Chitosan as a Coagulant and Precipitant of Algae Present in Backwater

Catherine Thomas, Jonathan Broussard, Victor Medina

Purpose

The purpose of this report is to highlight the current state of knowledge of algal flocculation by chitosan and identify data gaps that exist between specific algal characteristics and chitosan binding efficiency. The report herein will identify published relationships and correlations between the quality of backwaters and the prevalence of algae, determine a baseline for flocculation efficiency of microalgae, and identify ideal treatment instances for algal removal by way of chitosan flocculation and precipitation.

Background

Sediments high in organic content and nutrients are typically found in backwaters (Strauss et al., 2006). Slow-moving currents and elevated nutrient concentrations observed in backwaters encourages favorable conditions for the overgrowth of algae. Thus, backwaters are an ideal target area for algal removal to mitigate their migration downstream. Although algal harvesting technologies have become more efficient regarding algae-water separation, the primary factors limiting algal collecting activities are the high energy inputs required for physical separation, as well as their small size (ranging from 3-30 mm diameters). Flocculation and settling methods used for the separation of algae from water upon harvesting is not as energy intensive as physical separation methods such as centrifugation or filtration, nor requires excessive operational costs (Brayenkova et al., 2018). However, there are concerns of chemical contamination from many of the separation agents typically used (e.g. aluminum chlorohydrate, polyacrylamide, and ferric sulfate). As a result, chitosan, a natural, biodegradable material found in crustacean shells, has emerged as a favorable flocculating agent in the harvesting of microalgae (Chen et al., 2014).

Algae in Backwater

Phytoplankton is comprised of various classes of algae, some of which are harmful, that can bring about deleterious effects (i.e. oxygen depletion, light attenuation, and toxin production from harmful algal species) as a result of their overgrowth in aquatic ecosystems. The algal classes that comprise phytoplankton include blue-green, green, yellow-green, golden-brown, brown, and red algae; diatoms; euglenoids; and cryptomonads (Pal and Choudhury, 2014). Although the overgrowth of any class of algae is considered harmful if adverse effects are imposed upon the environment, toxin producing algae, are of particular concern as their toxins directly impact both aquatic and terrestrial organisms (USEPA, 2017a; Carmichael, 2001). It is noteworthy that harmful algal blooms (HABs) are indicative of an imbalance in the aquatic system, which is generally attributed to the presence of excess nutrients, nitrogen (N) and phosphorus (P). Backwaters are ideal for phytoplankton productivity due to significantly reduced currents that allows increased settling of suspended sediment. Increased light penetration through the water column as a result of decreased suspended solids encourages photosynthetic activity of HABs. Elevated concentrations of N and P, and total suspended solids are the main water quality indicators that precede algal bloom events in backwaters (Heisler et al., 2008).

<u>Water quality parameters affecting algal blooms</u>

Nutrients N and P both play important roles in algal growth trends. Phosphorus has been indicated in research as the limiting nutrient in HAB growth as studies conducted in selected freshwater systems has linked P loading to algal blooms (USEPA, 2017b). However, the nitrogen dynamics in water is key to understanding HAB growth trends as well (Davis et al., 2015). Nitrogen is essential for algae that lack the ability to fix nitrogen (such as *Microcystis aeruginosa*), and require both N and P to bloom; whereas the growth of nitrogen fixing algal species are primarily driven by P concentrations in water (Fay, 1992). The bioavailability of nitrogen in the form of ammonia (NH_4^+), urea (NH_2CONH_2), nitrite (NO_2^-), nitrate (NO_3^-), and atmospheric nitrogen (N_2) influences algal communities with NH_4^+ being the most efficient for algae to assimilate, and N_2 being least bioavailable as a considerable amount of energy is required that can limit growth (Davis et al., 2015). Phosphorus, on the other hand, is assimilated by algae in its inorganic, reactive form, orthophosphate (PO_4^{3-}). The uptake kinetics of PO_4^{3-} by algae is a two-stage process as P is both surface absorbed, as well as transported directly into the cell (Yao et al., 2011). Phosphorus is vital in the cell division process as algal cells have been observed to synthesize polyphosphate granules for cell wall construction (Solovchenko et al., 2019). The mechanism by which phytoplankton utilizes N and P are generally universal among the different classes of algae. In the case of N, there are several cyanobacterial species of the genera Anabaena, Microcystis, Planktothrix, and Nostoc that are N fixing, an thus do not require additional N from the water (Issa et al., 2014). Taken together, N and P contribute greatly to algal growth in backwater systems.

In addition to the effects of nutrients on algal blooms in backwater, turbidity is an important factor influencing algal growth. Depending on the type of aquatic system studied, the effect of turbidity on algae growth can yield inconsistent results. The turbidity observed in either the main river channel or flooded backwater areas are the result of a homogenously mixed media, and moderate to fast moving waters that together prevent algae colonization. In backwaters, where hydrologic connectivity occur seasonally, turbid waters have been reported to hinder algal growth of many species as light becomes the limiting factor (Wang, 1974). In such cases, the photosynthetic activity of the algae is reduced due to light attenuation; although this is not always the case as some algae species such as *Cylindrospermopsis* and *Planktothrix* are good competitors at low light (Berg and Suluta, 2015). During periods of hydrologic isolation, light penetration increases as the sediment settles, therefore allowing photosynthetic activity of the algae. In other instances, increased turbidity in larger water bodies caused by disturbances in the lake bed (ships, dredging, etc.), or either an input of sediment laden stormwater runoff have shown to contribute to algae growth. Although turbidity under these conditions is generally temporary, it tends to bring about the input or resuspension of excessive nutrients into the water column. Overall, algae growth in the natural environment is complex due to their remarkable range of growth and survival strategies, which makes pinpointing specific water quality parameters influencing bloom events challenging.

<u>Types of commonly phytoplankton found in backwaters</u>

There are numerous classes and species of phytoplankton that can be found in freshwater systems, however, not all species can thrive under the same conditions (Ferris et al., 2005). Species composition may shift as a result of changes in nutrient concentrations, light intensity, and temperature (Heisler et al., 2008). The most predominant types of phytoplankton commonly found in bloom form are those that can thrive in a range of conditions which include green algae, blue-green algae, and diatoms (Pal and Choudhury, 2014). Throughout this report, algal flocculation relative to the aforementioned algae genera will be of primary focus.

Algae Flocculation and Precipitation by Chitosan

The flocculation efficiency of microalgae is generally a function of size, stability, and surface characteristics of the algal cells (Beach et al., 2012). Flocculation of numerous genera of phytoplankton (including *Anabaena, Asterionella, Chaetocerosmuellari, Chlorella, Isochrysissp, Oscillatoria, Scenedesmus,* and *Spirulina*) have been investigated using ferric sulfate, alum, as well as natural materials such as clay and chitosan (Zhu et al., 2018). Chitosan is a material principally derived from the exoskeletons of crustaceans, as well as γ-polyglutamic acid, produced industrially via fermentation (Zhang et al., 2012). When considering an ex-situ application of a flocculant, a natural material is preferred to avoid secondary pollution, or any adverse effects to the existing ecosystem.

A preliminary investigation conducted at the US Army Engineer Research and Development Center (ERDC) evaluated flocculation agents chitosan, clays, and modified clays for their performance in the coagulation of algae. In this study, *Microcystis aeruginosa* (blue-green algae), collected from Lake Okeechobee, was employed as the test organism. The optical density (OD) of pre- and post- treated media was determined. The treatment process involved adding different doses of the flocculation agents to the algae samples, and mixing the samples on an orbital shaker to induce flocculation. Samples were allowed to settle after thorough mixing, and analyzed for OD. OD was measured at visible light absorption wavelengths of 660 nm and 665 nm.

Initial OD was determined for *M. aeruginosa,* and chitosan was added at concentrations between 0 and 100 mg/L to determine the most appropriate dosing range. Figure 1 shows the percent (%) difference between initial and final OD measurements for mixed and static samples. The % difference was calculated using the equation:

$$\% \, difference = \frac{\mathrm{OD}i - \mathrm{OD}f}{\mathrm{OD}i} \; x \; 100$$

Results from the broad range of chitosan dosing revealed that the most effective treatment range was between 2 and 18 mg/L. Chitosan addition between 50 and 100 mg/L yielded no difference in OD measurements between initial and final static samples.

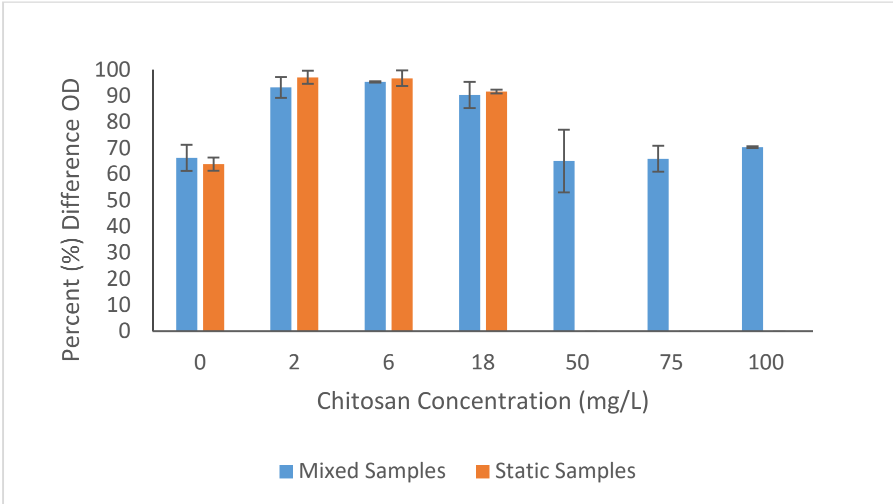

Figure 1. Percent OD difference in algal samples 24 hours after chitosan addition at concentrations between 0 and 100 mg/L.

A second batch test was conducted with algae testing a more narrow range of chitosan dosages (Figure 2). In this test, chitosan was evaluated at concentrations between 2 and 8 mg/L. After 24 hrs of treatment, percent OD differences were highest in algal samples treated with chitosan at concentrations between 4 and 8 mg/L.

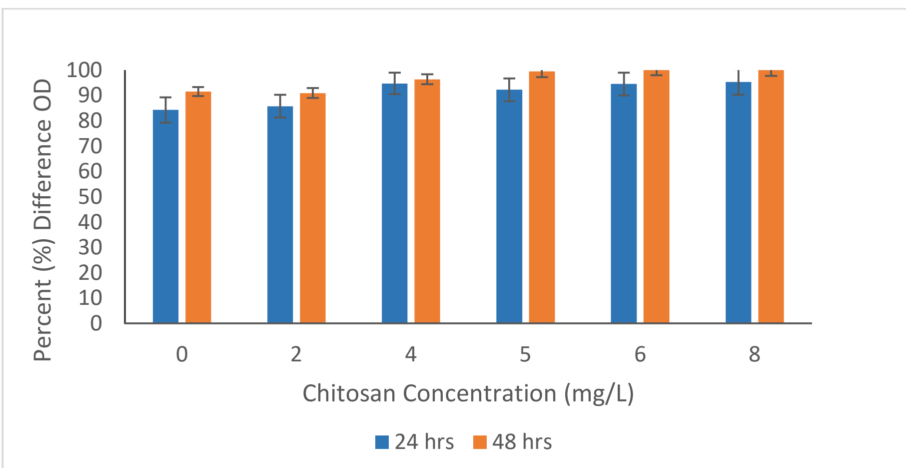

Figure 2. Percent OD difference in algal samples 24 and 48 hours after chitosan addition at concentrations between 0 and 8 mg/L.

A third batch test to evaluate chitosan flocculation efficiency was conducted on samples with a higher algal cell density. Percent differences in OD observed from samples after 24 hours of treatment suggests that 8 mg/L chitosan was most effective for algae precipitation. Relative to samples having a lower algal cell density, the treatment efficiency of chitosan was comparatively less for each dose.

Figure 3 shows the percent OD difference in algal samples of higher density after 24 hrs of chitosan treatment.

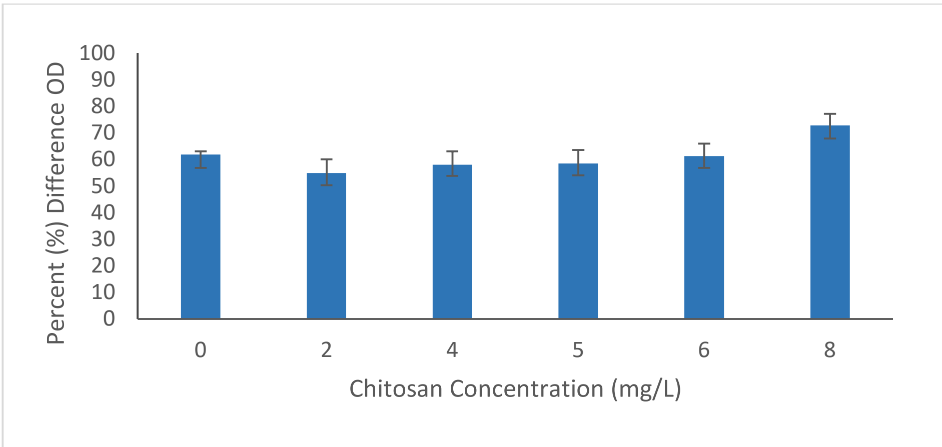

Figure 3. Percent OD difference in samples of higher algal density after 24 hrs of chitosan treatment.

In addition to treatments with chitosan, clays were also evaluated for their ability to flocculate and precipitate algae. Treatment with non-modified clay yielded a percent OD difference up to 65% when added at 350 mg/L. Modified clay exhibited a considerably greater ability to flocculate chitosan relative to non-modified clay. Algae samples treated with modified clay yielded a percent difference in OD up to 73% at a concentration of 50 mg/L. Figure 4 shows the percent OD differences in algal samples after 24 hrs of treatment with both modified and non-modified clay.

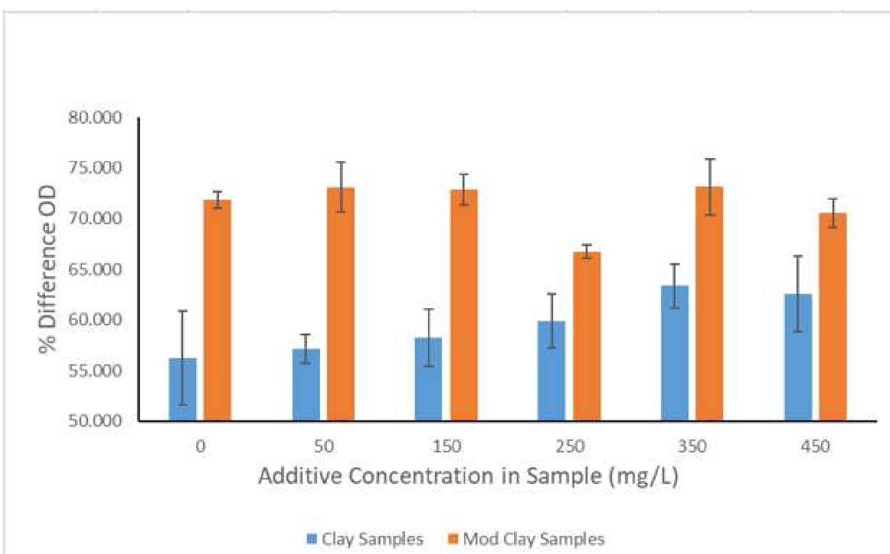

Figure 4. Percent difference in optical density of algae in samples 24 hrs following treatment with modified and non-modified clays.

Results from the preliminary studies suggest chitosan as a promising agent for algal flocculation. Although modified clay demonstrated the ability to flocculate algae similar to that of chitosan, it is important to note that samples dosed with modified clay yielded less algal resuspension into the water column after 24 hrs compared to samples treated with chitosan or clay. Considering the theoretical mechanism by which chitosan and clay particles bind to the algae membrane surface, the use of clays, particularly modified clays, may be of benefit when tested together to achieve a synergistic effect on algal precipitation (Figure 5).

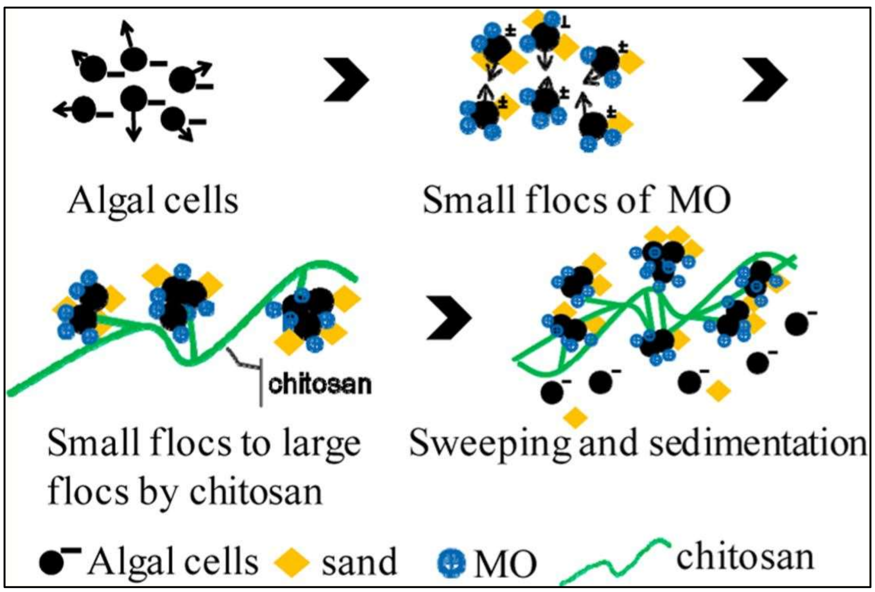

Figure 5. Conceptual representation of coagulant – ballast combination and flocculation mechanism. (Photo adapted from Li and Pan, 2013).

The flocculation capability of chitosan is attributed to adsorption, bridging, sweeping, and surface charge neutralization between chitosan monomers and the target cells (Li and Pan, 2013). However, pH is the primary factor affecting chitosan flocculation efficiency as chitosan exists in a linear arrangement surrounded with positive charged deacetylated units under acidic conditions, and in a coiled arrangement under alkaline conditions (Pugazhendhi et al., 2018). The effect of environmental conditions on algae flocculation by chitosan is yet two-fold. Environmental conditions must also be considered as morphological and physiological characteristics of algal cells can change considerably depending on factors such as light, temperature, pH, and nutrient levels (Gerardo et al., 2015). Because the flocculation process involves the aggregation of small particles through surface charge neutralization, the presence of algal cell extracellular organic matter (EOM) important to consider as EOM can change the cell surface charge (Darwesh et al., 2019). The amount and composition of EOM varies by species, growth stage, and environmental conditions (Pivokonsky et al., 2014). Larger scale studies are required to more accurately determine algae species-specific advantages and limitations of flocculation treatments by chitosan.

Discussion and Summary

Flocculation based processes for algae precipitation demonstrates promising efficiency and scalability. Chitosan can be potentially used as a primary flocculation agent in the environment as it is biodegradable, relatively inexpensive, and considered environmentally friendly (Matter et al., 2019). Though the capabilities of chitosan flocculation with multiple algae species have been observed in several studies (Pugazhendhi et al., 2019; Matter et al., 2019; Chen et al., 2014; Branyikova et al., 2018), data gaps in species-specific treatment longevity should be addressed. At present, controlled laboratory studies with chitosan has shown its promise in effectively binding unicellular microalgae (motile and non-motile). However, further research is needed to determine chitosan's treatment efficiency in large scale applications.

Technical Applications

Backwaters areas are ideal locations for demonstration scale testing, and possible treatment areas. During periods of hydrologic isolation when sediments begin to settle, and before algae cell proliferation accelerates, chitosan flocculation treatments can potentially bind the small algal cells and precipitate them into the sediment. Provided that flocs remain stable, it is likely that algal cells will remain dormant, or undergo cell death as photosynthesis cannot occur in cells precipitated into sediment. Li and Pan (2013) reported nearly total cell death in both motile and non-motile algal species after 20 hrs of treatment with chitosan and modified sand. During site selection for demonstration scaled testing, water quality parameters and algal cell density are to be considered for adjustments in flocculant dosage. Figure 6 shows a graphical illustration of the flocculation treatment in open water.

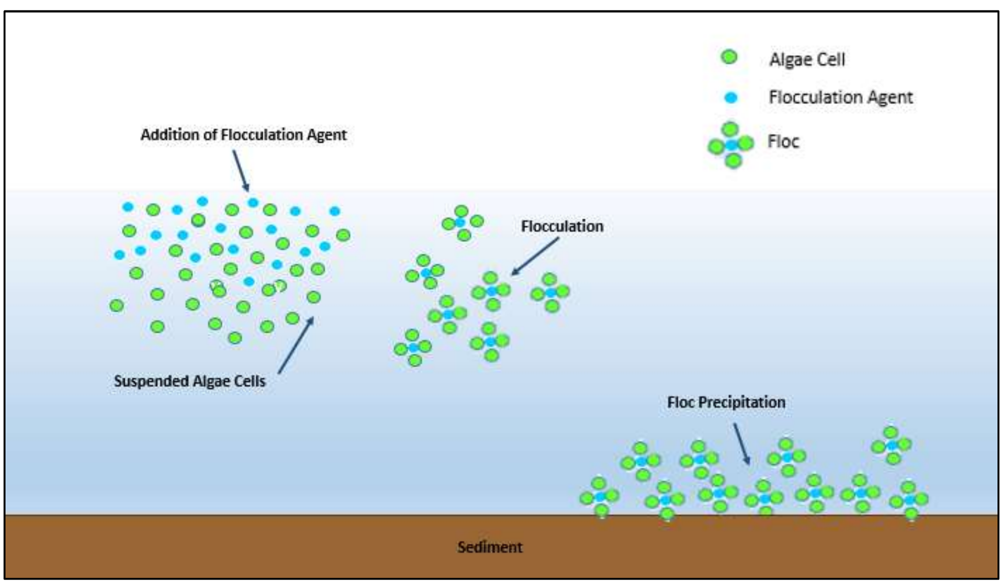

Figure 6.Schematic illustration of the anticipated behavior and fate of chitosan during the flocculation process in open water

Factors to consider when applying chitosan as a flocculent into open water includes the additives to be selected for optimizing the coagulation process, as well as the target algae species. Modified clays and sands have been noted as important additives to achieve high removal efficiency as chitosan-algae flocs alone can potentially be too small and remain suspended in the water. Also, depending on the algae species, wind induced currents may contribute to the escape of the algae cells from the floc, and its resuspension into the water column (Li and Pan, 2013).

Benefits to USACE

The application of algae flocculation by chitosan could be applied to bays, coves, and other areas where cyanobacteria are often concentrated. A cost effective means of algal sedimentation for backwater areas could considerably reduce their concentrations in the water columns, and thus prevent the spread of algal blooms throughout a USACE managed lake or reservoir. Findings from the present study provides valuable information that can lead to the implementation of minimally invasive removal technologies for cyanobacteria and its associated toxins. This application could be potentially optimized if combined with other treatment technologies (such as modified clays). This work would benefit USACE as the application of this technology is cost effective, and does not involve the addition of harsh chemical compounds into the environment.

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