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Aquatic Nuisance Species Research Program (ANSRP)

Rapid Algae Flotation Techniques

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Under Project 008284, “Research on Algae Flotation Techniques”

Abstract

Dissolved air flotation (DAF) is an effective technique for algae separation following the application of flocculants and coagulants. Some harmful algae produce mucilage or extracellular polymeric substances useful for flotation. This study evaluated natural polysaccharides to determine effects on algal flotation with DAF. Food-grade gums (xanthan gum, guar gum, gum arabic, gellan gum, and diutan gum) were tested with cyanobacteria cultures singly and in combination with commercial flocculants (including Tramfloc 222 and Tramfloc 300). Gum arabic alone had no effect when evaluated at concentrations between 10 mg/L and 5,000 mg/L. However, the combination of gum arabic and Tramfloc 300 yielded higher algal flocculation than Tramfloc 300 alone. The combination of xanthan gum (anionic) and guar gum (cationic) did not perform at the level of the combined xanthan gum and Tramfloc 222 in either flocculation or flotation of algae. Tramfloc 222 and xanthan gum; however, yielded effective flocculation seemingly resistant to changes in interfering factors such as turbulence, pH, and temperature. Furthermore, the combination of xanthan gum and Tramfloc 222 provided the most effective flotation and flocculation independent of pH effects. The results suggest that anionic polysaccharides can be used to increase the efficacy of cationic coagulants such as Tramfloc 222.

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Preface

This study was conducted for the Aquatic Nuisance Species Research Program (ANSRP) under Project Number 008284, “Research on Algae Flotation Techniques.” The technical monitor was Michael Greer, ANSRP Washington, DC 20314-1000.

The work was performed by the Materials and Structures Branch of the Research and Engineering Division, US Army Engineer Research and Development Center–Construction Engineering Research Laboratory (ERDC-CERL). At the time of publication, Ms. Ellen Hartman was acting branch chief; and Mr. Tim Shelton was division chief. The deputy director of ERDC-CERL was Ms. Michelle Hanson, and the director was Dr. Andrew Nelson.

COL Christian Patterson was commander of ERDC, and Dr. David W. Pittman was the director.

1 Introduction

1.1 Background

Rapid population growth has long been associated with environmental degradation (Commoner 1991) due to the depletion of sustainable natural resources, as well as the interruption of replenishing processes carried out in nature. Specifically, increased volumes from sewage treatment, industrial discharges, agricultural runoff, and urban areas all contribute to excessive nutrient loading into waterbodies (Khan and Mohammad 2013). Irreversible damage to aquatic ecosystems can result from the development of rural areas if mitigation plans are not developed to alleviate environmental stress. Further, removal of aquatic buffers and natural wetlands contribute nutrient loading into waterbodies as these environmental features act as natural buffers that filter surface water runoff. Resulting eutrophication can cause an overgrowth of algae, often referred to as harmful algal blooms (HABs) due to their debilitating effect on waterbodies. Commonly encountered toxin-forming strains of the blue-green algae *Microcystis* spp. that produce hepatotoxin microcystin and *Anabaena* spp. that produce the neurotoxin anatoxin, are also of great concern (Michalak et al. 2013). Dodds et al. (2009) projected an economic loss in the United States totaling over 2.2 billion dollars annually because of human-induced eutrophication. HABs continue to impair the water quality of US Army Corps of Engineers (USACE) managed water resources, which can disrupt the delivery of water for municipal, agricultural, and industrial uses. Additionally, USACE may have to devote national resources to mitigate the impact of HABs on recreation, navigation, and other activities that depend on clean and healthy water.

Several difficulties in addressing HABs are attributed to their rapid growth and toxin production. Chemical treatments can pose direct and indirect risks to the aquatic system. The chemical itself may have ecological toxicity effects, or it may damage the cells of toxin-producing algae species thereby releasing the toxins in the water. Physical removal methods, such as skimming, can be inefficient as they target removal of algae present only at the water surface. Thus, the process of algae separation has been optimized over the years into a beneficial practice dually targeted to remove algae and associated nutrients from the aquatic system.

In addition to the challenges of controlling and preventing nutrient loading into waterbodies, legacy phosphorus in sediment undergoes cycles of storage and release over long periods of time (Spears et al. 2011), though chemicals may be added to lock phosphorus in the sediment to slow this process.

In recent years, the concept of algae removal has been explored in the form of harvesting, where intact algae cells are removed from the water and recycled for further use in many applications. Presently, algae are being tested as a feedstock to produce clean biofuels and is of substantial economic significance compared to traditional fossil fuel sources of coal and gas, and even agricultural crops such as corn. While an algae feedstock is currently not projected to replace other sources of biofuel, the selection of a cost-effective means for algae harvesting can be a pivotal point in supplementing the biofuel production process. The cost-effectiveness of algae harvesting technology depends exclusively on biomass capture. Several methods of algae harvesting technologies were studied, including coagulation, flocculation, and centrifugation, with increasing attention directed towards refining the flocculation and coagulation process. This research will focus on identifying biobased constituents for flocculating microalgae in preparation for harvesting.

1.2 Algae separation mechanisms

The separation of algae for harvesting by way of dissolved air flotation (DAF) has been investigated for decades. Early research evaluating the DAF process did not demonstrate substantial algae removal when the algae cultures were tested without flocculating agents (Bare et al. 1975; Van Vuuren et al. 1965). Van Vuuren et al. (1965) were among the first to observe naturally occurring algae flotation, which lead to algae separation by flotation employing chemical flocculants and pressurized air during periods when algae were naturally respiring. Later, Bare et al. (1975) reported the success of algae separation with DAF when the natural flocculation of the algae occurred in the endogenous growth phase, although separation efficiencies varied with algae species and concentration.

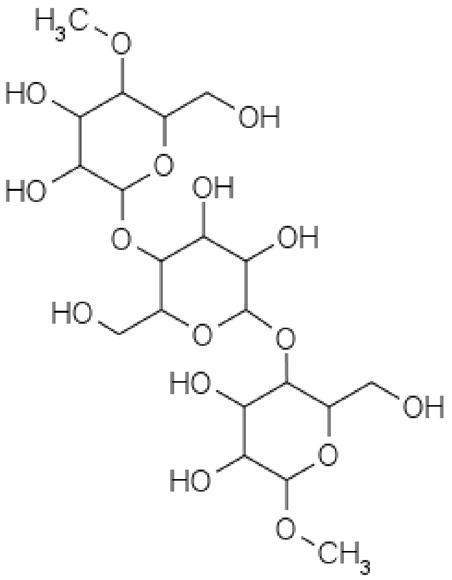
Many alga species exhibit growth strategies that can exploit different environments under different conditions, with each species possessing different adaptations (Steidinger and Garccés 2006). During the progression of the algae life cycle in many unicellular or filamentous algae, increased amounts of mucilage are produced from the cells. The mucilage, or extracellular polymeric substance (EPS) is typically secreted from algal pores (or in some instances filaments) and are mainly composed of polysaccharides. Due to its pseudoplastic nature, the EPS generally remains attached to the cell, although some of this substance can be released into the surrounding environment (Pereira et al. 2013). The natural aggregation of algal cells is driven by the EPS matrix secreted from the cell. Findings from a previous study investigating cellular aggregation abilities showed a decrease between 27.6% and 57.4% in cell amassing when the EPS matrix was extracted from cultured Microcystis (Xu et al. 2014). The presence of this substance can affect the extent to which binding agents can efficiently aggregate algal cells. This phenomenon is supported by Bare et al. (1975) as findings concluded that naturally separated algae in combination with chemically treated algae greatly improved removal efficiencies, an important factor to consider when optimizing methods to harvest algae using flocculating or coagulating agents.

To date, DAF is a proven and effective means of algae separation upon addition of flocculants and coagulants as a preliminary treatment. Generally, the types of flocculants used, particularly in wastewater treatment, include polyacrylamide-based flocculants, multivalent metal salts, chitosan, and tannins or starch compounds functionalized with quaternary ammonium groups (Muylaert et al. 2017; Roselet et al. 2016). In algae flocculation, the general function of coagulants (which tend to be positively charged to allow for interactions with the negatively charged surface of microalgal cells) is to induce coagulation by neutralizing the surface charge of particles or by forming bridges between the target cells (Muylaert et al. 2017). Flocculation increases particle sizes from microscopic to visibly suspended particles, which interact further with coagulants, bridge flocs and thereby increase separation kinetics. Li et al. (2018) explains that binding by way of bridge flocculation can be considerably stronger than coagulation by simple salts or electrolyte via charge neutralization, although coagulants can be applied post flocculation as a clarification step in particle separation. It is important to note that a flocculant must meet specific requirements when (1) being applied in open water and (2) harvesting algae for recycling purposes. In addition to a flocculating agent meeting criterion of cost-effectiveness, treatment efficacy, and availability, it must also be environmentally benign (Chatsungnoen and Chisti 2019).

1.3 Natural gums as a flocculant for algae

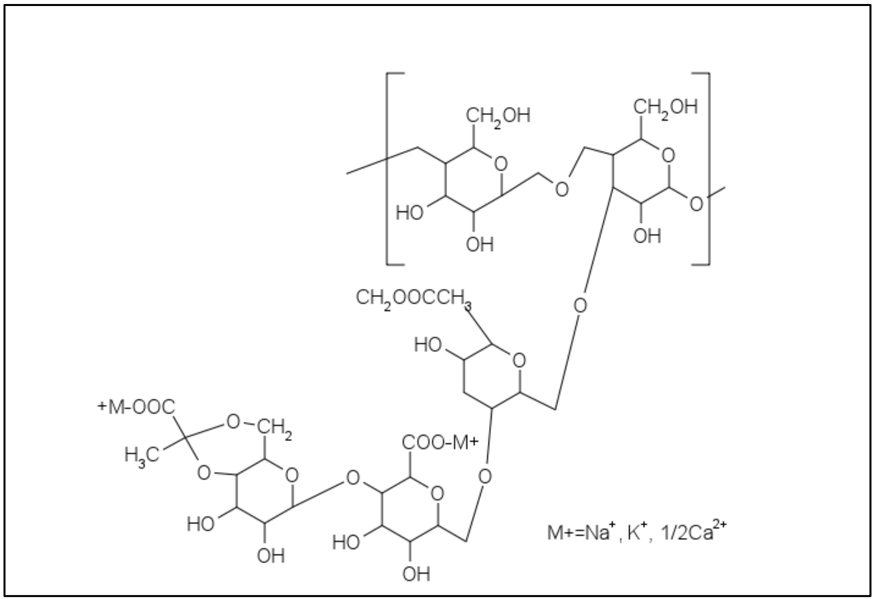
The gums evaluated in this study include gum arabic (GA) and xanthan gum (XG). GA is a branched-chain, complex polysaccharide, either neutral or slightly acidic, found as a mixed calcium, magnesium, and potassium salt of a polysaccharidic acid (Ali et al. 2009). As a natural exudate from the *Acacia senegalia* tree, GA is a high-molecular-weight polysaccharide with proteinaceous constituents (Figure 1). Dickinson et al. (1989) explains that most of the nitrogen content of GA is the fraction (<30%) of the compounds that functions in absorption. The sorption behavior of Acacia gum samples depends not only on the overall nitrogen content but also on the distribution of protein between low- and high-molecular-weight fractions and on the molecular accessibility of the protein component for rapid adsorption (Dickinson et al. 1989). GA is a negatively charged polyelectrolyte with several polysaccharide units linked to a common polypeptide chain, which provides its high surface activity and viscoelastic film-forming ability (Espinosa-Andrews et al. 2007). Thus, its function as an environmentally benign negatively charged flocculant for microalgae may include the addition of low dosed positively charged aluminum to achieve charge neutralization.

Figure 1. Chemical structure of gum arabic (GA).



XG is a branched, high-molecular-weight polysaccharide with acidic characteristics predominantly produced by *Xanthomonas campestris* (Bueno and Petri 2014; Geremia and Rinaudo 2005). Xanthan is an anionic gum that has been widely used due to properties such as high resistance to acidic and alkaline conditions (Rahmati et al. 2018; Long et al. 2013). Deprotonation of O-acetyl and pyruvyl residues at pH greater than 4.5 increases the negative charge density along xanthan chains. Physical crosslinking is then enabled and mediated by the presence of calcium ions (Bergmann et al. 2008, Dário et al. 2011). This leads to the presumption that the polysaccharide can effectively bind with the negatively charged cell surface of microalgae in electrostatic interactions (Figure 2).

Figure 2. Chemical structure of xanthan gum (XG).



Furthermore, the functions of GA and XG in alga flocculation are projected to occur via different mechanisms. GA or XG potentially require additives to facilitate flocculation.

1.4 Objective

The purpose of this work was to evaluate and manipulate the natural flotation mechanisms exhibited by harmful algae and cyanobacteria. Findings from preliminary work indicated improved efficiency from a pretreatment step applied in a water body upstream of the Harmful Algal Bloom Interception, Treatment, and Transformation System or similar surface algae mitigation systems (Page et al. 2020; Page et al. 2021). Specifically, the objectives were to (1) identify environmentally safe compounds that can coalesce algal colonies by enhancing the natural mechanism by which algal cells aggregate and (2) determine efficacy of the flocculating agents to float algal biomass to the water surface.

2 Technical Approach

2.1 Approach

Experimental design was used to evaluate and analyze experiments through the modification of input parameters and their impact on the dependent variables being studied. Orthogonal experimental matrices measure the impacts of the individual inputs independent of one another on the response variable.

Orthogonal experimental design employs a range of statistical tests to systematically investigate the effects of multiple factors on a response variable while minimizing the confounding of interactions among those factors. These tests primarily encompass analysis of variance (ANOVA) and regression techniques. ANOVA was employed to assess the significance of individual factors and their interactions, ensuring that each factor's contribution was accurately discerned by partitioning the total variance of the response variable. Regression analysis, particularly multiple linear regression, was employed to establish predictive models that quantitatively relate the factors to the response. The tests enabled the identification of significant factors and their optimal levels.

Rapid algal biomass flocculation and flotation were investigated and optimized using four iterative experimental design matrices. Each matrix consisted of selected desirable factors, identified interfering factors, and controlled constants. The manipulated factors were controlled at two levels apart from trial #4, which included a third level to identify model curvature.

2.2 Jar testing

Cyanobacteria samples collected from Lake of the Woods, Mahomet, Illinois, were cultured under laboratory conditions until desired algal concentrations were achieved. Jar tests were performed to evaluate the ability of natural, food-grade compounds to form aggregates of algal colonies that are subject to separation by DAF. Four laboratory trials were conducted. Table 1 lists the amendment and concentrations evaluated in this study.

Table 1. List of amendments evaluated in jar test experiments.

Anionic Additive	Experimental Ranges (mg/L)
Xanthan Gum	0.3 to 5
Gum Arabic	10 to 5000
Polyaluminum chloride 1400	100 to 200
Polyaluminum chloride 1430	100 to 200
Gellan Gum	0.3 to 5
Diutan Gum	0.3 to 5
Nonionic Additive	—
Mineral Oil	1 to 5
Cationic Additive	—
Tramfloc 222	0.1 to 5
Tramfloc 300	0.1 to 5
GFT5100	1 to 10
PolyDADMAC	0.1 to 1

All experimental treatments were performed using a four-station programmable, backlit Microfloc Platypus Jar Tester (Figure 3). Each jar was filled with 1 L* of algae-laden water for the experimental trials. The agitation program was set to three consecutive intervals: 30 s at 75 RPM, 120 s at 35 RPM, and 5 s at 15 RPM. Additives were consistently dosed at the start of the first interval in the program. All cationic additives were dosed after anionic or nonionic additives. Measurements were collected 120 s after addition of dissolved air from the DAF unit when visibility returned.

Dissolved air-flotation microbubbles were supplied by a Microfloc DAF Saturator Assembly, including a four-port distribution manifold (Figure 4). Microbubbles were introduced in the lower quarter of the jars immediately following the completion of the mixing procedures (Figure 3). Each experimental treatment was supplied microbubbles individually to prevent reduced air pressure across the manifold.

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* For a full list of the spelled-out forms of the units of measure used in this document and their conversions, please refer to *US Government Publishing Office Style Manual*, 31st ed. (Washington, DC: US Government Publishing Office, 2016), 248–52 and 345–47, https://www.govinfo.gov/content/pkg/GPO-STYLEMANUAL-2016/pdf/GPO-STYLEMANUAL-2016.pdf.

Figure 3. Four-station programmable, backlit Microfloc Platypus Jar Tester.

Figure 4. Microfloc dissolved air flotation (DAF) saturator assembly.

2.2.1 Laboratory trials

A total of four laboratory trials were conducted to evaluate the performance of environmentally safe, food-grade additives in the flocculation and flotation of algal colonies in water. In each trial, the treatment efficacy of food-grade additives was compared to the performance of commercially available flocculating agents that are highly effective in algae separation via DAF. Each trial consisted of an orthogonal testing matrix of factors set at two levels, low (-1) and high (+1). In some trials, a Block (B) factor was assigned to contain multiple noise or otherwise difficult to manipulate variables. The factor effects within a block were aliased together and evaluated apart from the main factor effects.

These reagents were used to establish a baseline by which the food-grade additives were compared (Table 2). The laboratory trials were conducted to down select the highest-performing additive for further evaluation in larger-volume reactors.

Table 2. Factors that remained constant in each laboratory trial.

Constants	
Mixing interval 1	30 s at 75 rpm
Mixing interval 2	120 s at 35 rpm
Mixing interval 3	5 s at 15 rpm
Measurement interval	120 s after DAF
Algae source	Shadow Lake, Mahomet
Algae species	Mixed; uncontrolled
Additive lot numbers	Constant for each material
DAF bubble size distribution	Greater than 1 micron

Trial #1 was a preliminary assessment in which GA was tested on algal cultures at concentrations between 10 and 5,000 mg/L. A cationic acrylamide copolymer, Tramfloc 300 (TF300), was also evaluated on algal cultures at 10 mg/L. Additionally, TF300 and GA were tested in combination at an application ratio of 1:2 under ambient conditions and no pH adjustment. To induce algae flotation and separation, 100 mL of DAF saturated air was added.

Laboratory trial #2, however, evaluated treatment combinations of Tramfloc 222 (TF222) and XG applied to an algae culture at a ratio of 2:0 under static conditions and ambient temperature (23°C) at pH 7. The reactors had 100 mL of DAF saturated air added. In a second test batch, cationic guar gum (CGG) and XG were also tested in combination in the algae culture at a ratio of 2:2 with 200 mL of DAF saturated air applied. Culture temperature was increased to 35°C, and pH was adjusted to 10 (Table 3).

Table 3. Experimental arrangement level settings for lab trial #2.

Factors	−1	1
Block (B)	—	—
→ pH	7	10
→ Temp (T) (°C)	23 (ambient)	35
→ Turbulence, additional	N	Y
Additive ratio (AR)	TF222:XG	CGG:XG
Additive concentration (AC) (mg/L)	2:0	2:2
DAF volume (DV) (mL)	100	200

In trial three, CGG, XG, TF222, PolyDADMAC* (PD), cationic starch GFT5100 (GF), and mineral oil (MO) were evaluated singly on algae cultures at concentrations of 0.5 mg/L, 0.3 mg/L, 0.5 mg/L, 0.1 mg/L, 0.5 mg/L, and 1.0 mg/L, respectively. In this trial, algae cultures were adjusted to pH 10, and subjected to 100 mL of DAF-saturated air (Table 4).

Trial #4 tested three treatment applications including XG, diutan gum (DG), and gellan gum (GG) tested at 0.5 mg/L in combination with TF222, BHR-P50 (BHR), polyaluminum chloride (PAC1400) and PAC1430 at concentrations of 0.25 mg/L–0.5 mg/L, 200 mg/L–400 mg/L, 100 mg/L–200 mg/L, and 100 mg/L–200 mg/L, respectively. Each test solution had an application of 100 mL of DAF saturated air (Table 5).

Table 4. Experimental arrangement for lab trial #3.

Level Settings	−1	1	Units
pH	8	10	—
XG	0	0.3	mg/L
CGG	0	0.5	mg/L
TF	0	0.5	mg/L
PD	0	0.1	mg/L
GF	0	0.5	mg/L
MO	0	1	mg/L

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* polydiallyldimethylammonium chloride

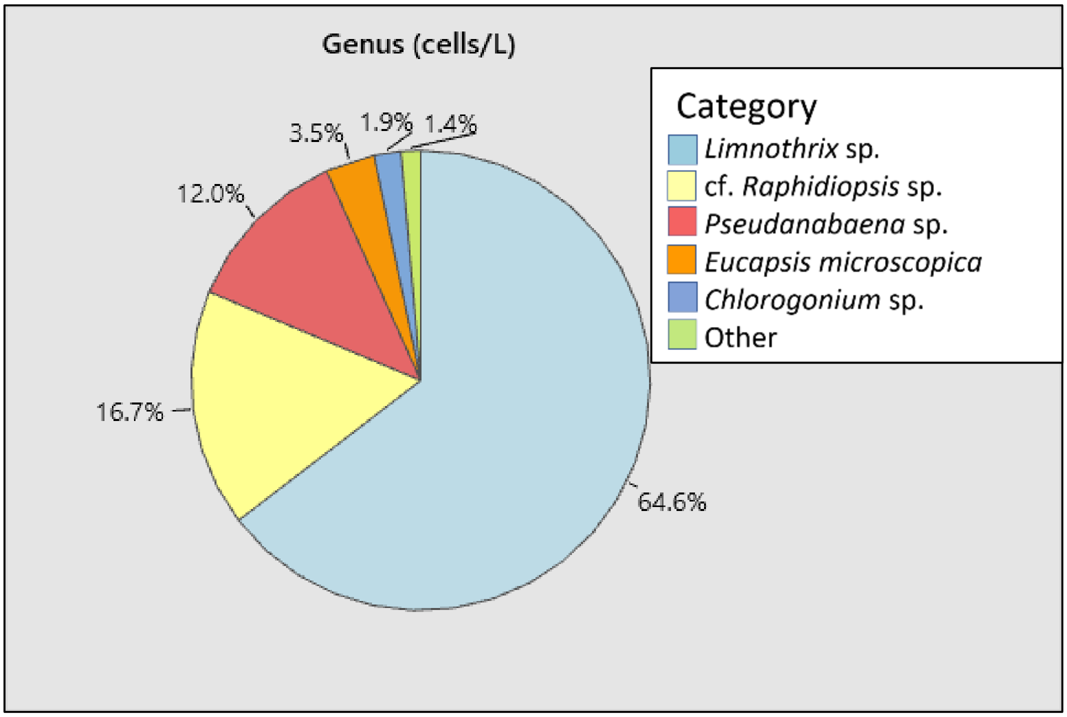
Table 5. Experimental arrangement for lab trial #4.

Level Settings	−1	0	1	
Gum	XG	DG	GG	Units
TF	0	0.25	0.5	mg/L
BHR	0	200	400	mg/L
PAC1400	0	100	200	mg/L
PAC1430	0	100	200	mg/L

2.2.2 Algae cultures

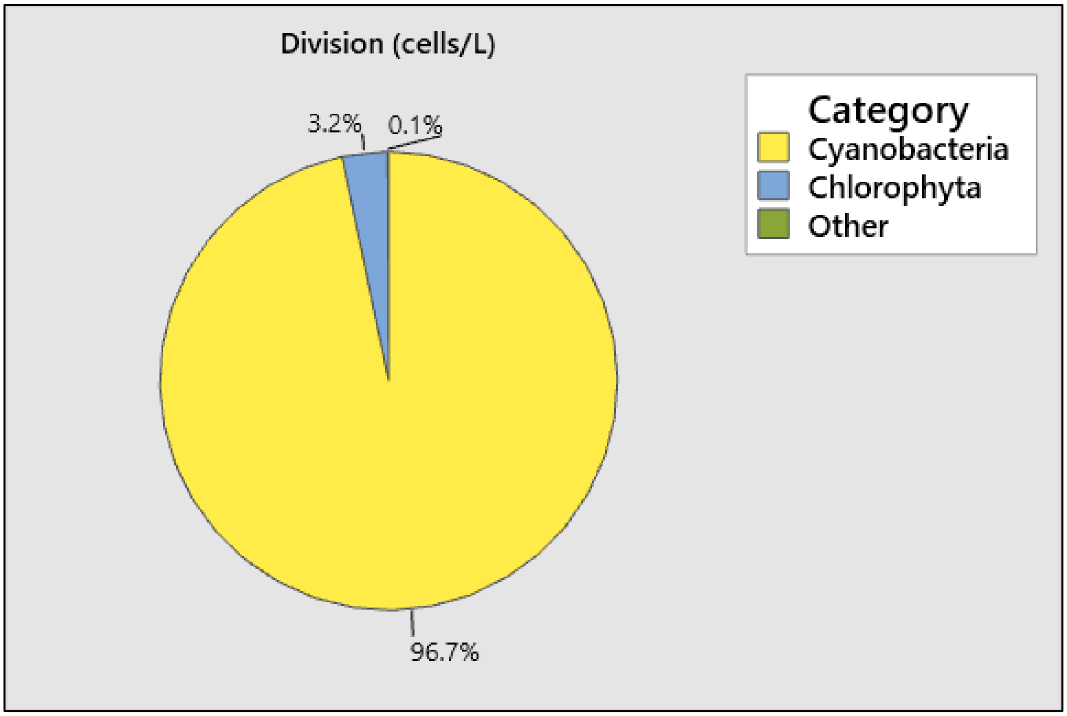
Mixed species cyanobacteria samples were collected and cultured from Lake of the Woods in Mahomet, Illinois. The samples were cultured under controlled laboratory conditions and maintained for experimentation (Figure 5).

Figure 5. Lab culture by genus.



The algae cultures were identified externally by BSA Environmental Services Inc. located in Beachwood, Ohio. The samples consisted primarily of the cyanobacteria *Limnothrix* sp. Much of the culture contained cyanobacteria over any other division of organism (Figure 6).

Figure 6. Lab culture by division.



2.3 Data Analysis

The matrices were recorded and analyzed using Minitab software. All experiments followed a two-level fractional factorial screening design. The data inputs for each factor were coded as low (-1) or high (+1) level to reduce bias. Automated stepwise regression was used in the Minitab software to determine the smallest p-value at each step ultimately resulting in the maximum of the test R^2 value. The selected regression equations for each lab trial are available in the Appendix. Pareto charts and accompanying desirability charts were generated from the model equation estimates to highlight the statistically significant factor and interaction effects.

Qualitative data collected through jar testing were transitioned to a semi quantitative state using an ordinal scale ranging from 1 through 5 (Table 6 and Table 7). Experimental results were evaluated twice by two individuals each and the results were averaged.

Table 6. Ordinal scale descriptions characterizing flocculation.

Flocculation Ordinal Scale	Description
5	Significant water clarity. Algae flocs are extremely dense and filamentous. Flocs are unable to be redispersed into the water through agitation.
4	Significant water clarity. Algae flocs are tighter but not dense. Few flocs can be redispersed through agitation.
3	Some clarity of water but noticeably green/opaque. Algae flocs are visible and somewhat loose. Flocs can be redispersed through agitation.
2	Water clarity is poor. Few algae flocs are visible and easily redispersed through agitation.
1	Water is green and considerably opaque. Nearly no algae flocs visible. Algae already dispersed in the water.

Table 7. Ordinal scale descriptions characterizing flotation.

Flotation Ordinal Scale	Description
5	All or nearly all visible algae flocs are located on the surface.
4	Most of the algae flocs are on the surface.
3	Some algae flocs are visible on the sides or bottom of the vessel.
2	Algae flocs are visible at every level below the surface.
1	Algae is still dispersed uniformly in the water column.

Other variables that could affect treatment performance included the following:

- Algae concentration
- Turbulence consistency
- Ambient temperature
- Individual colony size before treatment
- Ionic charge baseline before treatment
- Cooling rate of sample for high temp treatments (must be preheated before treatment)
- Microbubble size distribution and concentration
- DAF bubble flow rate into jar
- Cleanliness of jar before treatment
- Subjective measurement via visual ordinal scale

3 Results and Discussion

The preliminary assessment of algal flocculation and flotation in lab trial#1 showed that GA alone had no effect when evaluated at concentrations between 10 and 5,000 mg/L. The gelatinous aggregates of algae produced by GA demonstrated a tendency to adhere to surfaces and did not float efficiently under DAF conditions (Figure 7).

Figure 7. Electrostatically neutralized algae–gum arabic (GA) flocs observed after application of DAF saturated water.

The combination of GA and TF300 yielded visibly higher algal flocculation than TF300 alone (Figure 8 and Figure 9). The results show that low concentrations of the benign GA (or similar polysaccharides) could be used to increase the efficacy of low doses of cationic coagulants such as TF300. Since GA, as well as several other polysaccharides, are anionic, no ionic attraction is exhibited between the additive and suspended algal colonies as cyanobacteria in water also carries a net negative charge. Due to algae naturally producing small quantities of EPS, the addition of an anionic polysaccharide EPS substitute combined with the cationic attraction of the flocculant is likely to increase the mucus film formation and flocculation of the algae in solution.

Figure 8. From left to right is the control, Tramfloc (TF) 300, and TF300 + GA 30 s after dosing before the addition of DAF saturated water.

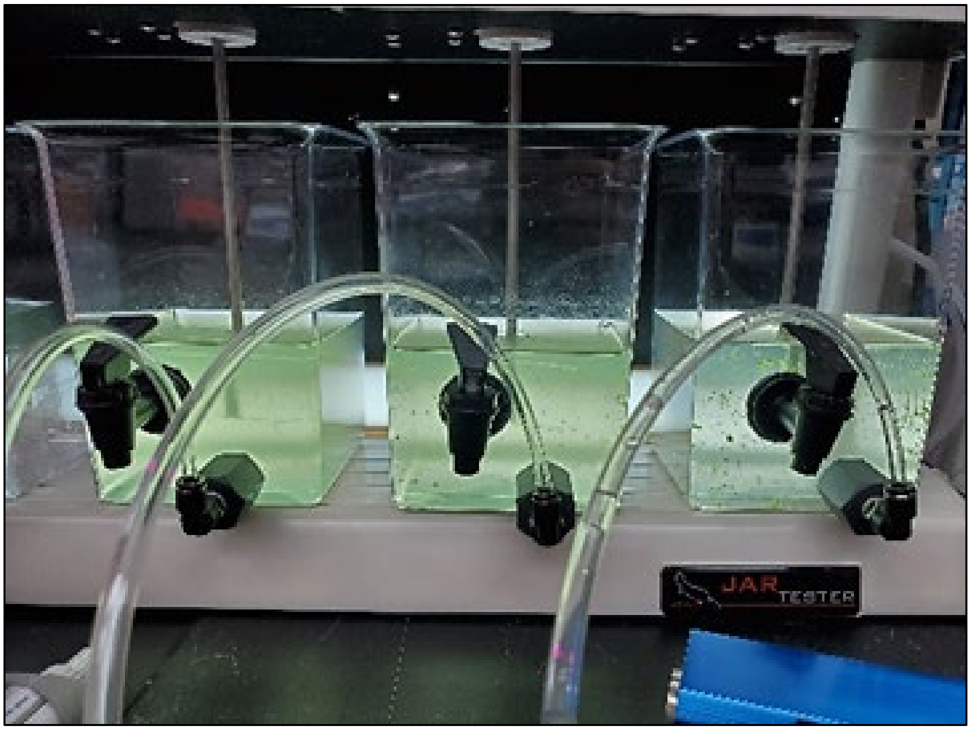
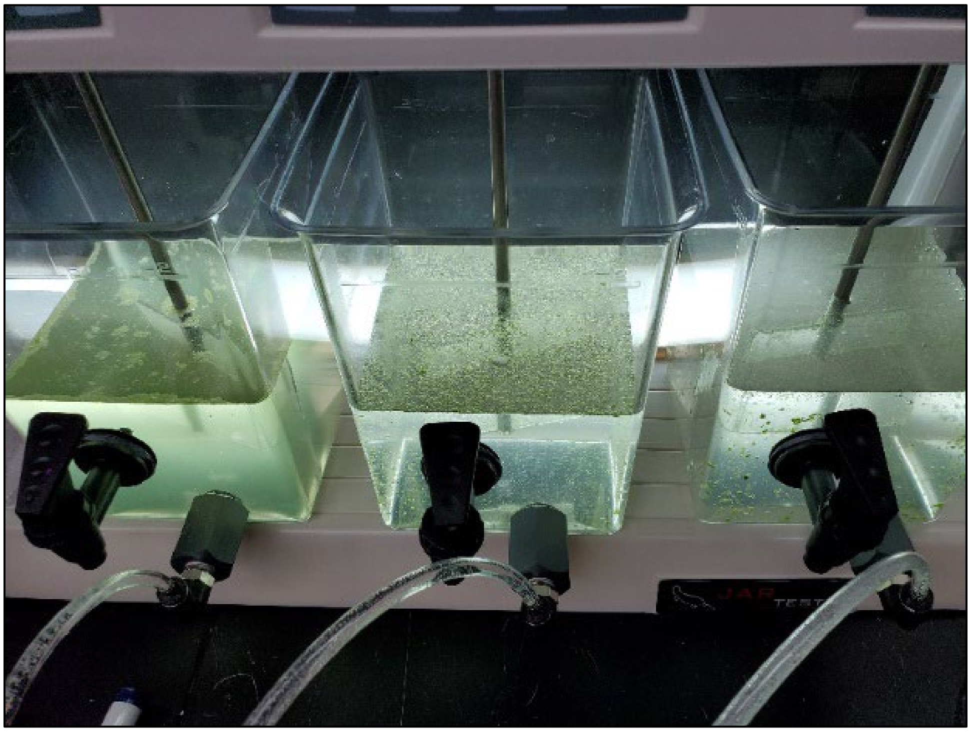
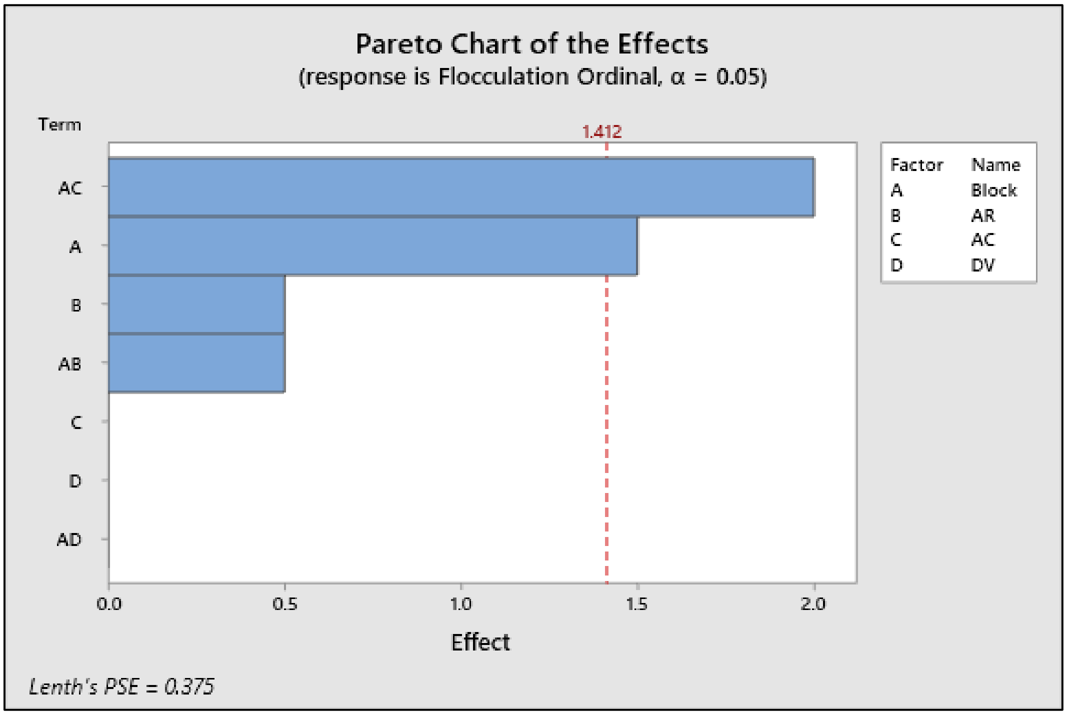


Figure 9. From left to right is the control, TF300, and TF300 + GA 100 mL of DAF saturated water added.



In laboratory trial #2, the combination of XG and CGG did not perform at the level of the combined XG and TF222 in either flocculation or flotation of algae. The additive concentration (AC)* performance alone was not statistically significant with noise from the block inhibiting ideal conditions for flocculation (Figure 10 and Figure 11). TF222, however, showed effective flocculation seemingly resistant to the effects of introduced interfering factors such as turbulence, pH, and temperature. No significant differences in flocculation efficiency were observed between 100 mL and 200 mL of DAF microbubble addition (Figure 11 and Figure 12).

Figure 10. Pareto chart depicting the ordinal flocculation effects from test additives: additive ratio (AR), additive concentration (AC), and dissolved air flotation volume (DV).



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* Terms A, B, C, and D correspond to the factors ‘Block’, additive ratio (AR), additive concentration (AC), and DAF volume (DV). Care should be taken not to confuse the abbreviation of additive concentration (AC) for the two factor interaction effect of terms A*C shown as AC.

Figure 11. First-order-effect plot for flocculation.

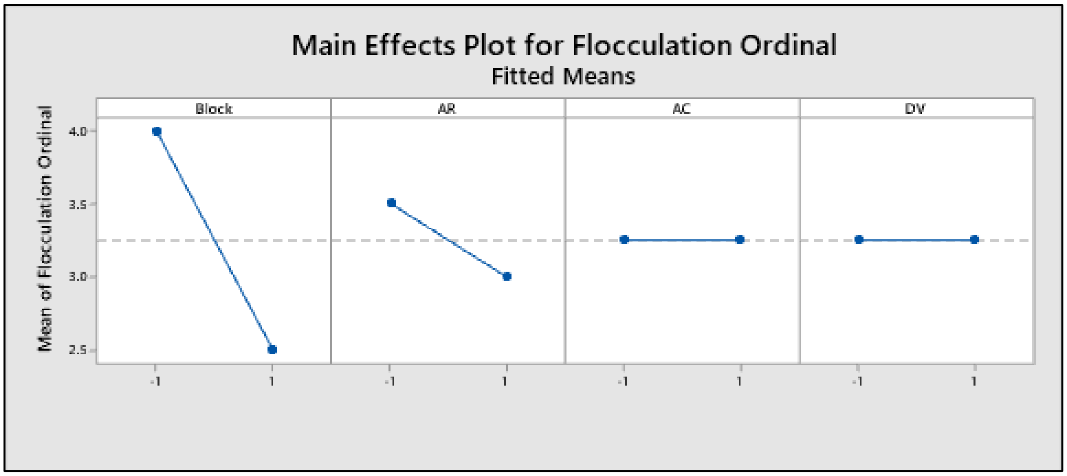
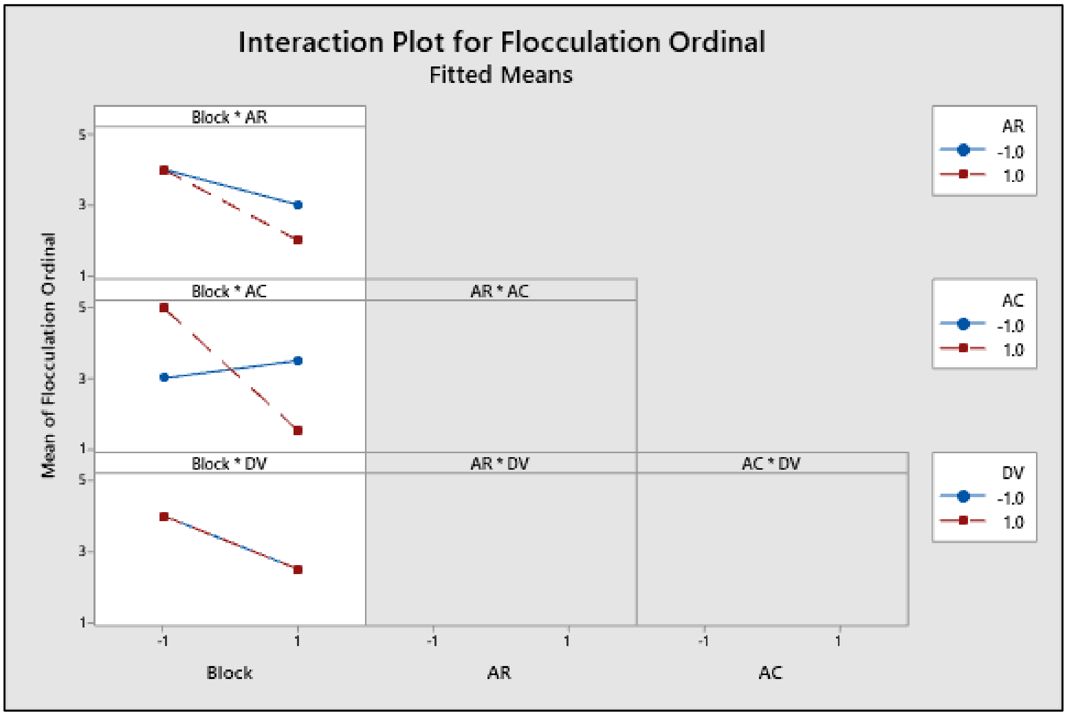


Figure 12. Second-order and cumulative effects for flocculation upon interaction of variables (additive ratio, additive concentration, and DAF volume).



The Pareto chart (Figure 10) indicates a significant effect on flocculation from the interaction between the AC factor and the block noise factor as well as the Block Noise alone. Optimizing the response suggests the artificially introduced noise had a major impact on successful flocculation (Figure 13). The block consisted of multiple variables and the weight of the effect of each cannot be determined from this experiment alone. It should be noted that decreasing the pH of the algae-laden water caused a visible but unstable flocculation effect without additional additives.

Although the Pareto analysis for flotation (Figure 14) did not identify any significant effects among the factors in this lab trial, there were treatments that exhibited favorable flotation. The interaction with the additive concentration factor warrants further investigation given that, in the absence of noise, the addition of XG produced a more favorable flocculation effect regardless of the cationic additive applied.

Figure 13. Desirability function plot for optimal flocculation and flotation.

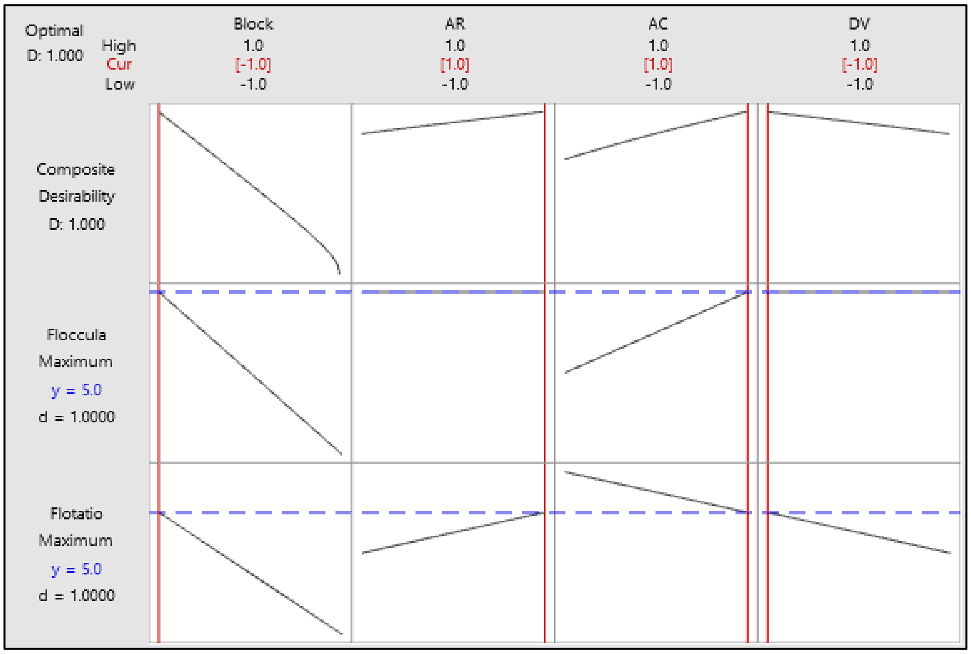
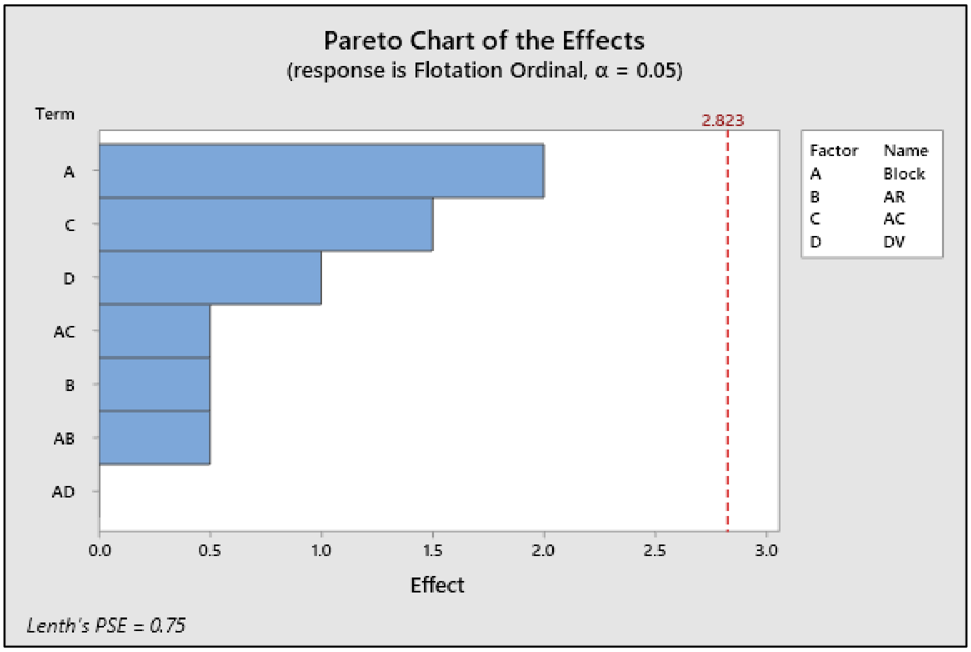


Figure 14. Pareto chart of effects by test variables on flotation.



During trial #3, the pH adjustment of cultures without additives was observed to modestly contribute to algae flocculation (Figure 15) compared to the unaltered control (Figure 16). Additional treatments in this trial further demonstrated the efficacy of XG in combination with a cationic polymer (TF222) to induce a desirable flocculation effect (Figure 17). Changes in pH did not affect flocculation and flotation efficacy of the two additives. The additive polyDADMAC did not improve flocculation as anticipated when evaluated singly or through interaction with XG. Neither mineral oil, GF, nor CCG yielded desirable effects for algal flocculation.

Figure 15. Algae-laden water adjusted from approximately pH 9.5 to 7.

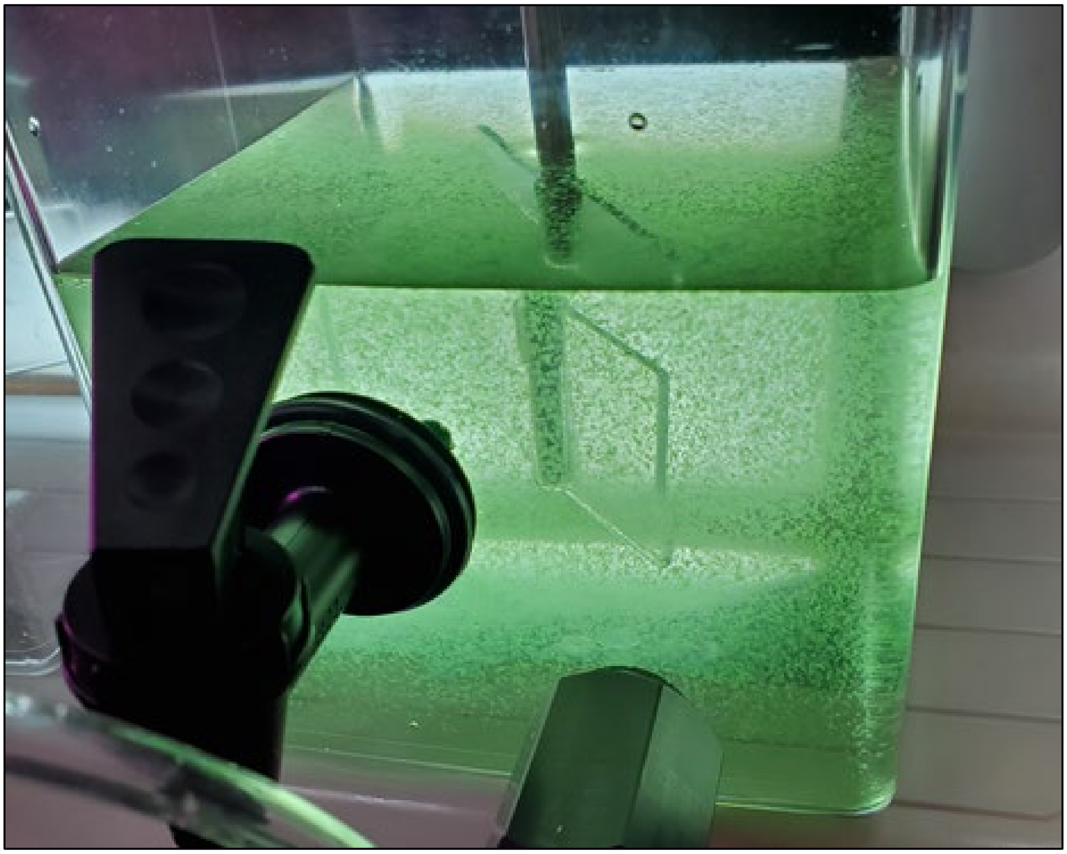


Figure 16. Control jar with no additives or treatment.

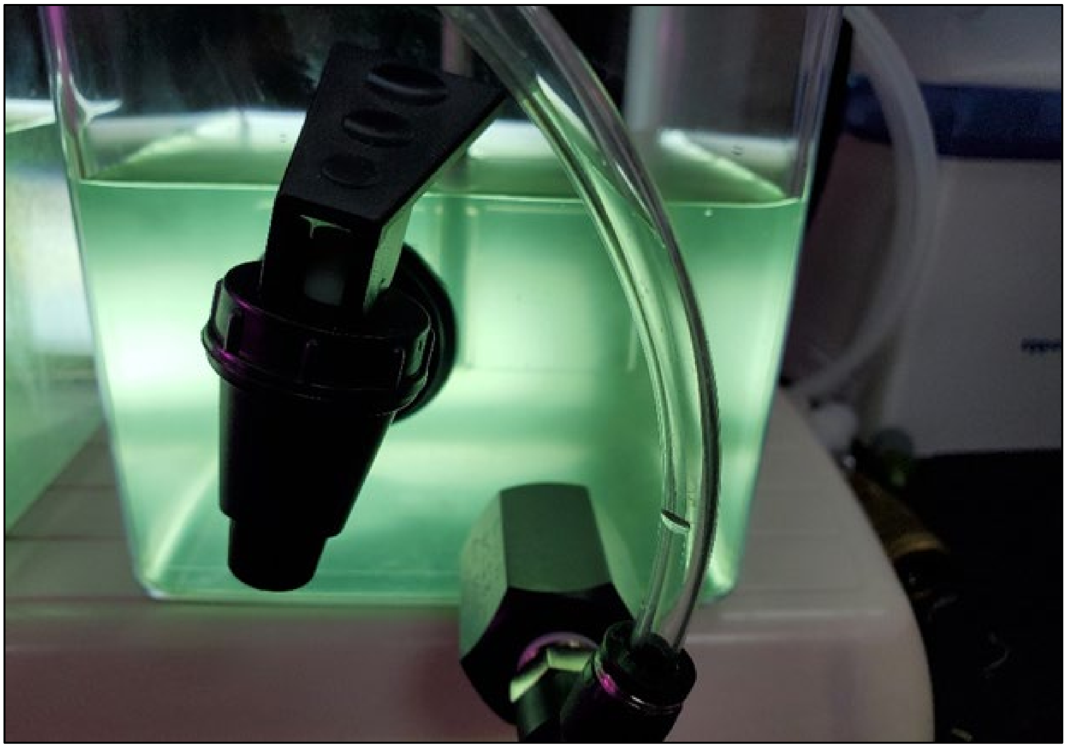
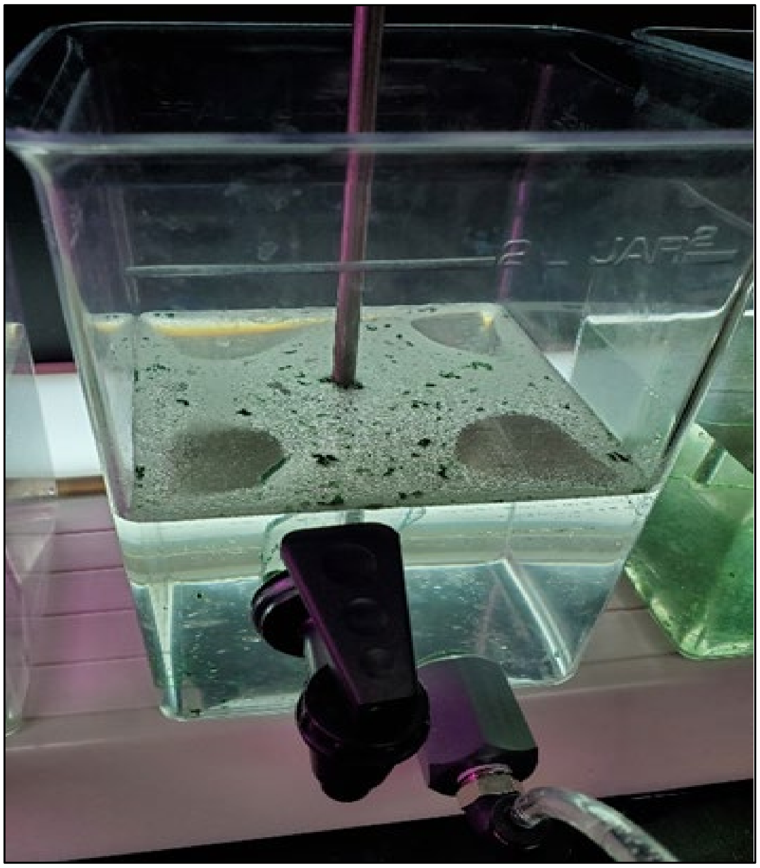


Figure 17. TF222 and XG after flocculation and flotation treatment.



The synergistic combination of XG and Tramfloc 222 provided the most effective flocculation independent of pH effects when applied at concentrations of 0.1 mg/L and 0.3 mg/L, respectively (Figure 18). The ordinal scale used to define flotation did not differentiate among the different additives (Figure 19). The flotation ordinal scale was unable to quantify biomass stability which was observed visually and qualitatively.

Turbidity was investigated to help quantify flocculation efficacy. The resulting data showed no significant response among the manipulated factors despite the visually observed effects (Figure 20).

Figure 18. Pareto chart of effects for flocculation. XG and Tramfloc 222 produced the greatest effect on algal flocculation.

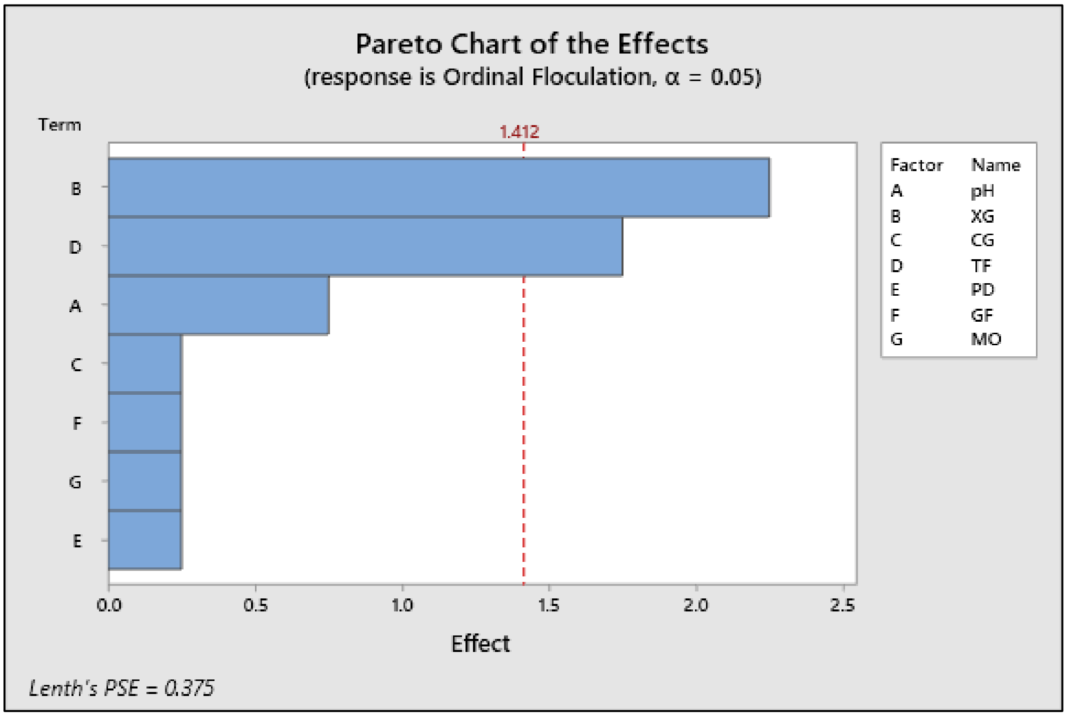


Figure 19. Pareto chart of effects for flotation.

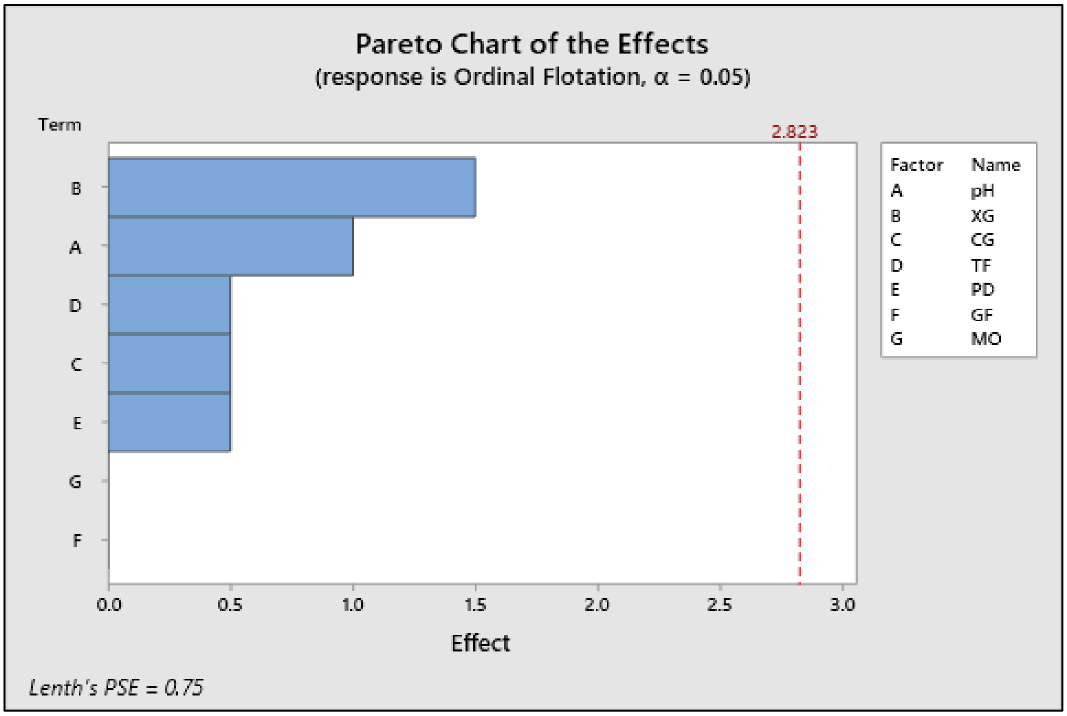
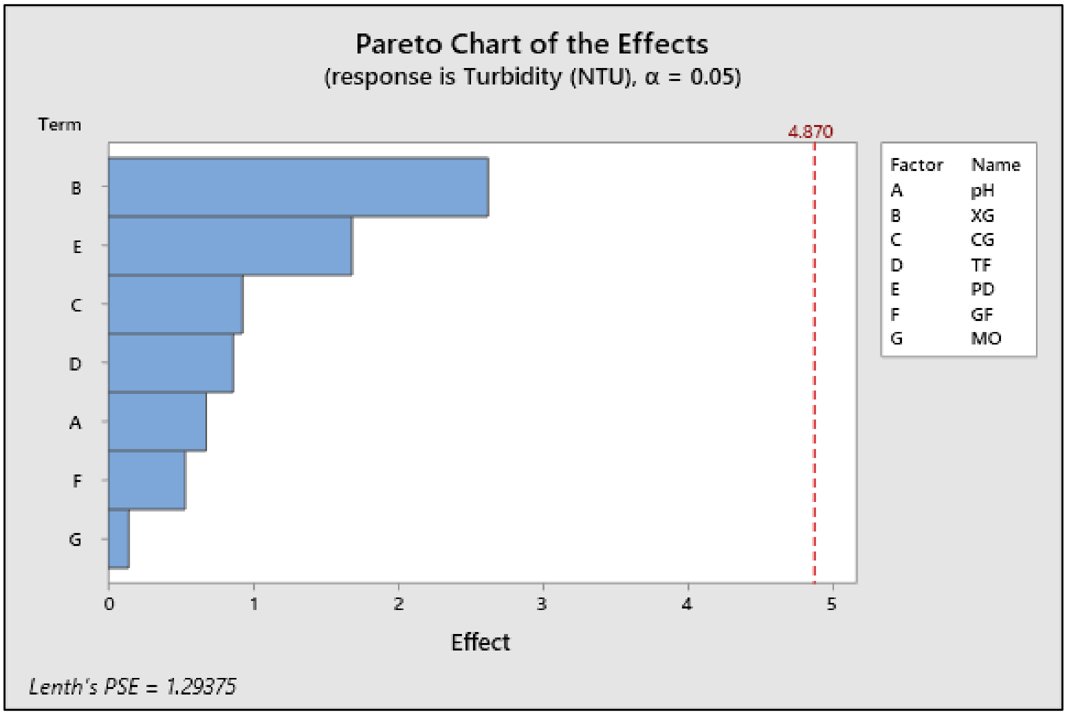


Figure 20. Pareto chart of effects for turbidity. Although modest, XG and polydadmac exhibited the greatest impact on turbidity relative to the other test variables and additives.



Results from lab trial #4 showed that industrial-grade polysaccharides chemically similar to XG (gellan gum and diutan gum) demonstrated comparable effects on algal flocculation synergy (Figure 21). Tramfloc 222 exhibited the greatest effect on algal flotation and flocculation. Interestingly, the polyaluminum chloride (PAC1430) and BHR demonstrated a moderate effect in algal flotation only (Figure 22). BHR was not as effective as TF222 in algae flocculation but exceeded its effect in algal flotation (Figure 23). Because the polyaluminum chloride, a major component of BHR, was evaluated in combination with gellan and diutan gums, it is unclear if the additive could be as effective as BHR when evaluated alone. Lab Trials in the Appendix contains further details regarding the experimental design and analyses.

Figure 21. Pareto chart of effects for flocculation.

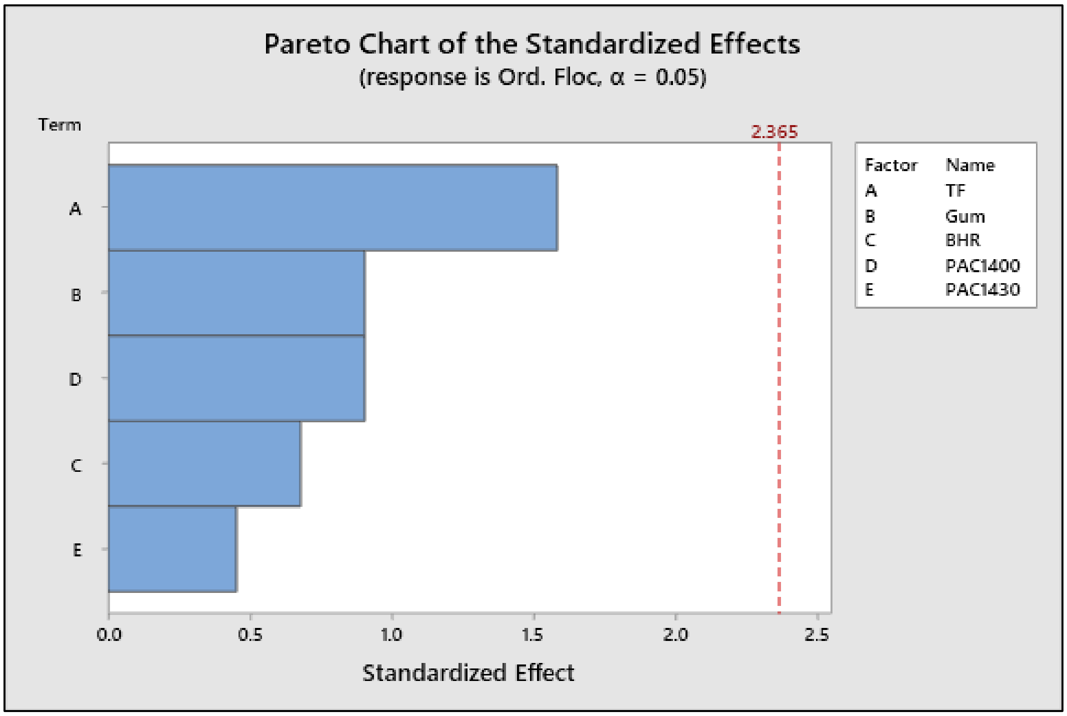


Figure 22. Pareto chart of trial #4 effects for flotation.

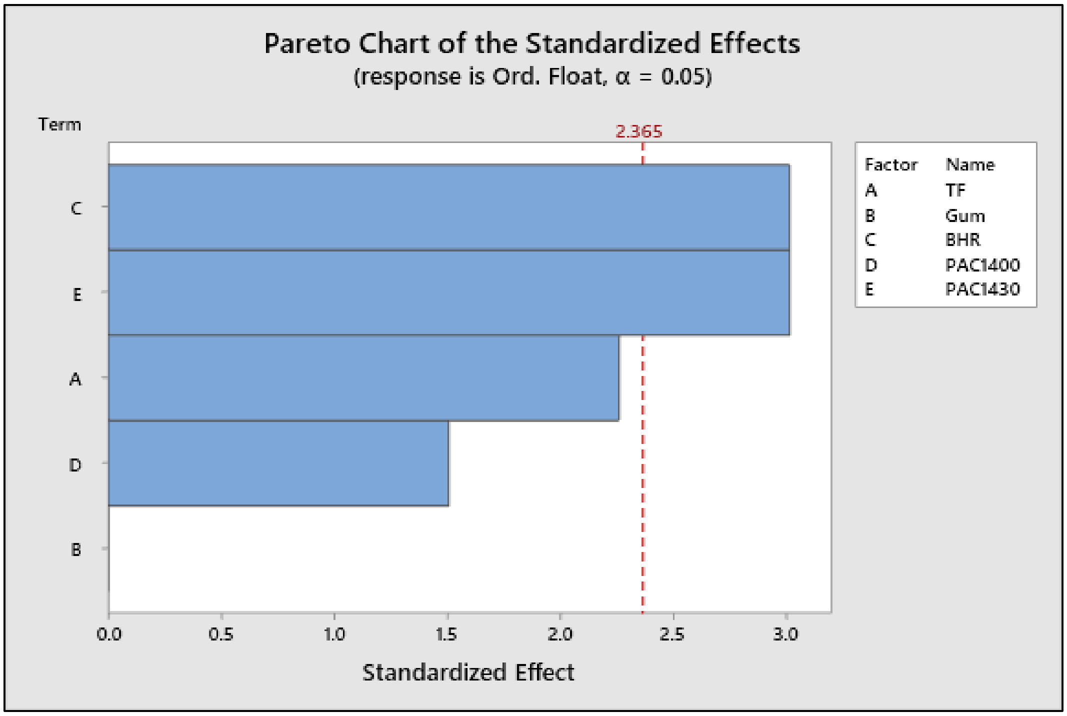
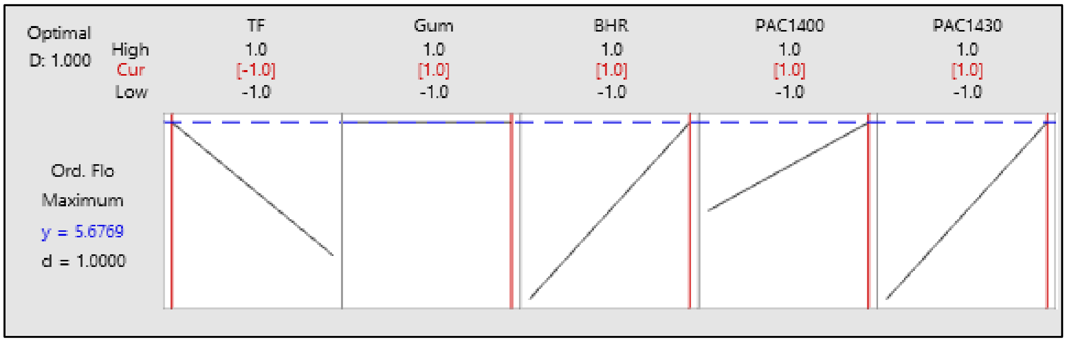


Figure 23. Dersirability function plot for optimal flotation.



4 Conclusions

The application of semitoxic cationic coagulants and environmentally safe, food-grade polysaccharides was shown to effectively form algae flocs that are subject to DAF. These unique combinations allow for lower effective doses of commercial cationic coagulants during algae concentration and improved harvesting. The synergistic combination of XG and cationic polyacrylamide, tradename Tramfloc 222, provided the most effective flotation and flocculation independent of pH effects. Global efforts to mitigate HABs in freshwater systems may rely heavily on chemical coagulation for biomass removal. The Army Corps of Engineers or other organizations can utilize the novel chemical synergies of XG and cationic polyacrylamide to produce robust, stable, and concentrated algal biomass capable of adsorbing dissolved air for high efficiency mitigation.

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Appendix: Lab Trials

Table A-1. List of chemical additives used in trial experiments.

Material	CAS#
BHR-P50	8042-47-5
Cationic acrylamide copolymer—Tramfloc/Tianfloc 222	69418-26-4
Cationic guar gum (CGG)	65497-29-2
Cationic starch— GFT5100	56780-58-6
Diutan gum (DG)	125005-87-0 595585-15-2
Gellan gum (GG)	71010-52-1
Gum arabic (GA)	9000-01-5.
Mineral oil	8042-47-5
Polyaluminum chloride—PAC1400	1327-41-9
Polyaluminum chloride—PAC1430	1327-41-9
Polydadmac	26590-05-6
Xanthan gum (XG)	11138-66-2

A.1 Lab Trial #2

A.1.1 Factorial regression: flocculation ordinal versus block, AR, AC, and DV*

Table A-2. Coded coefficients.

Term	Effect	Coef	SE Coef	*T*-Value	*P*-Value	Variance inflation factor (VIF)
Constant	—	3.25	—	—	—	—
Block	−1.5	−0.75	—	—	—	1
Additive ration (AR)	−0.5	−0.25	—	—	—	1
Additive concentration (AC)	0	0	—	—	—	1
Dissolved air flotation volume (DV)	0	0	—	—	—	1
Block-AR	−0.5	−0.25	—	—	—	1
Block-AC	−2	−1	—	—	—	1
Block-DV	0	0	—	—	—	1

---

* Terms A, B, C, and D correspond to the factors ‘Block’, additive ratio (AR), additive concentration (AC), and DAF volume (DV). Care should be taken not to confuse the abbreviation of additive concentration (AC) for the two factor interaction effect of terms A*C shown as AC.

Table A-3. Model summary.

Standard deviation	R^2	R^2 (adj)	R^2 (pred)
—	100.00%	—	—

Table A-4. Analysis of variance.

Source	Degrees of Freedom	Adjusted Sum of Squares	Adjusted Mean Squares	*F*-Value	*P*-Value
Model	7	13.5	1.92857	—	—
Linear	4	5	1.25	—	—
Block	1	4.5	4.5	—	—
AR	1	0.5	0.5	—	—
AC	1	0	0	—	—
DV	1	0	0	—	—
2-Way Interactions	3	8.5	2.83333	—	—
Block-AR	1	0.5	0.5	—	—
Block-AC	1	8	8	—	—
Block-DV	1	0	0	—	—
Error	0	—	—	—	—
Total	7	13.5	—	—	—

Table A-5. Regression equation in uncoded units.

Flocculation Ordinal	=	3.250 − 0.7500 Block − 0.2500 AR − 0.000000 AC − 0.000000 DV
		−0.2500 Block-AR − 1.000 Block-AC − 0.000000 Block-DV

Table A-6. Alias structure.

Factor	Name
A	Block
B	AR
C	AC
D	DV
Aliases	—
I + ABCD	—
A + BCD	—
B + ACD	—
C + ABD	—
D + ABC	—
AB + CD	—
AC + BD	—
AD + BC	—

A.1.2 Factorial regression: flotation ordinal versus block, AR, AC, and DV

Table A-7. Coded coefficients.

Term	Effect	Coef	SE Coef	*T*-Value	*P*-Value	VIF
Constant	—	3.5	—	—	—	—
Block	−2	−1	—	—	—	1
AR	0.5	0.25	—	—	—	1
AC	−1.5	−0.75	—	—	—	1
DV	−1	−0.5	—	—	—	1
Block AR	−0.5	−0.25	—	—	—	1
Block AC	−0.5	−0.25	—	—	—	1
Block DV	0	0	—	—	—	1

Table A-8. Model summary.

Standard deviation	R^2	R^2 (adj)	R^2 (pred)
–	100.00%	–	–

Table A-9. Analysis of variance.

Source	Degrees of Freedom	Adjusted Sum of Squares	Adjusted Mean Squares	*F*-Value	*P*-Value
Model	7	16	2.28571	—	—
Linear	4	15	3.75	—	—
Block	1	8	8	—	—
AR	1	0.5	0.5	—	—
AC	1	4.5	4.5	—	—
DV	1	2	2	—	—
2-Way Interactions	3	1	0.33333	—	—
Block*AR	1	0.5	0.5	—	—
Block*AC	1	0.5	0.5	—	—
Block*DV	1	0	0	—	—
Error	0	—	—	—	—
Total	7	16	—	—	—

Table A-10. Regression equation in uncoded units.

Flotation Ordinal	=	3.500 − 1.000 Block + 0.2500 AR − 0.7500 AC − 0.5000 DV − 0.2500 Block-AR
		−0.2500 Block-AC−0.000000 Block-DV

Table A-11. Alias structure.

Factor	Name
A	Block
B	AR
C	AC
D	DV
Aliases	—
I + ABCD	—
A + BCD	—
B + ACD	—
C + ABD	—
D + ABC	—
AB + CD	—
AC + BD	—
AD + BC	—

A.2 Lab trial #3

A.2.1 Factorial regression: ordinal flocculation versus pH, XG, CG, TF, PD, GF, and MO

Table A-12. Coded coefficients.

Term	Effect	Coef	SE Coef	*T*-Value	*P*-Value	VIF
Constant	—	3.375	—	—	—	—
pH	−0.75	−0.375	—	—	—	1
XG	2.25	1.125	—	—	—	1
CG	−0.25	−0.125	—	—	—	1
TF	1.75	0.875	—	—	—	1
PD	0.25	0.125	—	—	—	1
GF	0.25	0.125	—	—	—	1
MO	−0.25	−0.125	—	—	—	1

Table A-13. Model summary.

Standard deviation	R^2	R^2 (adj)	R^2 (pred)
—	100.00%	—	—

Table A-14. Analysis of variance.

Source	Degrees of Freedom	Adjusted Sum of Squares	Adjusted Mean Squares	*F*-Value	*P*-Value
Model	7	17.875	2.5536	—	—
Linear	7	17.875	2.5536	—	—
pH	1	1.125	1.125	—	—
XG	1	10.125	10.125	—	—
CG	1	0.125	0.125	—	—
TF	1	6.125	6.125	—	—
PD	1	0.125	0.125	—	—
GF	1	0.125	0.125	—	—
MO	1	0.125	0.125	—	—
Error	0	—	—	—	—
Total	7	17.875	—	—	—

Table A-15. Regression equation in uncoded units.

Ordinal Flocculation	=	3.375 − 0.3750 pH + 1.125 XG − 0.1250 CG + 0.8750 TF + 0.1250 PD
		+ 0.1250 GF − 0.1250 MO

Table A-16. Alias structure (up to order 3).

Factor	Name
A	pH
B	XG
C	CG
D	TF
E	PD
F	GF
G	MO
Aliases	—
I + ABD + ACE + AFG + BCF + BEG + CDG + DEF	—
A + BD + CE + FG + BCG + BEF + CDF + DEG	—
B + AD + CF + EG + ACG + AEF + CDE + DFG	—
C + AE + BF + DG + ABG + ADF + BDE + EFG	—
D + AB + CG + EF + ACF + AEG + BCE + BFG	—
E + AC + BG + DF + ABF + ADG + BCD + CFG	—
F + AG + BC + DE + ABE + ACD + BDG + CEG	—
G + AF + BE + CD + ABC + ADE + BDF + CEF	—

A.2.2 Factorial regression: ordinal Flotation versus pH, XG, CG, TF, PD, GF, and MO

Table A-17. Coded coefficients.

Term	Effect	Coef	SE Coef	*T*-Value	*P*-Value	VIF
Constant	—	2.5	—	—	—	—
pH	−1	−0.5	—	—	—	1
XG	1.5	0.75	—	—	—	1
CG	−0.5	−0.25	—	—	—	1
TF	0.5	0.25	—	—	—	1
PD	0.5	0.25	—	—	—	1
GF	0	0	—	—	—	1
MO	0	0	—	—	—	1

Table A-18. Model summary.

Standard deviation	R^2	R^2 (adj)	R^2 (pred)
—	100.00%	—	—

Table A-19. Analysis of variance.

Source	Degrees of Freedom	Adjusted Sum of Squares	Adjusted Mean Squares	*F*-Value	*P*-Value
Model	7	8	1.14286	—	—
Linear	7	8	1.14286	—	—
pH	1	2	2	—	—
XG	1	4.5	4.5	—	—
CG	1	0.5	0.5	—	—
TF	1	0.5	0.5	—	—
PD	1	0.5	0.5	—	—
GF	1	0	0	—	—
MO	1	0	0	—	—
Error	0	—	—	—	—
Total	7	8	—	—	—

Table A-20. Regression equation in uncoded units.

Ordinal Flotation	=	2.500 − 0.5000 pH + 0.7500 XG − 0.2500 CG + 0.2500 TF + 0.2500 PD
		+ 0.000000 GF + 0.000000 MO

Table A-21. Alias structure (up to order 3).

Factor	Name
A	pH
B	XG
C	CG
D	TF
E	PD
F	GF
G	MO
Aliases	—
I + ABD + ACE + AFG + BCF + BEG + CDG + DEF	—
A + BD + CE + FG + BCG + BEF + CDF + DEG	—
B + AD + CF + EG + ACG + AEF + CDE + DFG	—
C + AE + BF + DG + ABG + ADF + BDE + EFG	—
D + AB + CG + EF + ACF + AEG + BCE + BFG	—
E + AC + BG + DF + ABF + ADG + BCD + CFG	—
F + AG + BC + DE + ABE + ACD + BDG + CEG	—
G + AF + BE + CD + ABC + ADE + BDF + CEF	—

A.3 Lab Trial #4:

A.3.1 Screening design model: Ord. Floc versus TF, Gum, BHR, PAC1400, and PAC1430

Table A-22. Coded coefficients.

Term	Coef	SE Coef	*T*-Value	*P*-Value	VIF
Constant	2.615	0.388	6.75	0	–
TF	0.7	0.442	1.58	0.157	1
Gum	0.4	0.442	0.9	0.396	1
BHR	0.3	0.442	0.68	0.519	1
PAC1400	−0.4	0.442	−0.9	0.396	1
PAC1430	−0.2	0.442	−0.45	0.665	1

Table A-23. Model summary.

Standard deviation	R^2	R^2 (adj)	R^2 (pred)
1.3978	40.73%	0.00%	0.00%

Table A-24. Analysis of variance.

Source	Degrees of Freedom	Adjusted Sum of Squares	Adjusted Mean Squares	*F*-Value	*P*-Value
Model	5	9.4	1.88	0.96	0.499
Linear	5	9.4	1.88	0.96	0.499
TF	1	4.9	4.9	2.51	0.157
Gum	1	1.6	1.6	0.82	0.396
BHR	1	0.9	0.9	0.46	0.519
PAC1400	1	1.6	1.6	0.82	0.396
PAC1430	1	0.4	0.4	0.2	0.665
Error	7	13.6769	1.9538	—	—
Total	12	23.0769	—	—	—

Table A-25. Regression equation in uncoded units.

Ord. Floc	=	2.615 + 0.700 TF + 0.400 Gum + 0.300 BHR − 0.400 PAC1400 − 0.200 PAC1430

Table A-26. Alias structure (up to order 2).

Factor	Name
A	TF
B	Gum
C	BHR
D	PAC1400
E	PAC1430
Aliases	—
I + 0.77 AA + 0.77 BB + 0.77 CC + 0.77 DD + 0.77 EE	—
A	—
B	—
C	—
D	—
E	—

A.3.2 Screening design model: ord. float versus TF, Gum, BHR, PAC1400, and PAC1430

Table A-27. Coded coefficients.

Term	Coef	SE Coef	*T*-Value	*P*-Value	VIF
Constant	3.077	0.233	13.23	0	—
TF	−0.6	0.265	−2.26	0.058	1
Gum	0	0.265	0	1	1
BHR	0.8	0.265	3.02	0.019	1
PAC1400	0.4	0.265	1.51	0.175	1
PAC1430	0.8	0.265	3.02	0.019	1

Table A-28. Model summary.

Standard deviation	R^2	R^2 (adj)	R^2 (pred)
0.838628	78.52%	63.18%	16.37%

Table A-29. Analysis of variance.

Source	DF	Adj SS	Adj MS	*F*-Value	*P*-Value
Model	5	18	3.6	5.12	0.027
Linear	5	18	3.6	5.12	0.027
TF	1	3.6	3.6	5.12	0.058
Gum	1	0	0	0	1
BHR	1	6.4	6.4	9.1	0.019
PAC1400	1	1.6	1.6	2.28	0.175
PAC1430	1	6.4	6.4	9.1	0.019
Error	7	4.9231	0.7033	—	—
Total	12	22.9231	—	—	—

Table A-30. Regression equation in uncoded units.

Ord. Float	=	3.077 − 0.600 TF − 0.000 Gum + 0.800 BHR + 0.400 PAC1400 + 0.800 PAC1430

Table A-31. Alias structure (up to order 2).

Factor	Name
A	TF
B	Gum
C	BHR
D	PAC1400
E	PAC1430
Aliases	—
I + 0.77 AA + 0.77 BB + 0.77 CC + 0.77 DD + 0.77 EE	—
A	—
B	—
C	—
D	—
E	—

A.3.3 Response optimization: ordinal flotation

Table A-32. Parameters.

Response	Goal	Lower	Target	Upper	Weight	Importance
Ord. Float	Maximum	1	5	—	1	1

Table A-33. Solution.

Solution	TF	Gum	BHR	PAC1400	PAC1430	Ord. Float Fit	Composite Desirability
1	−1	1	1	1	1	5.67692	1

Table A-34. Multiple Response Prediction.

Variable	Setting	—	—	—	—
TF	−1	—	—	—	—
Gum	1	—	—	—	—
BHR	1	—	—	—	—
PAC1400	1	—	—	—	—
PAC1430	1	—	—	—	—
Response	—	Fit	SE Fit	95% CI	95% PI
Ord. Float	—	5.677	0.637	(4.171, 7.183)	(3.187, 8.167)

Abbreviations

AC	Additive concentration
ANOVA	Analysis of variance
AR	Additive ratio
BHR	BHR-P50
CGG	Cationic guar gum
DAF	Dissolved air flotation
DG	Diutan gum
DOE	Design of experiments
DV	Dissolved air flotation volume
EPS	Extracellular polymeric substance
GA	Gum arabic
GF	GFT5100
GG	Gellan gum
HAB	Harmful algal bloom
MO	Mineral oil
PAC	Polyaluminum chloride
PD	PolyDADMAC
TF	Turbulence, additional
TF222	Tramfloc 222
TF300	Tramfloc 300
USACE	US Army Corp of Engineers
XG	Xantham gum

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14. ABSTRACT

Dissolved air flotation (DAF) is an effective technique for algae separation following the application of flocculants and coagulants. Some harmful algae produce mucilage or extracellular polymeric substances useful for flotation. This study evaluated natural polysaccharides to determine effects on algal flotation with DAF. Food-grade gums (xanthan gum, guar gum, gum arabic, gellan gum, and diutan gum) were tested with cyanobacteria cultures singly and in combination with commercial flocculants (including Tramfloc 222 and Tramfloc 300). Gum arabic alone had no effect when evaluated at concentrations between 10 mg/L and 5,000 mg/L. However, the combination of gum arabic and Tramfloc 300 yielded higher algal flocculation than Tramfloc 300 alone. The combination of xanthan gum (anionic) and guar gum (cationic) did not perform at the level of the combined xanthan gum and Tramfloc 222 in either flocculation or flotation of algae. Tramfloc 222 and xanthan gum; however, yielded effective flocculation seemingly resistant to changes in interfering factors such as turbulence, pH, and temperature. Furthermore, the combination of xanthan gum and Tramfloc 222 provided the most effective flotation and flocculation independent of pH effects. The results suggest that anionic polysaccharides can be used to increase the efficacy of cationic coagulants such as Tramfloc 222.

15. SUBJECT TERMS
Algae; Biomass; Coagulation; Flocculation; Flotation; HAB; Polysaccaride; Xantan gum

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