Eco-Friendly and Effective Flocculation Method to Remove Algae from Water

Shrika Kantipudi[1] and Leya Joykutty[#]

[1]Mentor High School
[#]Advisor

ABSTRACT

Algal blooms are rapid growth of microscopic algae in the water that are harmful to the environment because they block sunlight from reaching plants under the surface, deplete the water of useful nutrients, and release carbon emissions. Flocculation is a method that is used to remove algae and it works by binding and agglomerating suspended particles in water to form large particles to assist in their settling. However, most flocculants are chemical flocculants and may harm water quality. Bio-flocculation based on natural flocculants has been studied in drinking water treatment plants (DWTPs) as an eco-friendly alternative technology to conventional flocculants for both turbidity and HABs removal. The total solids assay, sedimentation kinetics assay, and pH testing will be used to measure the effectiveness of flocculants and water quality. These assays were tested with three types of algae: *Chlorella vulgaris*, *Spirulina*, *Scenedesmus obliquus,* and five types of flocculants: Aluminium Sulfate, Copper Sulfate, Chitosan, *Moringa oleifera*, and *Strychnos potatorum Linn*. The data collected so far in the study show that the chemical flocculants and bio-flocculants have a similar effectivity at flocculating algae from the water. The findings suggest that there is no significant difference between the bio-flocculants and the chemical flocculant. and that all of the flocculants will be effective. pH testing results have shown that Chitosan, *Moringa oleifera*, and *Strychnos potatorum Linn* affect the water quality the least which makes them the more environmentally friendly flocculants.

Introduction

Algal blooms are the rapid growth of microscopic algae in the water and they result in colored scum on the surface. Harmful Algal Blooms (HABs) in water supply systems have become a major concern for water utilities around the world. The increased nutrient inputs caused by anthropogenic activities and global warming are primarily responsible for the increased frequency of algal blooms. Furthermore, algal bloom proliferation can be aided by a combination of environmental factors such as water temperature, thermal stratification, salinity, light intensity, stagnation and residence time, nutrient concentration increase, and eutrophication. Eutrophication is when polluted runoff containing nutrients such as phosphorus and nitrogen, is released into the water. Since phosphorus and nitrogen are nutrients for algae, this leads to an increase in algal blooms. Due to an increase in pollution, the number of algal blooms is only increasing. The occurrence of these harmful algal blooms (HABs) has increased as a result of water eutrophication and climate change, endangering human health and the environment in terms of water supply. Algal blooms block the sunlight from reaching the plants underneath the surface which causes the plants to lack nutrients. They also deplete the water of useful nutrients for other plants and aquatic organisms. Also, when algae die, they sink to the bottom of the water body and release carbon emissions which are harmful to the environment (World Health Organization, 2021).

Some types of algae that are common in algal blooms are *Chlorella vulgaris*, *Spirulina*, and

Scenedesmus obliquus. *Chlorella vulgaris* (*C. vulgaris*) is a spherical unicellular eukaryotic green algae that present a thick cell wall (100–200 nm) as its main characteristic. (*Chlorella vulgaris*. ScienceDirect Topics. (n.d.).). *Spirulina* is a cyanobacteria and it is particularly recognizable for its 0.2-0.5mm long spiral shape (Anna, 2019). *Scenedesmus obliquus* is a unicellular green algae that is found in freshwater environments. Some chemical compounds like DNAN, NQ, NTO, and RDX, and the heavy metals Zn, Ni, and Cu in *Scenedesmus Obliquus* are toxic to aquatic organisms. *Scenedesmus Obliquus* also takes in chemicals, holds them in, and releases them at a later time which is why this can be dangerous when working with chemical flocculants (Wunschiers, 1996).

Many methods have been tried to remove algae from the water, but not many are successful. Flocculation is one of the environmentally friendly methods that is still being researched. Flocculation is the process of separating a solution; it is frequently used to remove sediment from a fluid. When a flocculant is added to water it binds and agglomerates suspended particles in water to form large particles to assist in their settling. Commonly used chemical flocculants are copper sulfate and aluminum sulfate. While they are effective in removing algae from the water, concerns have been forming about whether they are harmful to water quality.

Copper sulfate kills algae while also causing toxin release. Aluminum sulfate or alum precipitates and accumulates at the bottom of water treatment, endangering aquatic life. It can also inhibit aquatic organisms from regulating salt concentrations and clogging fish gills (ChemREADY, 2022).

Bio-flocculation based on natural flocculants has been studied in drinking water treatment plants (DWTPs) as an eco-friendly alternative technology to conventional flocculants for both turbidity and HABs removal. Bioflocculants can be a substitute for chemical flocculants in order to reduce environmental damage and health hazards. Bioflocculants are organic and biodegradable and are derived from living things or their byproducts; as a result, they have little to no negative effects on human health and the environment. As an alternative to the widely used chemical flocculants, some bioflocculants obtained from various sources have already been researched and shown to be effective for application to drinking water treatment procedures (Kurniawan, 2020). Some examples of natural flocculants include chitosan, moringa extract, and indupa extract. Chitosan is a sugar that comes from the outer skeleton of shellfish and it is mainly used as a medicine . Chitosan has a strong flocculation effect because it is only sparingly soluble in water at a pH of 9, which makes it an effective flocculant (WebMD, 2005). Moringa powder comes from the moringa leaves of *Moringa oleifera* and it has a lot of vitamin C. Since Moringa seeds contain water-soluble proteins, they could be used as an effective bio-flocculant. The seeds contain calcium, protein, iron, and amino acids (Cadman, 2020). The Indupa extract comes from the seed of nirmali tree *Strychnos potatorum* Linn shows coagulation properties in clarifying turbid water. This property was attributed to the presence of anionic polyelectrolytes having -COOH and free -OH surface groups that are present in the seed protein (MediLexicon International. (n.d.)).

Research Question/Hypothesis

Are natural / Bioflocculants more effective in collecting algal blooms than chemical flocculants?

Materials List

Model Organisms

Chlorella vulgaris
Spirulina
Scenedesmus obliquus

Equipment

Aquarium tanks
Fritz A
Fritz B Bubblers
Spectrophotometer
pH strips
Beakers
Hot plate
Filter paper

Flocculants for Assays

Aluminum Sulfate
Copper Sulfate
Chitosan
Moringa oleifera seed extract
Strychnos potatorum Linn seed extract

Procedures

Phase-I: Growing Algae

Algal Species Culture and Cultivation

Procedure for Fresh Water Medium: 1. KNO3 (3g per liter), NaH2PO4*2H20 (0.26g per liter), KH2PO4 (0.74g per liter), HEPES (2.38g per liter), H3BO3 (61.80ug per liter), EDTA-Na2 (37 mg per liter), ZnSO4*7H20 (3.20mg per liter), MnCl2*4H20 (13 mg per liter), and CuSO4*5H2O (1.83mg per liter) added to demineralized water

2. Set the pH of the solution to 6.8 using 4 M HCl
3. Dispense 100 mL of this medium into 300 ml Erlenmeyer flasks and seal with cotton and an aluminum cap and autoclave for 20 minutes at 121 °C
4. After cooling, add MgSO4*7H2O (0.4g per liter), CaCl2*2H2O (13 mg per liter), Vitamin B12 (1ug per liter), D-biotin (1ug per liter), and Thiamine-HCl (200ug per liter)

Algae Pre-Growth and Inoculum Preparation: Wash hands, put on a mask, and use a disinfectant spray on the materials before beginning the experiment to follow Covid-19 safety guidelines. Make sure there is no one in a range of six feet from the experimentation area before beginning.

1. Take the 2.5-gallon aquariums and make sure they are clean.

2. Pour 100 liters of distilled water into each tank

3. Add 2 mL of Fritz A and 2 ml of Fritz B to the tank

4. Add 1 tube of *Chlorella Vulgaris* to a tank, and allow it to grow for 4 weeks

5. Do the same to *Spirulina* in another tank and let it grow for 4 weeks

6. Do the same to *Scenedesmus obliquus* in another tank and let it grow for 4 weeks

7. Put a bubbler next to each tank, put the tubing in, and leave them on.

8. Stir the algae every day to prevent them from falling to the bottom (Stir by using an orbital shaker with 180 rpm).

Phase-II: Flocculation

Total Solids Assay

1. Weigh an empty beaker
2. Fill the beaker with a known volume of the solution of algae
3. Evaporate the water in an oven and completely dry the residue
4. Then, weigh the beaker with the residue and subtract the weight of the beaker from the weight of the residue and beaker combined

pH Testing Assay

1. Collect 15 ml of algae into a beaker

2. Add 0.1 grams of the desired flocculant into the beaker

3. Stir for 10 minutes and then let rest for 10 minutes

4. Collect the supernatant into another beaker

5. Test and record the pH of the supernatant

Sedimentation Kinetics Assay

1) Take the samples of the microalgal suspensions and dilute in a cuvette

2) After mixing, leave the suspension to settle at 27°C in the dark in a spectrophotometer.

3) Measure the temperature and pH of all samples in the beginning and at the end of the sedimentation period and see if they were constant respectively at 27°C and pH 7.
4) During the settling period, measure the turbidity of the sample at 750 nm at the same height in the cuvette to determine the recovery.
5) Take turbidity (OD750(t_0)) of sample at time zero and take turbidity (OD750(t)) of sample at time t.

Results and Data Analysis

Sedimentation Kinetics Assay

This experiment was conducted to determine which flocculant was more effective over a certain time period.

All experimental groups that had been exposed to copper sulfate experienced a statistically significant increase in flocculation measured in the sedimentation kinetics assay.

This shows that copper sulfate is the most effective flocculant. The most effective bio-flocculant was shown to be Indupa extract which was more effective than aluminum sulfate, which is the most common flocculant. ($p = 0.05$, two-sample t-test, Figure 1,2,3).

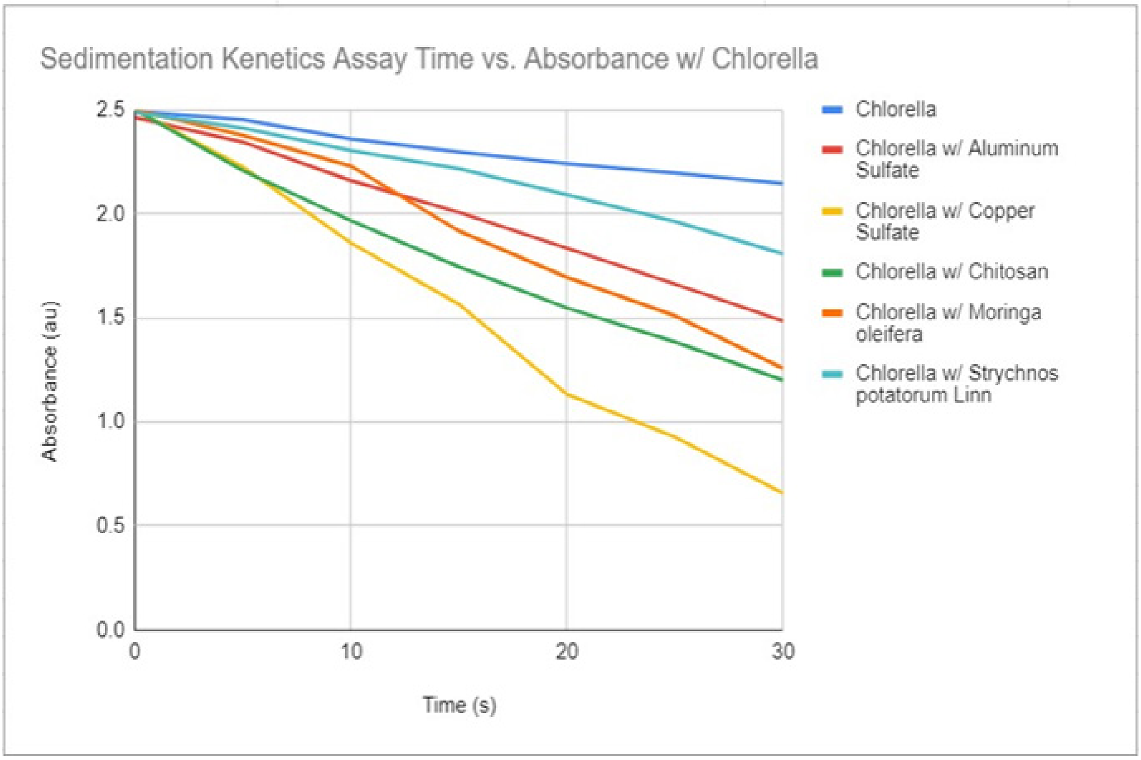


Figure 1. *Chlorella vulgaris* sedimentation kinetics assay with *Moringa oleifera*, *Strychnos potatorum Linn*, Chitosan, Aluminum Sulfate and Copper sulfate

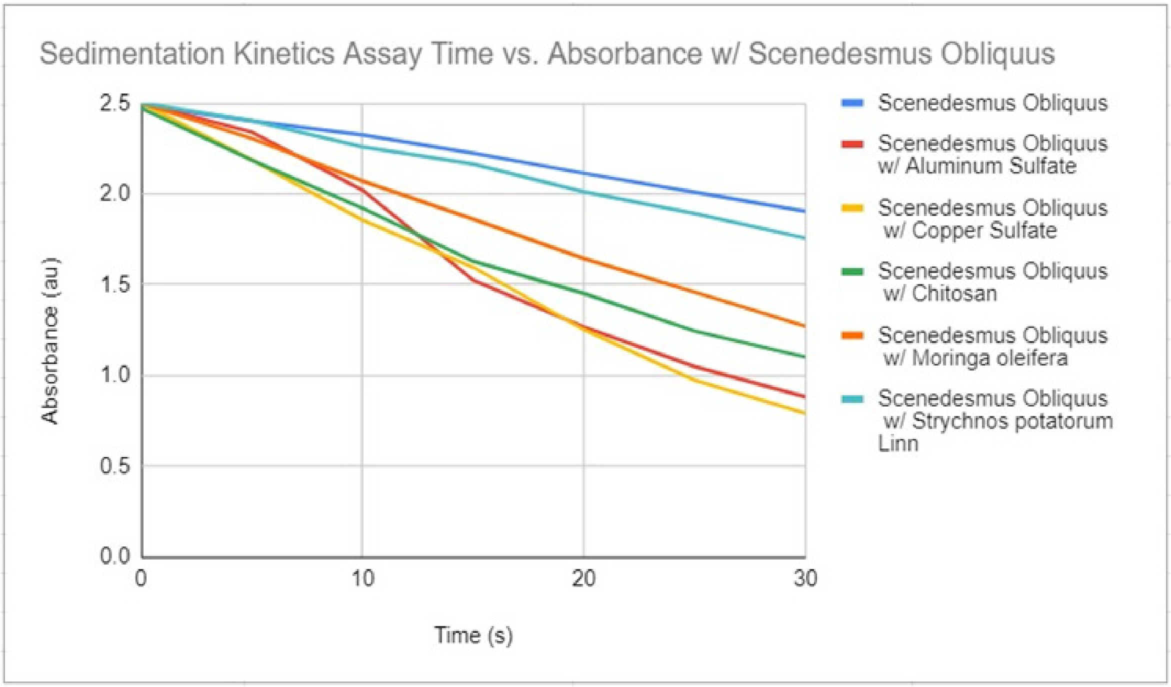


Figure 2. *Scenedesmus Obliquus* sedimentation kinetics assay with *Moringa oleifera*, *Strychnos potatorum Linn*, Chitosan, Aluminum Sulfate, and Copper sulfate

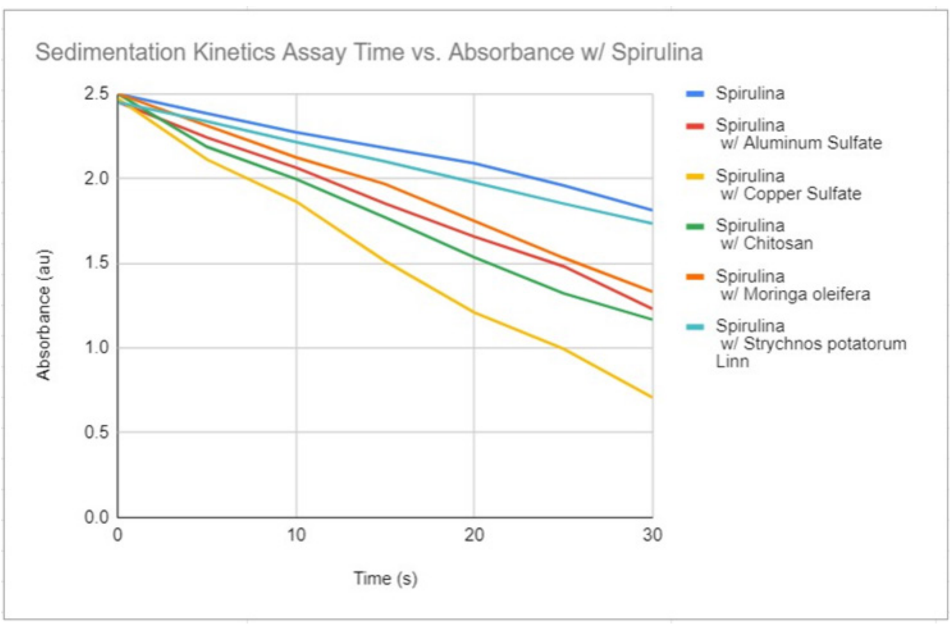
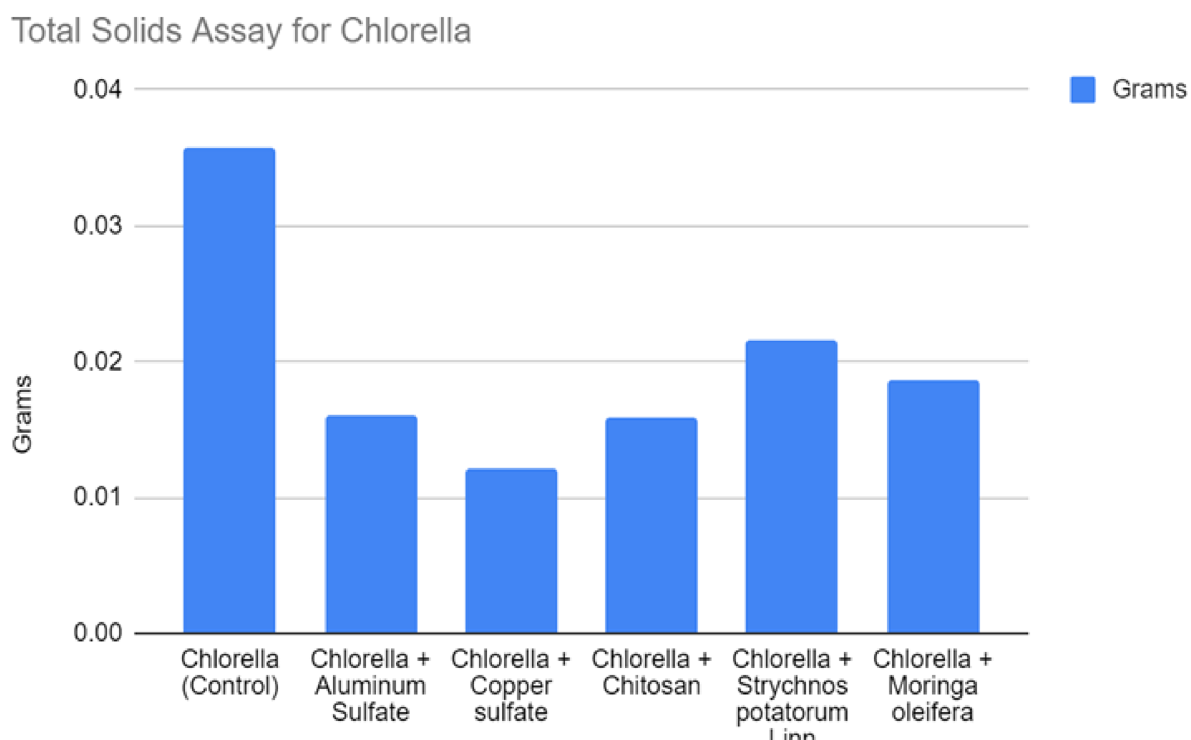


Figure 3. Spirulina sedimentation kinetics assay with Moringa oleifera, Strychnos potatorum Linn, Chitosan, Aluminum Sulfate, and Copper sulfate

Figure 10. Setup used in Sedimentation Kinetics assay

Total Solids Assay

This experiment was conducted to determine which flocculant was more effective at creating sediment at the bottom of the solution. All experimental groups exposed to Chitosan experienced a statistically significant decrease of algae in the supernatant measured in the total solids assay. This shows that chitosan is the most effective at creating sediment. The most effective chemical flocculant was shown to be copper sulfate. Both of these flocculants were more effective than aluminum sulfate, which is the most commonly used flocculant. ($p<0.05$, two-sample t-test, Figure 4, 5, 6).



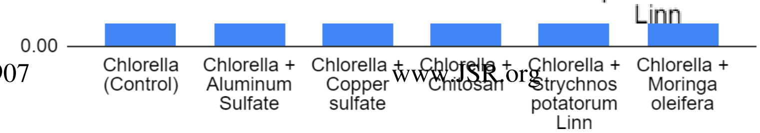


Figure 4. *Chlorella vulgaris* Total Solids Assay with *Moringa oleifera*, *Strychnos potatorum Linn*, Chitosan, Aluminum Sulfate and Copper sulfate

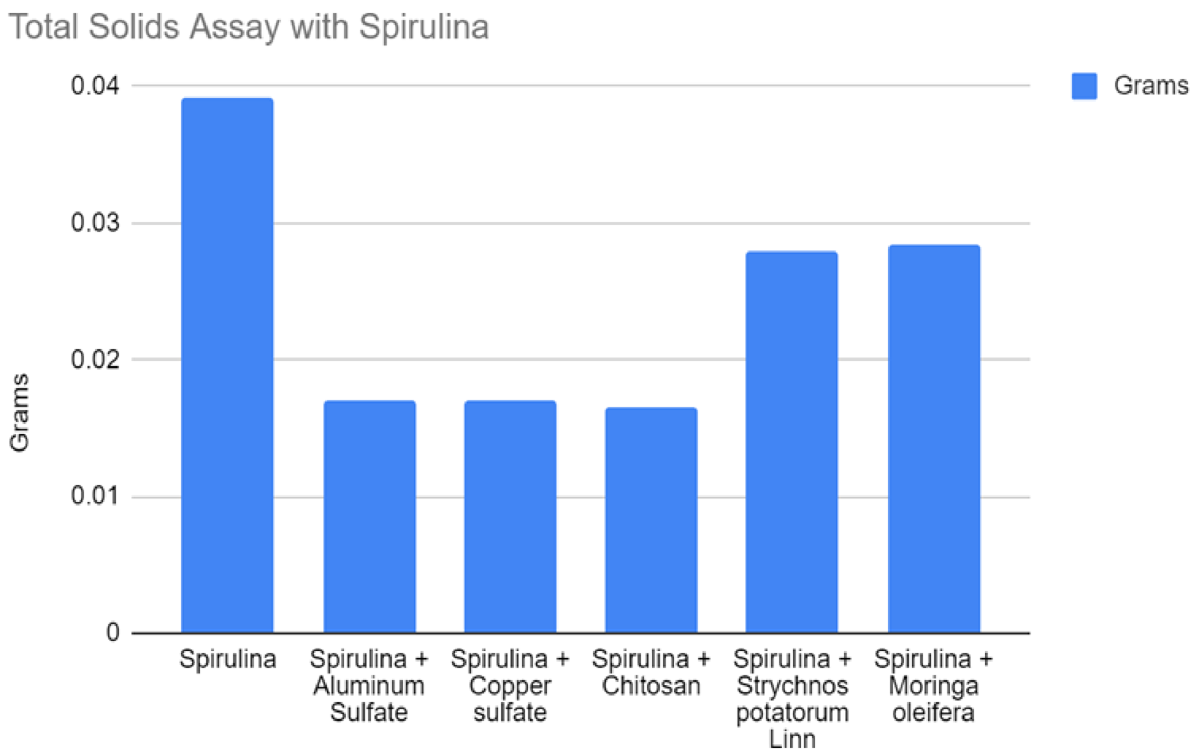


Figure 5. Spirulina Total Solids Assay with Moringa oleifera, Strychnos potatorum Linn, Chitosan, Aluminum Sulfate, and Copper sulfate

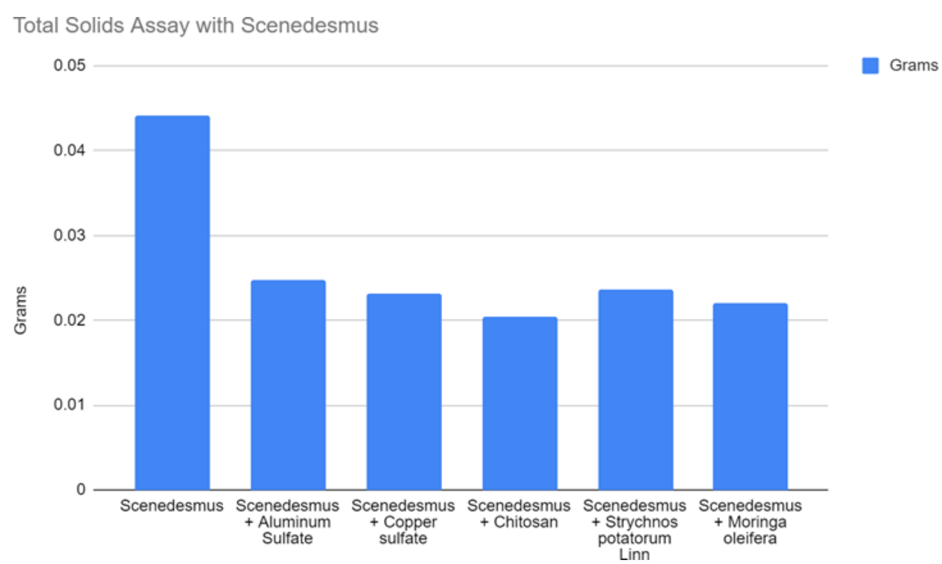


Figure 6. *Scenedesmus Obliquus* sedimentation kinetics assay with *Moringa oleifera*, *Strychnos potatorum Linn*, Chitosan, Aluminum Sulfate, and Copper sulfate

Figure 11. Setup used in Total Solids assay

pH Testing Assay

This experiment was conducted to determine which flocculant is more environmentally friendly and releases the least amount of chemicals into the water. All experimental groups exposed to moringa extract and indupa extract had an average pH closest to 7 which was measured in the pH assay. This shows that moringa extract and indupa extract are the most environmentally friendly because the water in the supernatant was closest to 7 which is water's normal pH. Chitosan was the most environmentally friendly flocculant after moringa extract and indupa extract, averaging a pH of 7.75. All the bioflocculants had a significantly closer pH to 7 than the chemical flocculants. This shows that bioflocculants are more environmentally friendly than chemical flocculants ($p<0.05$, two-sample t-test, Figure 7, 8, 9.

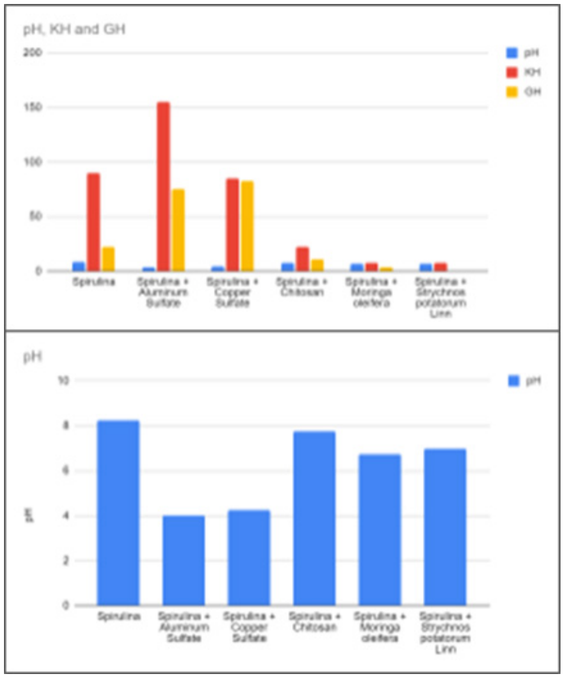


Figure 7 a & Figure 7b. Spirulina pH Assay with Moringa oleifera, Strychnos potatorum Linn, Chitosan, Aluminum Sulfate, and Copper sulfate

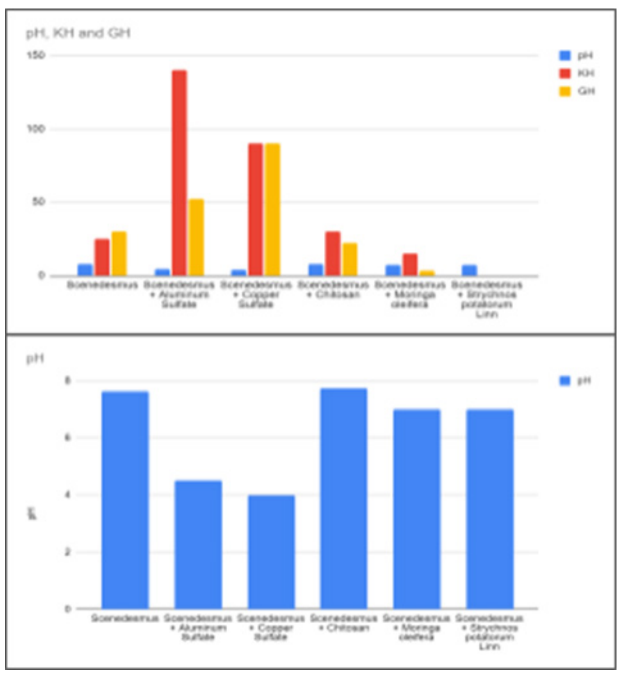


Figure 8a & Figure 8b. Scenedesmus pH Assay with Moringa oleifera, Strychnos potatorum Linn, Chitosan, Aluminum Sulfate, and Copper sulfate

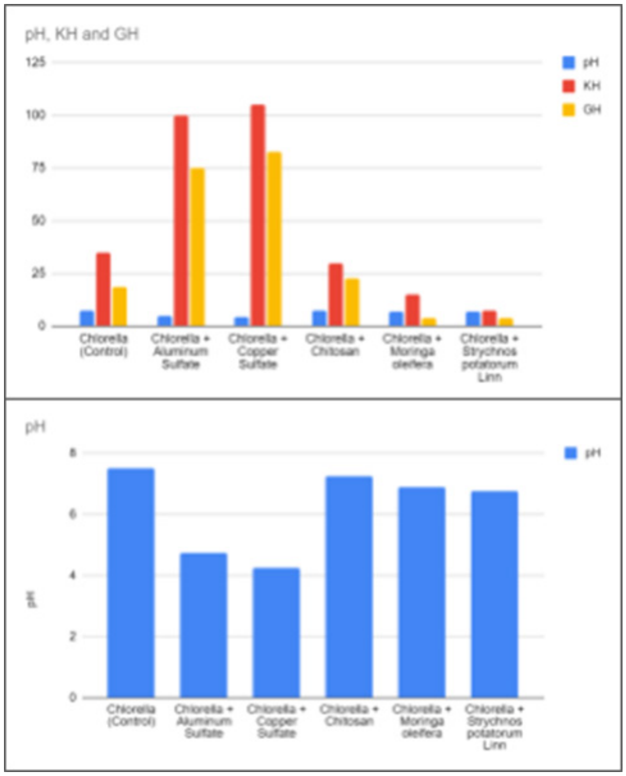


Figure 9a & Figure 9b. Chlorella pH Assay with Moringa oleifera, Strychnos potatorum Linn, Chitosan, Aluminum Sulfate, and Copper sulfate

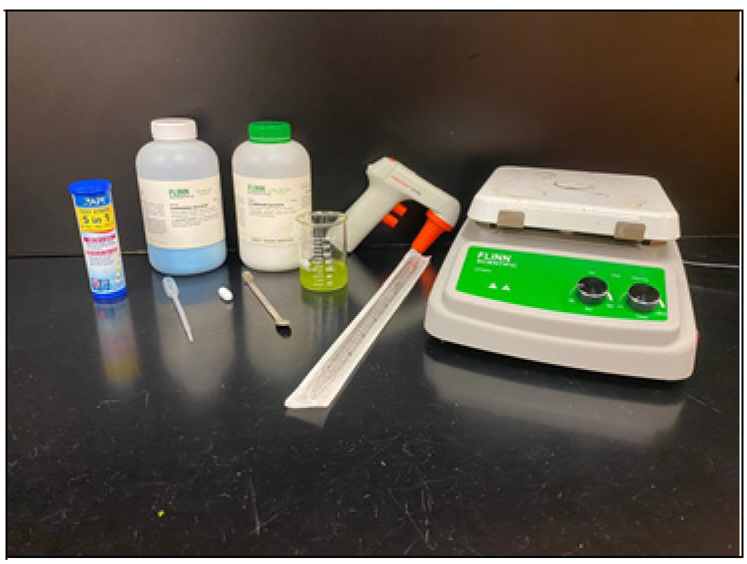


Figure 12. Setup used in pH assay

Conclusion and Discussion

The purpose of this experiment was to observe how effective chemical flocculants are compared to bioflocculants within a given time frame. The hypothesis of the total solids assay, the sedimentation kinetics assay, and the pH assay was tested in four trials. A one-tailed student's t-test at a five percent significance level was used to compare the total solids assay, the sedimentation kinetics assay, and the pH assay data to each group's respective control. The data did not support the hypothesis for the sedimentation kinetics assay because there was no statistically significant difference between the chemical flocculants and bio-flocculants and they were both equally effective in flocculating algae from the water. The data supported the hypothesis for the total solids assay because chitosan was the most effective flocculant in creating sediment at the bottom of the solution. A one-tailed t-test was used and all the data proved to be significant with $p<0.05$. These results show that although aluminum sulfate and copper are effective they harm water quality and are not good for the environment. They also show that there is no significant difference between the effectiveness of the bioflocculants and chemical flocculants meaning that both types of flocculants will be effective in removing algae from the water. Overall, the results also show that chitosan, a bioflocculant, is more effective than aluminum sulfate which is the most commonly used chemical flocculant.

Error Analysis

After the data was collected, the researcher looked for mistakes that may have been done during the procedure. For example, for the total solids assay, the researcher has to collect the algae left over at the surface of the water. There could have been an error in the collecting process leading to a miscalculation of the data. In addition, the researcher could have measured the sedimentation kinetics assay incorrectly since it is a visual assay. All the beakers might not have been stirring in the same RPM and the spectrophotometer might not have been functioning properly. This could have altered the results and the averages calculated for this experiment. However, the researcher conducted multiple trials to ensure that there is minimal room for error.

Limitations

Limiting factors to the validity of this experiment include, model organism growth restriction, susceptibility to human, and machine error. Growing the algae took over 3 months and my project originally had 4 types of algae but one of them died twice. This added a time constraint on the project due to the 3 months spent growing the algae. Machine error could also have limited this project due to the fact that the machine may have been calibrated incorrectly and the maximum absorbance was 2.5. However, the researcher conducted many trials to assure the minimum amount of error.

Future Research

In future experimentation, the specific bio-flocculant that is used to flocculate the algae can be different. For example, instead of using moringa extract as the flocculant, other alternatives are maize and Polyacrylamide gel. This project can also be done with different concentrations of flocculants to analyze which concentration is the most optimal for flocculation. Researchers could also add another step to this project. After flocculating, researchers could take the sediment and use it to produce bioplastic. They could do many assays such as tensile strength.

Applications

This investigation has many implications for the treatment of algae in wastewater. Algal blooms are a major problem in today's world due to the major increase in chemical waste runoff ithe nto water. There has been a 51 percent increase in the use of chemical fertilizers since 2000. Current treatments for algal blooms are inefficient and expensive, proving that algal blooms will continue to be a problem if not prevented soon. Algal blooms also cause many environmental disasters such as the death of many fish and underground plants. The treatment used in this experiment is a cost-effective, environmentally friendly way to remove algal blooms from wastewater. They do not create more of a hassle for water cleaning companies because this process is free of harmful chemicals.

Acknowledgments

I would like to thank my advisor for the valuable insight provided to me on this topic.

References

1. U.S. National Library of Medicine. (n.d.). Home - books - NCBI. National Center for Biotechnology Information. Retrieved January 15, 2023, from https://www.ncbi.nlm.nih.gov/books
2. Chlorella vulgaris. Chlorella vulgaris - an overview | ScienceDirect Topics. (n.d.). Retrieved January 15, 2023, from https://www.sciencedirect.com/topics/engineering/chlorella-vulgaris
3. Anna. (2019, December 11). Spirulina, Heavy Metal Detox. Apogee Spirulina. Retrieved January 15, 2023, from https://apogeespirulina.com/spirulina-heavy-metal-detox/
4. *Scenedesmus obliquus. Scenedesmus Obliquus - an overview | ScienceDirect Topics. (n.d.). Retrieved January 15, 2023, from https://www.sciencedirect.com/topics/engineering/scenedesmus-obliquus*
5. *Flocculants and coagulants for wastewater treatment. ChemREADY. (2022, July 31). Retrieved January 15, 2023, from https://www.getchemready.com/wastewater-treatment/flocculants-coagulants-wastewater- treatment/*
6. Kurniawan, S. B., Abdullah, S. R. S., Imron, M. F., Said, N. S. M., Ismail, N. 'I., Hasan, H. A., Othman, A. R., & Purwanti, I. F. (2020, December 12). Challenges and opportunities of Biocoagulant/Bioflocculant application for drinking water and wastewater treatment and its potential for sludge recovery. International journal of environmental research and public health. Retrieved January 15, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7764310/#B14-ijerph-17-09312
7. WebMD. (n.d.). Chitosan: Overview, uses, side effects, precautions, interactions, dosing and reviews. WebMD. Retrieved January 15, 2023, from https://www.webmd.com/vitamins/ai/ingredientmono-625/chitosan
8. MediLexicon International. (n.d.). Moringa: Benefits, side effects, and risks. Medical News Today. Retrieved January 15, 2023, from https://www.medicalnewstoday.com/articles/319916
9. Yadav, K. N., Kadam, P. V., Patel, J. A., & Patil, M. J. (1970, January 1). Strychnos potatorum: Phytochemical and pharmacological review. Pharmacognosy Reviews. Retrieved January 15, 2023, from https://doi.org/10.4103/0973-7847.125533
10. Okaiyeto, K., Nwodo, U. U., Okoli, S. A., Mabinya, L. V., & Okoh, A. I. (2016, April). Implications for public health demands alternatives to inorganic and synthetic flocculants: Bioflocculants as important candidates. MicrobiologyOpen. Retrieved January 15, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4831466/

11. panelVictorAjaoabPersonEnvelopeHarryBruningbHuubRijnaartsbHardyTemminkab, A. links open overlay, VictorAjaoabPersonEnvelope, a, b, HarryBruningb, HuubRijnaartsb, HardyTemminkab, Highlights•Possible to combine wastewater treatment with EPS production as bioflocculants.•EPS comprised a mixture of high, & flocculants, A. N. (2018, May 22). Natural flocculants from fresh and saline wastewater: Comparative properties and flocculation performances. Chemical Engineering Journal. Retrieved January 15, 2023, from https://www.sciencedirect.com/science/article/pii/S1385894718309379
12. U.S. National Library of Medicine. (2020, December 12). Challenges and opportunities of Biocoagulant/Bioflocculant application for drinking water and wastewater treatment and its potential for sludge recovery. International journal of environmental research and public health. Retrieved January 15, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7764310/
13. American Chemical Society. (n.d.). Retrieved January 16, 2023, from https://pubs.acs.org/doi/10.1021/es305234d
14. Salim, S., Bosma, R., Vermuë, M. H., & Wijffels, R. H. (2011, October). Harvesting of microalgae by bio-flocculation. Journal of applied phycology. Retrieved January 15, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3172406/