ERDC/TN ANSRP-22-2
July 2022

Chitosan as a Coagulant and Precipitant of Algae Present in Backwater

by Catherine Thomas, Jonathan Broussard, and Victor Medina

PURPOSE: The purpose of this technical note (TN) is to highlight the current state of knowledge of algal flocculation by chitosan and identify data gaps existing between specific algal characteristics and chitosan binding efficiency. Published relationships and correlations between the quality of backwaters and the prevalence of algae, a baseline for flocculation efficiency of microalgae, and ideal treatment instances for algal removal by way of chitosan flocculation and precipitation will be identified.

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) because 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 allow increased settling of suspended sediment. Increased light penetration through the water column due to 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).

Water quality parameters affecting algal blooms. Nutrients N and P 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 have 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 lacking 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 and 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, and 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 occurs 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.

Types of commonly phytoplankton found in backwaters. There are numerous classes and species of phytoplankton found in freshwater systems, however, not all species can thrive under the same conditions (Ferris et al. 2005). Species composition may shift because 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 including green algae, blue-green algae, and diatoms (Pal and Choudhury 2014). Throughout this TN, algal flocculation relative to blue-green algae will be of primary focus as microcystin toxins released from the *Microcystis* genera are of great concern.

LITERATURE BACKGROUND: 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.

ERDC study methods. A preliminary investigation conducted at the US Army Engineer Research and Development Center (ERDC) evaluated flocculation agent chitosan, clay, and a modified clay 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 treatment process involved adding different doses of the flocculation agents (chitosan and clays) to the algae samples and mixing them on an orbital shaker to induce flocculation. In the first batch experiment, algae samples were treated with chitosan under continuous mixing and static (no mixing) conditions to compare the binding affinities of the chitosan to algal cells in each instance. Thereafter, two additional batch tests evaluated chitosan flocculation of algae at: (1) a broad chitosan application range at concentrations between 0 and 100 mg/L to determine the most appropriate dosing amounts, and (2) a narrow chitosan application range between 0 and 8 mg/L to further determine optimal dose amounts relative to algal density. A final batch test evaluated clays at dosing concentrations between 0 and 450 mg/L to compare flocculation efficiency with that observed in chitosan batch tests. The optical density (OD), measured in Absorbance Units (AU), was determined via visible spectrophotometry at wavelengths between 660 nm and 665 nm for pre- and post- treated media.

ERDC study results. Results from algae batch experiments evaluating a broad range of chitosan dosages revealed the most effective concentrations were between 2 and 18 mg/L for samples tested under continuous mixing and static conditions (Figure 1). The initial OD measurement for algae test cultures at 0 hr was 0.02 AU with chitosan applications yielding OD reductions up to 90%. Optical density measurements for static and mixed samples with chitosan amendments were between 0.002 and 0.003 AU. However, an important point to note is the reduction in OD by 70 and 75% for static and mixed samples, respectively, after 24 hr in algae samples with no chitosan amendment. OD measurements for samples amended with chitosan at 18 mg/L under static conditions were comparable to those observed in mixed samples; thus suggesting that an over loading of chitosan may have occurred.

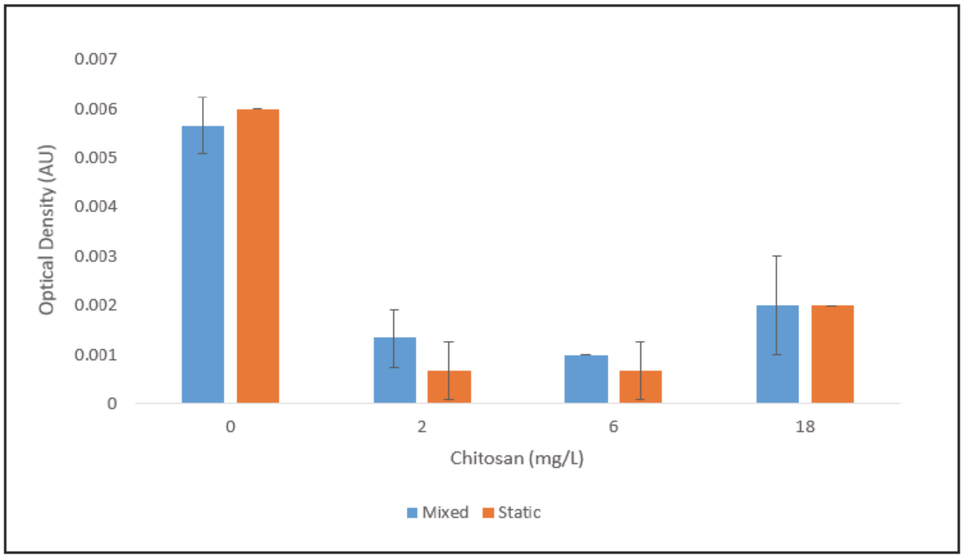

Figure 1. Optical density measurements for algae samples 24 hr after chitosan treatments at concentrations between 0 and 18 mg/L under mixed and static conditions.

A second batch test with algae evaluated a narrower range of chitosan dosages with concentrations between 0 and 8 mg/L under continuous mixing conditions (Figure 2). The initial OD measurement of algae samples with no chitosan amendment was 0.035 AU at 0 hr. The most effective chitosan dose was observed to be 8 mg/L as OD measurements at 24 and 48 hr were 0.001 and 0.000 AU, respectively, which yielded an OD reduction between 96 and 100%.

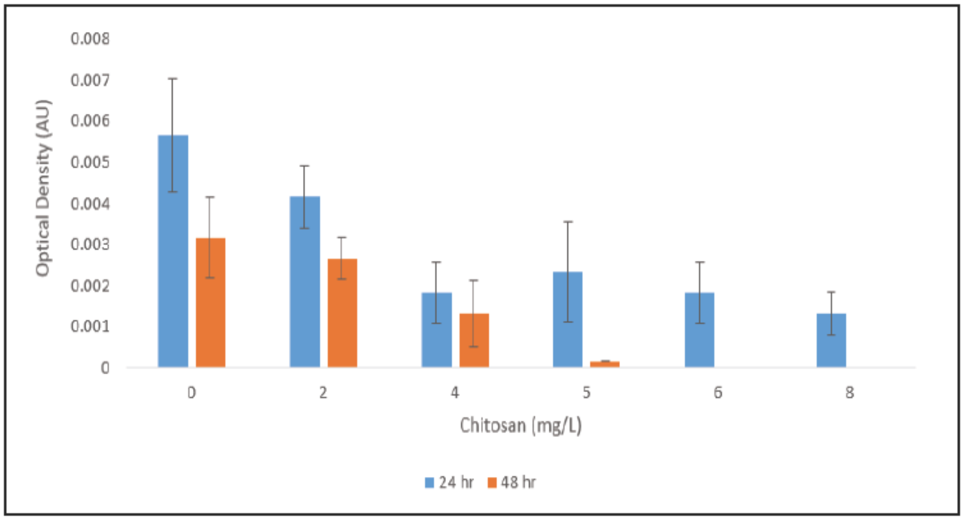

Figure 2. Optical density measurements for algae samples at 24 and 48 hr after chitosan treatments at concentrations between 0 and 8 mg/L.

A third batch test to evaluate chitosan flocculation efficiency was conducted on samples with a higher algal cell density with an average OD measurement of 0.45 AU. Algae samples amended with chitosan at concentration ranges between 0 and 8 mg/L yielded OD measurements between 0.19 and 0.12 AU, with the most effective dosage at 8 mg/L (Figure 3). Compared to algae samples having a lower cell density, the treatment efficiency of chitosan was comparatively less for each dose as OD measurements for chitosan treatments between 2 and 6 mg/L were greater than or equal to algae sample with no chitosan addition.

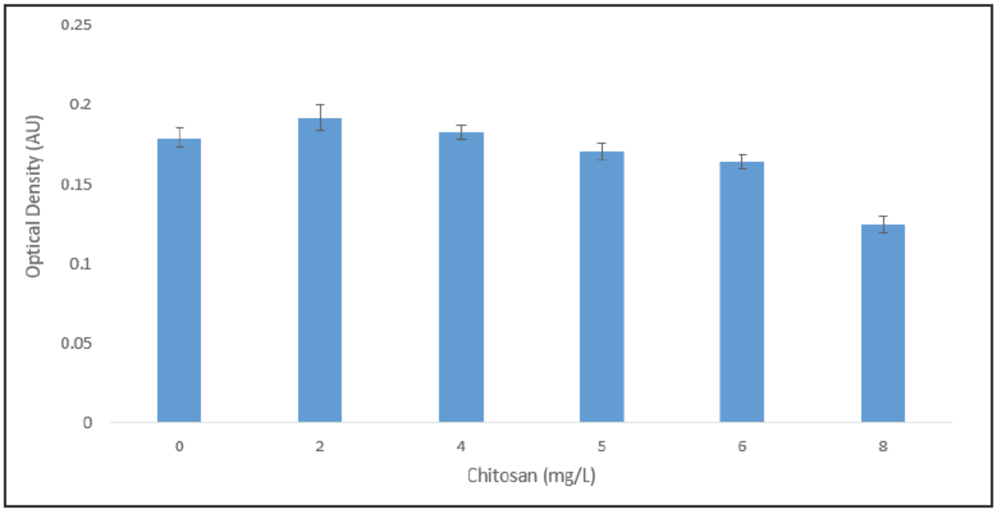

Figure 3. Optical density measurements for high algal biomass samples at 24 hr after chitosan treatments at concentrations between 0 and 8 mg/L.

In addition to treatments with chitosan, clays were also evaluated for their ability to flocculate and precipitate algae. Modified and non-modified clays were comparable in their flocculation efficiency after 24 hr relative to samples with no clay amendments. Clay amendments yielded OD measurements greater than or equal to the samples with no clay amendments. Modified clay at 50 ppm after 24 hr yielded a marginally lower OD measurement at 0.16 AU compared to samples with no modified clay amendments, as the OD measurement was 0.17 AU after 24 hr (Figure 4). Clay amendments were applied to samples with a relatively high cell biomass having an initial OD measurement of 0.61 AU at 0 hr.

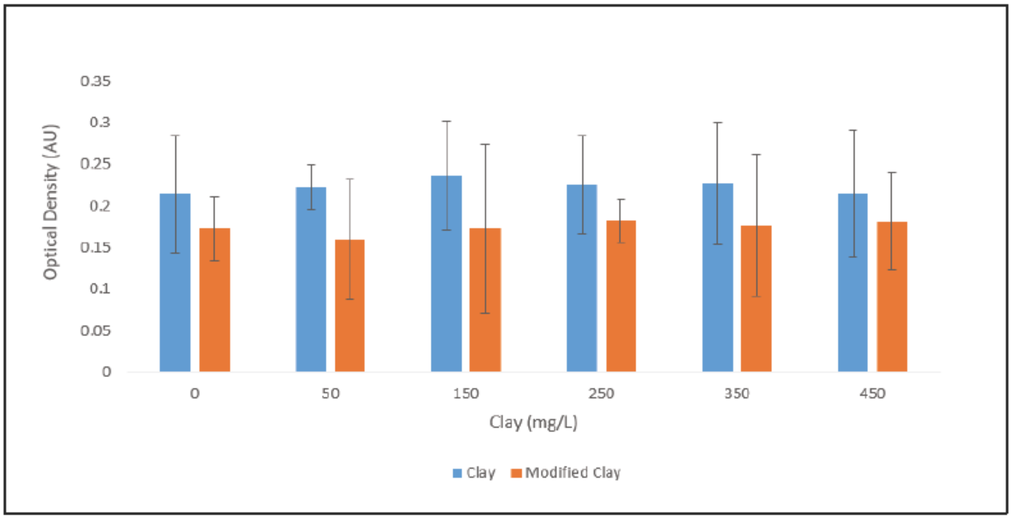

Figure 4. Optical density measurements for algae samples at 24 hr after clay treatments at concentrations between 0 and 450 mg/L.

Results from the preliminary studies suggest chitosan as a promising agent for algal flocculation. Although modified clay demonstrated a marginal ability to flocculate algae, the theoretical mechanism by which chitosan and clay particles bind to the algae membrane surface may be of benefit when tested together to achieve a synergistic effect on algal precipitation (Figure 5).

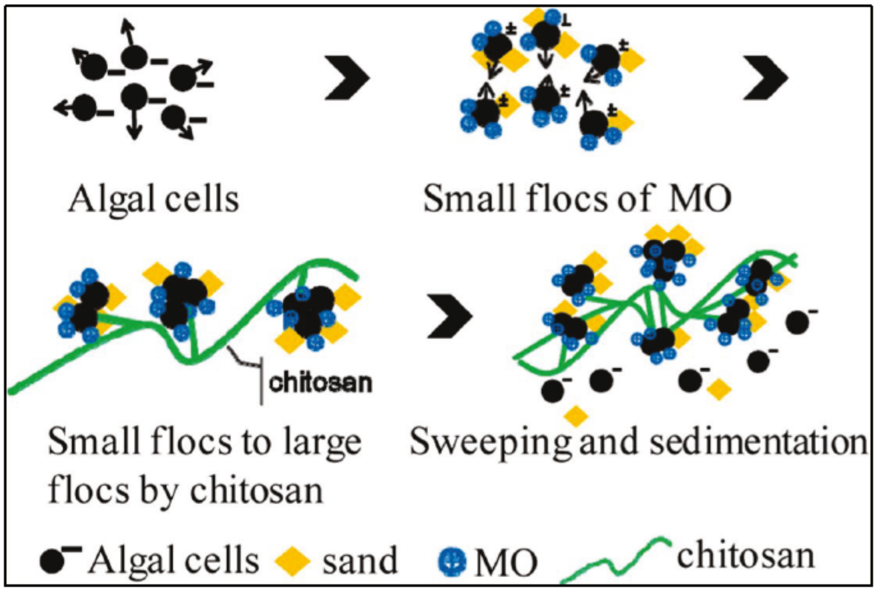

Figure 5. Conceptual representation of algal flocculation by chitosan and sand modified with Moringa oleifera (MO). (Photo adapted from Li and Pan 2013).

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. 2019). Environmental conditions must be considered as morphological and physiological characteristics of algal cells since they 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) is important to consider since it 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 determine algae species-specific advantages and limitations of flocculation treatments more accurately 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: Backwater 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 motile and non-motile algal species after 20 hr of treatment with chitosan and modified clay. During site selection for demonstration scaled testing, water quality parameters and algal cell density should be considered for adjustments in flocculant dosage. Figure 6 shows a graphical illustration of the flocculation treatment in open water.

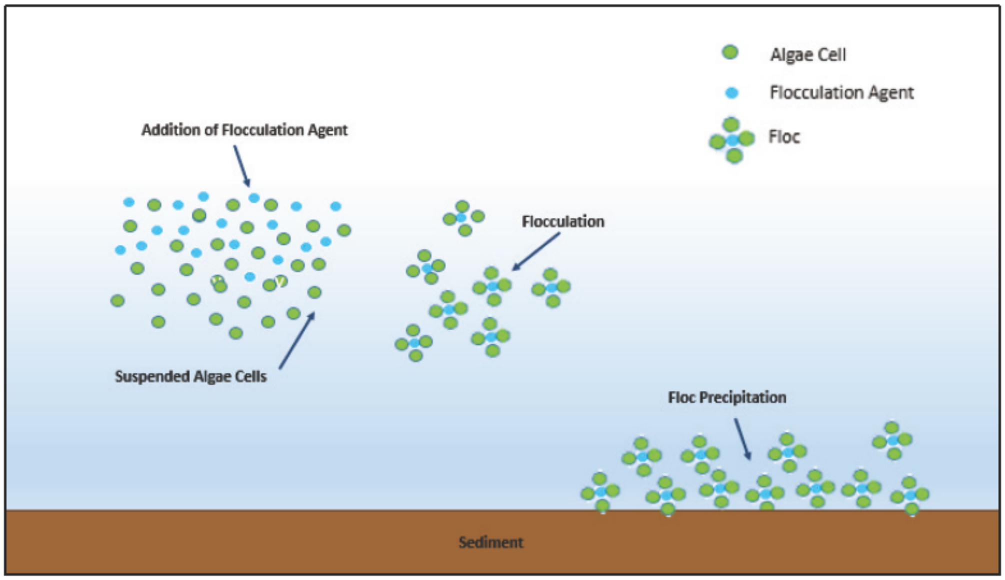

Figure 6. Schematic illustration of the anticipated behavior and fate of chitosan during the flocculation process in open water.

Factors to consider when applying chitosan as a flocculent into open water include 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 US Army Corps of Engineers (USACE) managed lake or reservoir. Findings from the present study provide 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.

REFERENCES

Beach, E., M. Eckelman, Z. Cui, L. Brentner, and J. Zimmerman. 2012. "Preferential technological and life cycle environmental performance of chitosan flocculation for harvesting of the green algae *Neochloris oleoabundans*." *Bioresource Technology* 121:445–449.

Berg, M., and M. Sutula. 2015. *Factors affecting the growth of cyanobacteria with special emphasis on the Sacramento-San Joaquin Delta*. Southern California Coastal Water Research Project (SCCWRP) Technical Report #869.

Branyikova, I., G. Prochazkova, T. Potocar, Z. Jezkova, and T. Branyik. 2018. "Harvesting of Microalgae by Flocculation." *Fermentation* 93(4): 1-12.

Carmichael, W. 2001. "Health Effects of Toxin-Producing Cyanobacteria: The CyanoHABs." *Human and Ecological Risk Assessment: An International Journal* 7(5): 1393-1407.

Chen, G., L. Zhao, Y. Qi, and Y. Cu. 2014. "Chitosan and Its derivatives applied in harvesting microalgae for biodiesel production: An outlook." *Journal of Nanomaterials* 2014:1-9.

Darwesh, O., I. Matter, M. Eida, H. Moawad, and Y. Oh. 2019. "Influence of nitrogen source and growth phase on extracellular biosynthesis of silver nanoparticles using cultural filtrates of Scenedesmus obliquus." *Applied Science* 9:1465.

Davis, T. W., G. S. Bullerjahn, T. Tuttle, R. M. McKay, and S. B. Watson. 2015. "Effects of increasing nitrogen and phosphorous concentrations on phytoplankton community growth and toxicity during planktothrix blooms in Sandusky Bay, Lake Erie." *Environmental Science & Technology* 49(12), 7197-7207.

Fay, P. 1992. "Oxygen relations of nitrogen fixation in cyanobacteria." *Microbiology and Molecular Biology Reviews* 56 (2): 340-373.

Ferris, M., K. Sheehan, M. Kuhl, K. Cooksey, B. Wigglesworth-Cooksey, R. Harvey, and J. Henson. 2005. "Algal Species and Light Microenvironment in a Low-pH, Geothermal Microbial Mat Community." *Applied and Environmental Microbiology* 71(11): 7164–7171.

Gerardo, M., S. Van Den Hende, H. Vervaeren, T. Coward, and S. Skill. 2015. "Harvesting of microalgae within a biorefinery approach: A review of the developments and case studies from pilot-plants." *Algal Research* 11: 248–262.

Heisler, J., P. Gilbert, J. Burkholder, D. Anderson, W. Cochlan, W. Dennison, C. Gobler, Q. Dortch, C. Heil, E. Humphries, A. Lewitus, R. Magnien, H. Marshall, K. Sellner, D. Stockwell, D. Stoecker, and M. Suddleson. 2008. "Eutrophication and harmful algal blooms: A scientific consensus." *Harmful Algae* 8(1):3-13.

Issa, A., M. Abd-Alla, and T. Ohyama. 2014. *Nitrogen fixing cyanobacteria: Future prospect, advances in biology and ecology of nitrogen fixation*. DOI: 10.5772/56995. https://www.intechopen.com/books/advances-in-biology-and-ecology-of-nitrogen-fixation/nitrogen-fixing-cyanobacteria-future-prospect.

Li, L., and G. Pan. 2013. "A universal method for flocculating harmful algal blooms in marine and fresh waters using modified sand." *Environmental Science & Technology* 47(9):4555-4562.

Matter, I., V. Bui, M. Jung, J. Seo, Y. Kim, Y. Lee, and Y. Oh. 2019. "Flocculation harvesting techniques for microalgae: A review." *Applied Science* 9(15):3069.

Pal, R., and A. Choudhury. 2014. A Brief Introduction to Phytoplanktons in *An Introduction to Phytoplanktons: Diversity and Ecology*, eds. Ruma Pal and Avik Kumar Choudhury, pp 1-39. Springer New Delhi. ISBN 978-81-322-1838-8.

Pivokonsky, M., J. Safarikova, M. Baresova, L. Pivokonska, and I. Kopecka. 2014. "A comparison of the character of algal extracellular versus cellular organic matter produced by cyanobacterium, diatom and green alga." *Water Research* 51:37-46.

Pugazhendhi, A., S. Shobana, P. Bakonyi, N. Nemestóthy, A. Xia, J. R. Banu, and K. Kumar. 2019. "A review on chemical mechanism of microalgae flocculation via polymers." *Biotechnology Reports* 21:e00302.

Solovchenko, A., I. Goldberg, S. Selyakha, L. Semenova, T. Ismagulova, A. Lukyanov, I. Mamedov, E. Vinogradov, O. Karpova, I. Konyukhov, S. Vasilieva, P. Mojzes, C. Dijkema, M. Vecherskaya, I. Zvyagin, L. Nedbal, and O. Gorelova. 2019. "Phosphorus starvation and luxury uptake in green microalgae revisited." *Algal Research* 43: 101651.

Strauss, E., W. Richardson, J. Cavanaugh, L. Bartsch, R. Kreiling, and A. Standorf. 2006. "Variability and regulation of denitrification in an Upper Mississippi River backwater." *Journal of the North American Benthological Society* 25(3): 596-606.

USEPA. 2017a. *The Effects: Dead Zones and Harmful Algal Blooms*. Nutrient Pollution. https://www.epa.gov/nutrientpollution/effects-dead-zones-and-harmful-algal-blooms.

USEPA. 2017b. *Indicators: Phosphorus*. National Aquatic Resource Surveys. https://www.epa.gov/national-aquatic-resource-surveys/indicators-phosphorus.

Wang, W. 1974. *Effect of Turbidity on Algal Growth*. Urbana, IL: Illinois State Water Survey. ISWS-74-CIR121. http://hdl.handle.net/2142/94428.

Yao, B., C. B. Xi, H. Shouliang, H. Su, and H. Liu. 2011. "A model and experimental study of phosphate uptake kinetics in algae: Considering surface adsorption and P-stress." *Journal of Environmental Sciences* 23(2): 189-198.

Zhang, H., J. Zhu, X. Zhu, J. Cai, A. Zhang, Y. Hong, J. Huang, L. Huang, and Z. Xu. 2012. "High-level exogenous glutamic acid-independent production of poly-(γ-glutamic acid) with organic acid addition in a new isolated Bacillus subtilis C10." *Bioresource Technology* 116: 241-246.

Zhu, L., Z. Li, and E. Hiltunen. 2018. "Microalgae Chlorella vulgaris biomass harvesting by natural focculant: Effects on biomass sedimentation, spent medium recycling and lipid extraction." *Biotechnology for Biofuels* 11:183.