Microplastic Contamination of Water: Effects of Novel Bio-Composites Comprising of Okra and Aloe Vera.

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ABSTRACT

Mechanical and physicochemical degradation of plastic waste leads to the formation of plastic fragments called microplastics that are widely polluting our water and food chains. The current methods of removing microplastics from water involve use of chemical flocculants, which result in non-biodegradable by-products, making this removal technique harmful to the environment. The purpose of this research was to develop alternatives using natural flocculants made out of Abelmoschus esculentus (Okra) and Aloe Vera with variations including zinc oxide and non-toxic resin. Novel bio-flocculants when tested displayed high levels of microplastic flocculation when Okra based bio-composites were used. The Fourier Transform Infrared (FTIR) Spectroscopy confirmed the lack of toxic byproducts created in the process of bio-flocculation. Scanning Electron Microscope (SEM) scans provided evidence for physical change of the microplastics that enabled their effective removal from water samples. In conclusion, bio-flocculants offer a safer, promising and economical alternative to the present-day agents that can be implemented in the current water treatment systems without creating harmful by-products.

INTRODUCTION

First pioneered in 1907, anthropogenic long-chain polymeric materials, or plastics, have since been extensively used in all aspects of modern life due to their high versatility. Plastics come in a variety of forms and can be strong enough to be reused or weak enough to serve a one-time purpose. United Nation Environment Program (1) found that 50% of plastics are one-time-use and either get recycled responsibly or thrown into the oceans and environment. Every year, over 100 million animals die due to plastic pollution, usually through plastic consumption (2). Mechanical and physicochemical degradation of plastic waste leads to the formation of plastic fragments called microplastics (smaller than 5mm). The abundance and size of microplastics cause them to have numerous detrimental impacts on the environment and other organisms.

Impacts of Microplastic Pollution of Water

The concerns over microplastics are about the potential harms that can impose on organisms and humans. The environmental impacts range from physical, chemical and biological impacts. Physical impacts mainly include entanglement and ingestion of plastic debris. Entanglement of microplastics mainly happens to comparatively large marine organisms. On the other hand, ingestion of microplastics can be found throughout almost all the trophic levels, including zooplankton taxa. Microplastics are largely consumed by aquatic species, which are also consumed by humans, causing a ‘trophic transfer’ of microplastics. After ingestion, microplastics cause toxicity to humans and living organisms through several pathways and mechanisms. The polymeric compounds and additives such as copper ions used during plastic production also contribute to the toxicity of microplastics. More importantly, various toxins in waters that are initially absorbed onto microplastics may subsequently be desorbed inside of human and animal bodies. Additionally, microplastics can be a vector for water-borne hydrophobic pollutants including polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT). PCBs are well known to be carcinogenic, mutagenic, and/or teratogenic. DDT can lead to adverse neurological effects and immunodeficiency. Microplastics also act as a harbor for pathogenic bacterial species. Microplastics can also cause alterations in locomotion, intestinal damage, and metabolic profiles as seen in populations of adult zebrafish, which displayed drastic changes to limbs and tails in hatched offspring (3). In terrestrial environments, microplastics can lower the quality of soil and water. They can also cause stunted growth and malnutrition of plants. The lack of treatment of water at source causes microplastics to enter the water system and agricultural irrigation systems. This has led to microplastics being consumed through agricultural produce and drinking water. A recent study (4) indicated that 77% of humans are estimated to have microplastic levels in blood streams and endocrine organs, making microplastic pollution an urgent environmental crisis.

Current Solutions Addressing Microplastic Contamination

There are solutions that have been implemented in the past, although there is yet to be an effective and safe solution to removing microplastics. Chemical flocculants, the current standard, land up causing even more pollution as the resultant sludge is non-biodegradable loaded with chemicals and heavy metals. Research indicates that the most commonly used flocculants are metal based like aluminum sulfate (alum), ferric chloride, and ferric sulfate. Consequently, the sludge created as a result of these treatments is non-biodegradable and can have adverse impacts on neurological health and human development. Along with that, 80% of plastic waste is directly dumped into the oceans through runoff from textiles, city dust, and tires (5). This water rarely gets treated yet microplastics make it through the food chain through agriculture to human bodies. Plastic degrading bacterial species are a theoretical solution, but their implementation at a large scale is impractical and does not address the issue of how to clean the byproducts created in the process. Hence, there is a great need for non-toxic, biodegradable, and promising removal methods for microplastics.

Purpose of Current Research

The foundation of this research focused on the use of Okra and Aloe Vera as natural flocculants for removal of microplastics. The project was planned to systematically address microplastic pollution of water at all three levels: laboratory, water treatment, and water body level. *Level 1*: focused on the development of solutions at the laboratory level that included testing powdered forms of bio-flocculants and metal oxides (primarily Zinc oxide). Results from this experiment aided in the formulation of optimal bio-composites. *Level 2:* focused on developing novel bio-composites made of bio-flocculants. These bio-composites were then tested for their flocculation properties. *Level 3:* focused on the development of sturdier bio-composites with use of non-toxic resin to potentially create bioplastic like biomaterials for use in aquatic environments for example, fishing systems.

RESULTS

Level: 1 Testing of Biomaterial powders at laboratory level

Flocculation properties of various biomaterial powders were tested. Floc is essentially a flake which is produced by the agglomeration of suspended solids. Across all trials, Okra powder led to more flocs compared to Aloe. Zinc oxide seemed to increase flocculation when added to these powders. Photographic (Figure 1) and graphical data (Figure 2a & 2b) indicate the ability of Okra to act as strong bio-flocculant. The trials were conducted at varying amounts of Okra and a direct relationship was noticed between Okra quantity and increased flocculation of microplastic beads.

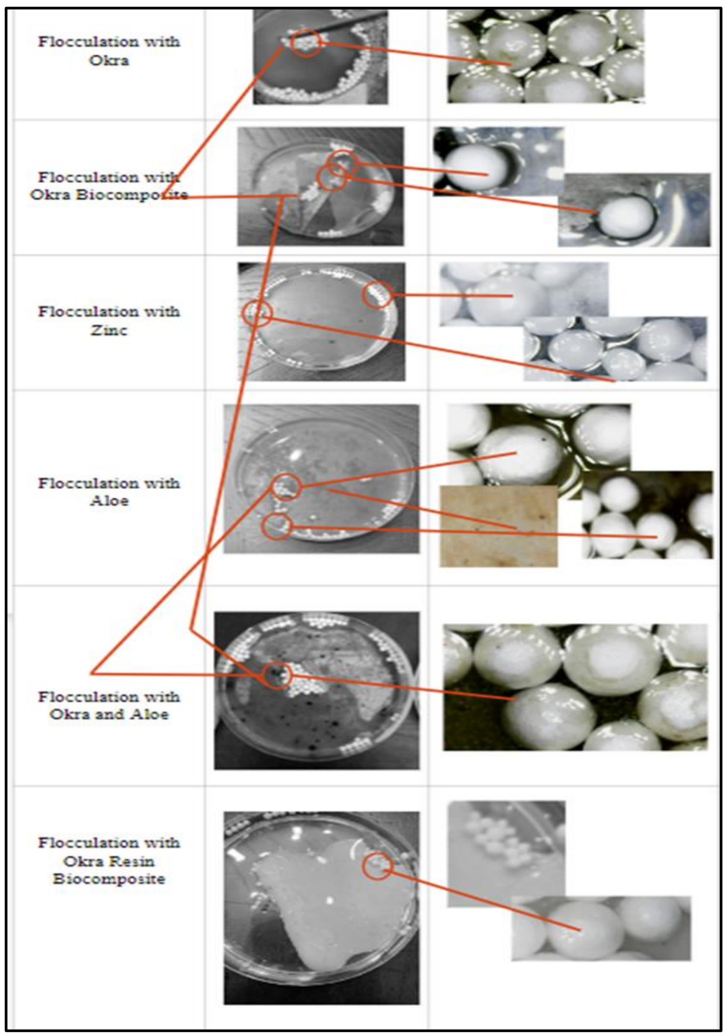


Figure 1: Photographic Data indicating flocculation for various Level 1 trials.

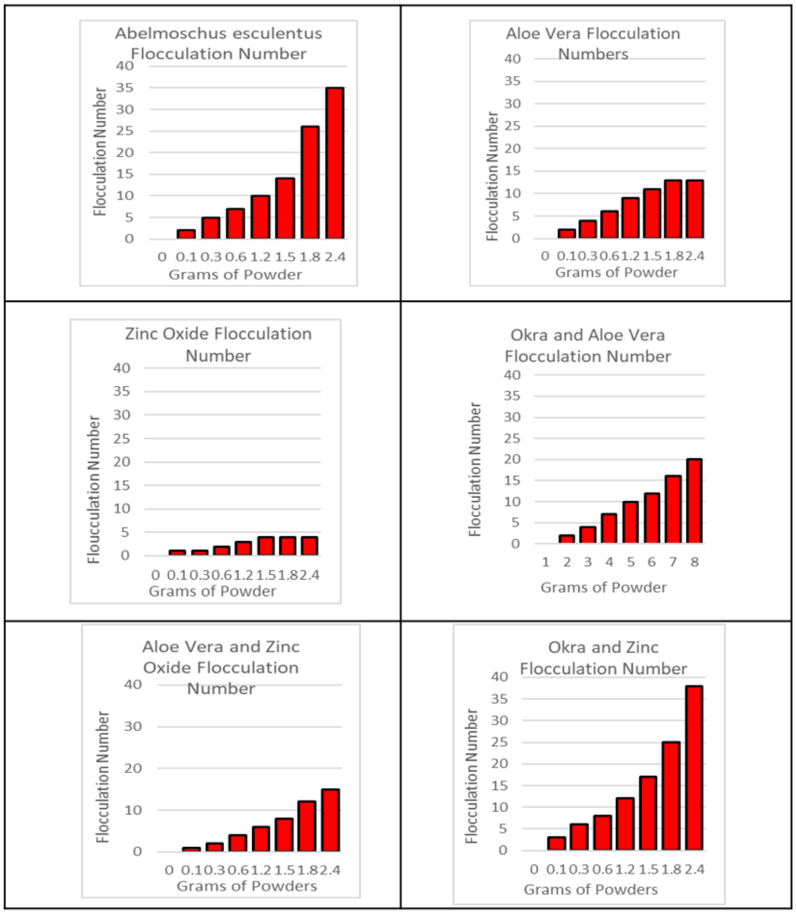


Figure 2a: Flocculation levels for Okra and Aloe Vera dependent on amount of powder

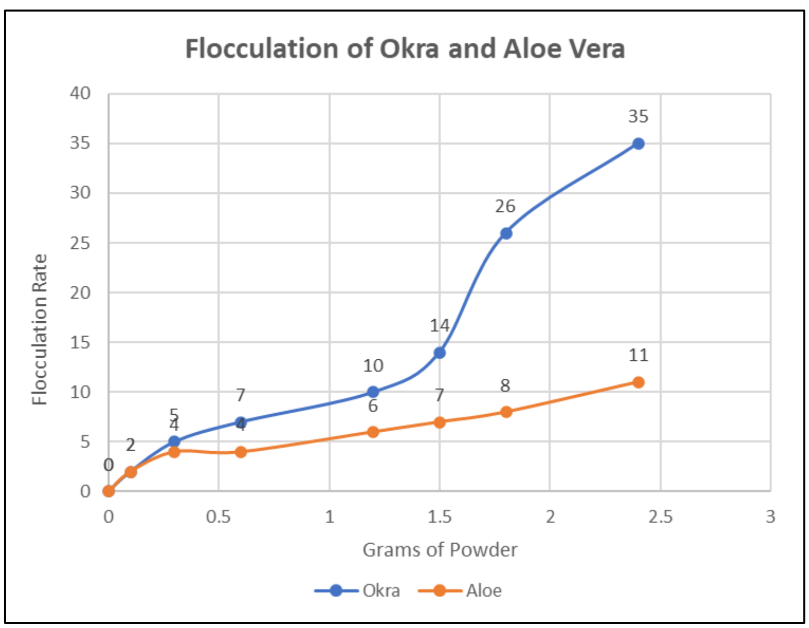


Figure 2b: Flocculation levels for Okra and Aloe trials

Level 2: Development of bio-flocculant materials followed by testing

Level 2 of the project focused on bio-composite preparation and testing. This was a stepwise process of starting with lower amounts of certain ingredients and increasing and amending till a good sturdy bio-composite was derived. Samples 1 to 6 (Table 1 & Figure 3) indicate the progressions of these trials. Bio-composite 6 was finally a version that was strong in nature and similar in texture to raw paper. These were then tested for their bio-flocculation properties (Figure 4). Bio-composites 4, 5 & 6 displayed good flocculation numbers without degrading too soon in water. These materials showed promising ability to function as bio-flocculants in water systems. The flocculation seen in Figure 4 shows how the floc sizes were mostly smaller, due to the binding of the biomaterials aided by cornstarch and agar agar. However, considering the duration of time that the bio-composites were tested in water samples, the relative disintegration was low. Still sturdier bio-composite options were warranted and hence explored in Level 3 of this project.

Trial # and Name	Okra (g)	Okra type	Water (g)	Agar (g)	Zinc Oxide (g)	Glycerol (g)	Aloe Vera (g)
Bio composite #1	100g	Fresh	140 ml	20g	none	20g	0 g
Bio composite #2	100g	Fresh	140 ml	30g	none	0g	0g
Bio Composite #3	100g	Fresh, finely chopped	100 ml	30g	10g	20g	0g
Bio Composite #4	100g	Fresh, finely chopped	25 ml	30g	5g	20g	0g
Bio Composite #5	100 g	Fresh, finely chopped	40 ml	10 g	10 g	30 g	20 g
Bio Composite #6	100 g	Fresh, finely chopped	30 ml	10 g	10 g + 5 grams of cornstarch- made a big difference in binding	30 g	20 g

Table 1 - Biomaterial Composition and Trials

Figure 3- Bio-composites prepared in Level 2

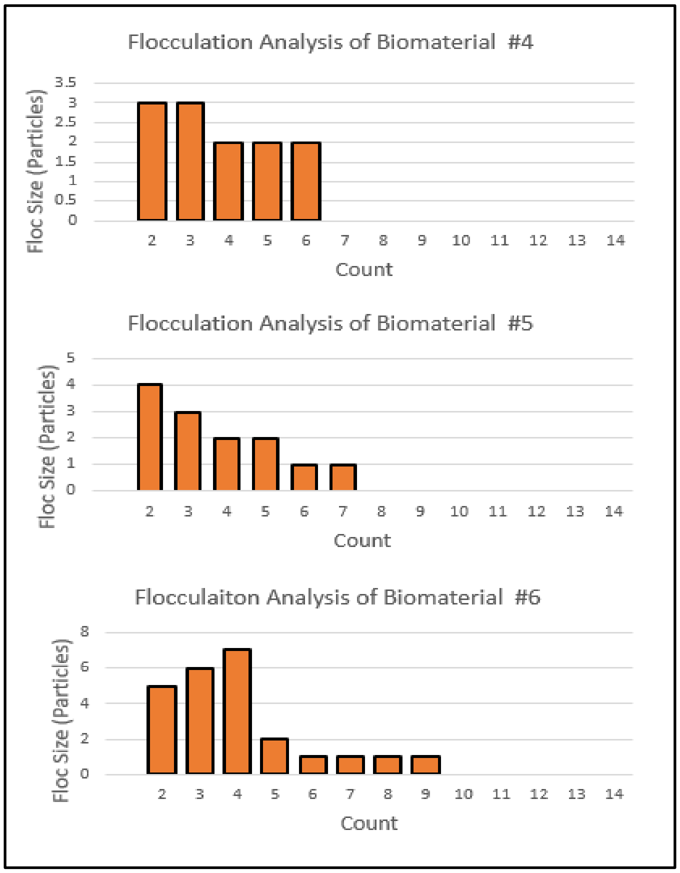


Figure 4: Flocculation Analysis for Bio-composites

Level 3: Development of durable bio-composites followed by testing.

Formulation of bio-composites using food grade epoxy resin was the focus of Level 3 (Table 2). Several different versions were developed using fresh and powdered Okra, powder Aloe and combinations of these. Bio-composite #7, #8 and #10 as shown in Table 2, were promising in terms of their durability while still offering the flocculation properties of the bio-composite. In some samples, like #9 and #10, the resin simply acted as a binding agent while in #7 and #8 its purpose was both binding and coating and there was no breakdown of biomaterials. In samples 9 and 10 however, biomaterial particles were seen to be floating after 3 months of trial, yet they were small and few enough to be negligible. The difference in sturdiness and mechanical qualities of various samples can be seen in the mechanical analysis section. The relative disintegration of bio-composites is consistent with its flocculation; bio-composites 9 and 10 had higher flocculation rates than 7 and 8, and Okra biomaterials had the most as seen in bio-composite # 9.

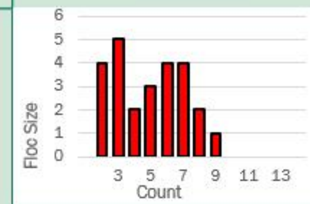
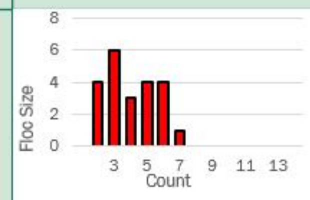
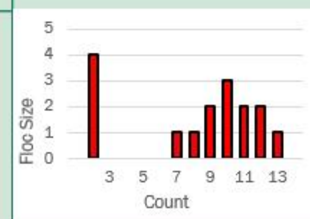
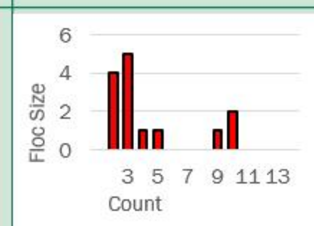
Trial # and Name	Okra (g)	Okra type	Resin (ml)	Aloe Vera (g)	Image	Floc Charts
Bio composite #7	15 g	Powder	20 ml	0 g		
Bio composite #8	0 g	-	20 ml	15 g, powder		
Bio Composite #9	30 g	Fresh, finely chopped	30 ml	0g		
Bio Composite #10	5 g	Powder	30 ml	25 g, powder		

Table 2 - Formulation of Bio-composites using resin and testing

Mechanical Analysis of Bio-composites

The novel bio-composites developed were tested for several key mechanical properties like tensile strength, texture, hardness, opacity and degradability in water to ensure how suitable these would be in the water environments for extended periods of time. Comparison (Table 3 & 4) of various versions indicate that bio-composites 5 & 6 were most sturdy in terms of mechanical properties.

Resin- reinforced bio-composites versions 7-10 were also analyzed for mechanical properties. Version 8 had the most plasticity, strength and least degradability while version 10 was most translucent and had low degradability. These bio-materials with versatile properties have potential to replace plastics used in aquatic fishing environments for varied purposes.

Trial #	Degradability in water	Tensile Strength	Hardness
Bio composite #1	High, 2 hours	Low- little to no bonds between materials	0.4 kg/m^2
Bio composite #2	High, 1 hour	Low	0.6 kg/m^2
Bio Composite #3	Reasonable, 27 hours	Low	1.0 kg/m^2
Bio Composite #4	Low, 5 days	Brittle, immediately breaks	7.3 kg /m^2
Bio Composite #5	Low, 1 month	Strong, no tensile strength, still brittle	8.6 kg/m^2
Bio Composite #6	Low, 1 month	Strong, no tensile strength, still brittle	9.5 kg/m^2
	Elongation at break for these bio composites was 0 cm		

Table 3: Mechanical Properties of Biomaterial Bio-composites developed in Level 2

Trial #	Degradability in Water	Tensile Strength	Opacity	Hardness
Bio composite #7	Low	High plasticity and resistant to applied stress, low elasticity	Low	Plasticity
Bio composite #8	Low	High plasticity and resistant to applied stress, low elasticity	Higher than #7, but low	Plasticity
Bio Composite #9 or *BC #9	Minimal, small flakes of biomaterial after 13-14 days	Reasonable, brittle and can break, elongation at break is 0 cm	Very low and inconsistent	High, around 10 kg/m^2
Bio Composite #10	Low	High, low plasticity and low elasticity	High	High

Table 4: Mechanical Properties of Resin-reinforced Bio-composites developed in Level 3

Scanning Electron Microscope (SEM)

A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample. The resulting images show information about what the object is made of and its physical features. As a practical and useful tool, SEM has a broad range of applications, across several industries and sectors. It can analyze both man-made and naturally occurring materials. An SEM image produced from the intensity of back-scattered electrons and the beam position can show the distribution of different elements in the sample. Elements that are heavier and reflect more electrons will appear brighter in the image so back-scattered electrons can show contrasts in chemical composition. Figure 5 shows the SEM images of microplastic beads interacting with various biomaterials.

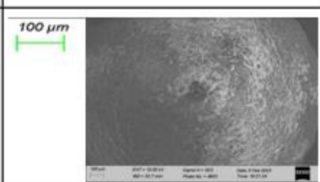
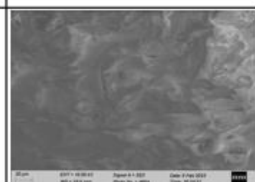
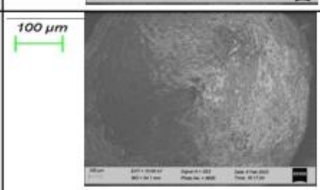
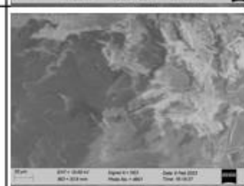
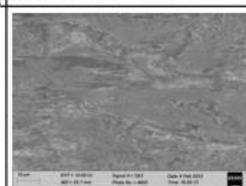
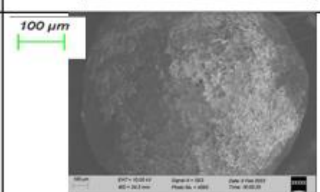
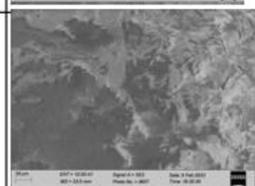
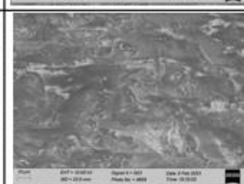
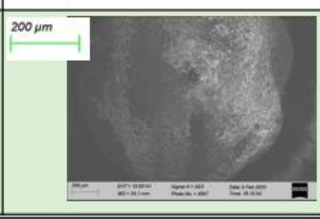
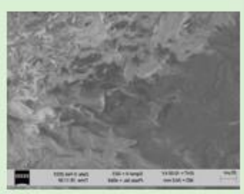
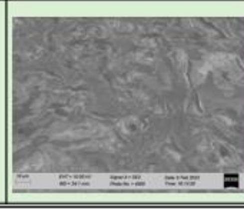
Trial	Microplastic scan	20 um	10 um	Interpretation
No biomaterial				The microplastic with no treatment is the darkest sample showing the limited reflectivity of the material.
Okra Powder				The rough surface shows the treatment of okra on the sample, although the SEM interpretation would include it as part of the microplastic surface.
Aloe Vera powder				The deposits of the aloe vera are obvious when seen at 20 um and 10 um, and the distorted shape of the microplastic represents degradation and deposits.
Zinc Powder				The light shades of SEM scans represent the high reflectivity of zinc treated microplastics. Increased contrast explains why zinc enhances results in all tests. Shape significantly distorted,

Figure 5: SEM Image Data and interpretation

Fourier Transform Infrared Spectroscopy

All infrared spectroscopies act on the principle that when infrared (IR) radiation passes through a sample, some of the radiation is absorbed. Reading the spectrum is a matter of determining which groups and bonds correspond to which peaks. Simple reference tables for the various groups can help.

Results from FTIR readings of Okra, Zinc oxide and a combination of the powder-solutions can be seen in Figure 6. Water was used as a blank to calibrate the FTIR. Strong similarities were observed between the biomaterials and water readings representing no chemical byproduct formed due to the flocculation methods. This is contrasted to current flocculants which result in metallic compound byproducts leaving the resultant sludge unusable. This also confirmed the biomaterial is non-reactive and environment friendly. The FTIR readings serve as a foundation of this research for the development of bio-composites using these biomaterials. These materials allowed for more light transmission making it more suitable for water treatment, given that marine life will not be affected. This also supports the use of this research in real-world scenarios as it has a low absorbance rate, which means light would pass through for the growth of organisms at a water body level.

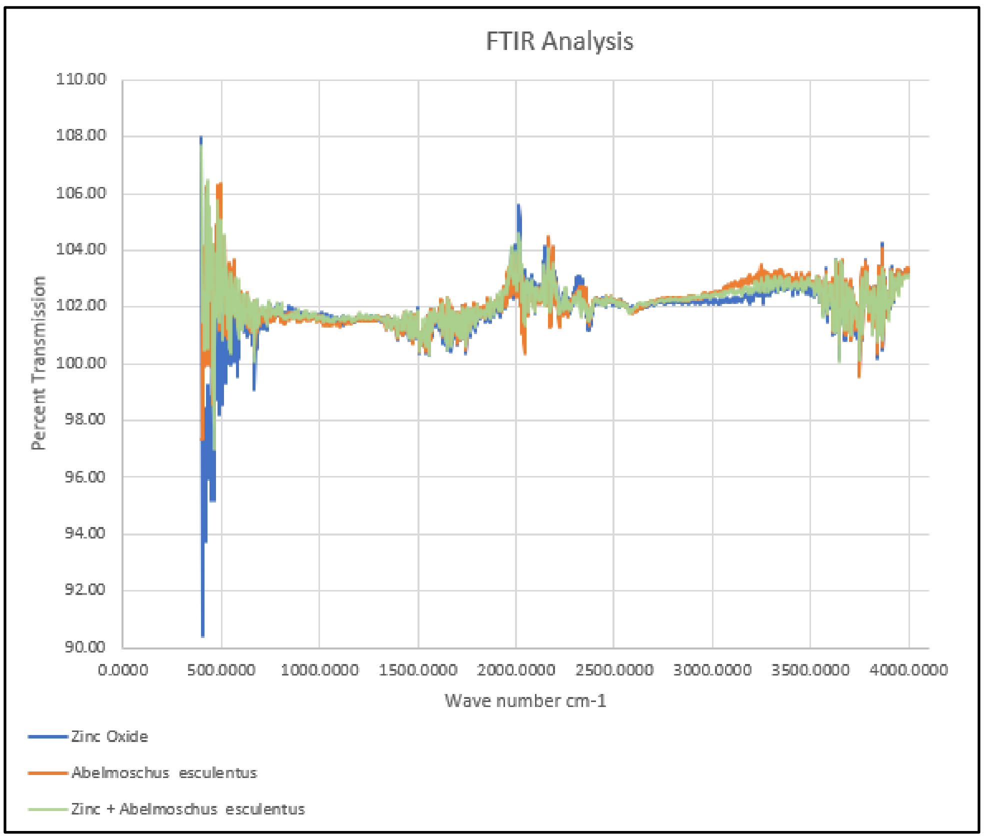


Figure 6: FTIR data for various bio-flocculation trials

DISCUSSION

In the current project, there was a definite trend indicating Okra as a powerful bio-flocculant for microplastic removal from water. Okra when present alone or with Aloe and zinc oxide showed high levels of flocculation of microplastics owing to its strong binding properties. The carbohydrate-based mucilage produced by Okra is the main reason behind its flocculation property. This mucilage is composed of anionic polysaccharides giving Okra its gelatinous properties. Additionally, the microscopic images also showed some kind of cohesion between Okra particles and adhesion with microplastic particles further explaining its flocculation property. Aloe Vera mucilage consists mainly of immobilized aqueous phase with several solids like polysaccharides suspended in there. This mucilage is not as gelatinous as the Okra mucilage and explains the milder flocculation properties of Aloe compared to Okra. Zinc oxide has a polar tendency and non- toxic properties, making it a good candidate for experimentation to increase interaction between water and biomaterial particles. Biomaterials developed using Okra, agar-agar and corn starch had a paper-like texture. These sheets could potentially be introduced in wastewater and then after a few weeks be removed along with all the flocs. The bio-flocculants can be used in the current water management systems making this a very practical and economical solution. Resin contributed to the tensile strength of biomaterials by creating stronger bonds and a sturdier product. These resin- reinforced biomaterials could be potentially used as bio-plastics in aquatic environments for purposes like fishing systems and replace current plastics suspended in ocean water for months creating further microplastic load. Plant materials like Okra are very commonly grown in the United States. As a crop it has a high yield but needs to be used within a given time window due to its high moisture content. As a result, large quantities of Okra get wasted that can be put to this alternative use as bio-flocculants.

CONCLUSIONS

1. Okra demonstrated strong flocculation properties across varying types and sizes of microplastics. Okra was an effective flocculant and caused thick patches of microplastics trapped in its gelatinous slurry called flocs.
2. Aloe had a mild flocculating effect that became stronger with addition of Zinc oxide and Okra.
3. Zinc Oxide added to the effectiveness of the flocculation process only to a certain degree. The presence of zinc significantly lowered the percent transmission and since this transmission in the FTIR is based on chemical bonds, it depicted the intensity of zinc oxide-water solutions.
4. Okra based composites showed great promise in terms of strength, durability and effectiveness. The addition of agar and glycerol strengthened the novel bio-composites.
5. Resin contributed to the mechanical strength, durability and plasticity of the bio-composite making it suitable for varied purposes and uses, including aquatic fishing systems.
6. Finally, these natural flocculants can be used in the current water treatment infrastructure without major changes, and this makes the solution even more viable and economical (Figure 7).

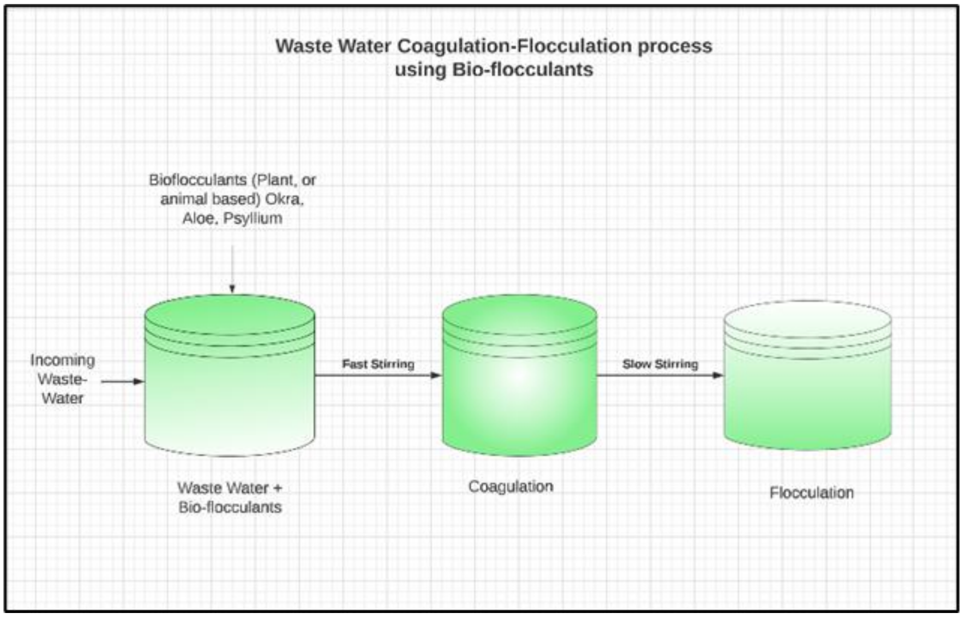


Figure 7: Thematic representation of Use of Bio-flocculants in Wastewater Treatment System

METHODS

Level 1: This experiment tests the chemical and physical properties of Aloe Vera, Okra, and zinc oxide powders and their interactions. Observations concerning internal and external flocculation of Okra and Aloe Vera, water-powder interactions, and floc analysis are the main aims of this experiment. A Magnetic stirrer was used to maintain consistency and trials were conducted in controlled petri dishes and test tubes with the presence of visible light. Results were analyzed using FTIR as well as SEM for microplastics.
Level 2: This experiment tests the mechanical and chemical properties of the bio-composites developed over several trials. The trials were novel and required many improvisations in biomaterials and methods, such as the addition of cornstarch and agar-agar. Analysis included SEM images and microscope images for bio-composites.
Level 3: This experiment expands on Level 2 through the creation of sturdier options that fulfill ideal mechanical criteria for real-world applications in terrestrial and marine ecosystems. The use of resin to coat and produce bio-composites helps fulfill this criterion. Additionally, analysis was done using the SEM for microplastics and microscopic images for the bio-composites.

Data and Statistical Analysis Methods

Visual and numerical Data: The use of microscopes helped collect visual data of the microplastics before and after they were introduced to bio-flocculants as well as the flocculation of the sample. SEM was used to study structural properties of the bio-composites and samples. The visual data and flocculation numbers were compared between the trials. Bar graphs and related statistical analyses were used to depict the results.

FTIR Data- FTIR Spectroscopy was used to analyze microplastics and bio-composites. The samples' transmission and absorption percentages can be observed by taking a spectroscopy scan of each trial. This was done by the use of an FTIR scanner. Graphing transmission of plastics and samples helped compare and contrast the properties.
Mechanical Analysis - Bio-composites developed were compared for mechanical properties like hardness, opacity, degradability and tensile strength. These were presented in a tabular form for ease of comparison and contrast.

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