiment was done to find out whether there is any difference in flocculation, if pH is changed after chitosan addition. The flocculation efficiency gets reduced in all pH levels tested.

The effect of turbidity and algal species was checked in another series of tests. As concentration of suspension increased the efficiency decreased. According to Tenney et al. (1969) a definite stoichiometric relationship exists between algal cell concentration and requisite cationic polymer dosage for optimal flocculation. The efficiency was higher in *S.elongatus* when compared to *C.pyrenoidosa* and a natural bloom dominated by green algae. This difference in flocculation efficiency may be due to algal cell size and concentration.
The presence of algae in source waters not only causes problems in the treatment process, but also complicates the treatment and disposal of sludge or further processes in algal recovery. Because of incorporation of algae, water treatment plant sludge becomes more complex and behaves like a mixture of inorganic and organic sludge. Sludge dewatering can be influenced by many factors such as particle size distribution, shape, specific surface area, density, particle charge, bound water content, $pH$, and organic content. Pan et al. (1999) studied the dewatering characteristics of ‘algae containing alum sludge’. The presence of organic materials in the source water produces smaller flocs with more water content, resulting in poor dewaterability of alum sludge. The alum-algae flocs in the present study are also small and the sludge volume index for different alum-algae sludge was in the range of 124 mL/L - 157 mL/L. The SVI for ‘chitosan-algae’ floc was in the range 60 mL/L - 89 mL/L and flocs were larger and feathery when compared to alum-algae sludge. A normal sludge with good settling characteristics generally has a SVI of less than 100 mL/L (Standard methods, 1999).

Concentrating and storing algae in a moist form provide high nutritional value (D'Souza et al., 2002; Heasman et al., 2000; Knuckey et al., 2006). Moist algal concentrates can potentially provide a more cost-effective alternative to fresh algal cultures as well as simplify hatchery procedures. Centrifugation is the conventional method for algal concentration, especially in hatcheries. Algal concentrates produced by centrifugation have been fed to bivalves and prawn larvae with promising results (D'Souza et al., 2000; Heasman et al., 2000; Robert et al., 2001). Centrifugation of algal cells is an energy demanding one and is volume dependent. (Knuckey et al., 2006). An alternative to centrifugation is flocculation. For an algal floc to be an acceptable aquaculture feed it must be nontoxic and it should also be capable of being de-flocculated to release the trapped cells for planktonic feeders such as oysters and larval prawns.
The results of present study reveal that there is no change in the biochemical composition of the three algal species in flocculated and centrifuged algal biomass. This data favour that chitosan flocculation for algal removal for various purposes. The experiments by Knuckey et al., (2006) demonstrated a proof-of-concept for a commercial application of algal concentrates prepared by flocculation, especially for use at a remote nursery without on-site mass-algal culture facilities. The process was rapid, simple and inexpensive, and relatively cost neutral with increasing volume when compared to concentration by centrifugation.

Chitosan assay revealed that it is not algicidal in nature. Algal cells remained intact, but as clumps throughout the assay. The polymer adsorption is the first necessary step in flocculation and stabilization. The type and amount of adsorbed polymer and the conformation of the adsorbed layer will determine whether flocculation or stabilization will occur and if so by what mechanism (Somasundarana et al., 2005). The mechanism of chemically induced algal flocculation by cationic polymers, is interpreted in terms of bridging phenomenon between the discrete algal cells and linearly extended polymer chains, forming a three dimensional matrix that is capable of subsiding under quiescent conditions (Tenney et al., 1969). The combination of flocculation and adsorption data of Strand et al. (2003) on the interaction between chitosans and bacterial suspensions clearly showed that charge neutralization was not the main flocculation mechanism and emphasized bridging as one dominating mechanism for flocculation. Therefore, chitosan flocculation may be used as an ex-situ water quality management tool; but process optimisation of chitosan flocculation is complicated.
4.5 References


