THE DEVELOPMENT OF CHITOSAN AND IRON OXIDE NANOPARTICLES FUNCTIONALIZED WITH CHITOSAN SOLUTION TO HARVEST HARMFUL ALGAL BLOOMS IN THE BEIRA LAKE, SRI LANKA

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ABSTRACT

Harmful algal blooms (HABs), which include both prokaryotic and eukaryotic organisms, result in the from excessive growth of algae colonies that can produce hazardous impacts on human beings, fish, shellfish, aquatic mammals, and birds. In the presence of nutrients in the water bodies, harmful algal blooms can develop rapidly and drastically deteriorate the quality of water. Therefore, it is important to prepare a solution to harvest HABs from surface waters. This study was carried out to develop the best method to harvest HABs using Chitosan and Magnetic Iron Oxide nanoparticles. Magnetic Iron oxide nanoparticles were synthesized using the co-precipitation method and functionalized with chitosan. The prepared magnetic coagulant was then used in jar test experiments to find the optimum conditions required for the maximum flocculation efficiency of algae, using water samples collected from the Beira Lake, in Sri Lanka. A series of different concentration combinations were used to test the optimum condition. The reduction efficiency of the parameters including *Microcystis* spp and *Spirulina* spp cell density was evaluated. The magnetic iron oxide nanoparticle functionalized with chitosan coagulant significantly reduced cell densities by 87% of *Microcystis* spp, and 86% of *Spirulina* spp than the chitosan coagulant. It was found that the magnetic coagulant; from the coagulation/ flocculation treatment using magnetic iron oxide nanoparticles functionalized with chitosan coagulant, could be used as an efficient treatment to reduce the cell density of harmful algal blooms, in the water samples collected from the Beira Lake.

Key words: Harmful Algal Blooms, Iron Oxide Nanoparticles, Chitosan, Coagulant

1. INTRODUCTION

Freshwater, a vital natural resource on Earth, is unevenly distributed. Only 2.5% of the world's water is freshwater, and less than 1% of that is readily available for development and various uses (Bashar Bhuiyan et al., 2013). Natural waterbodies like lakes and reservoirs and other surface water sources get highly polluted due to population growth, globalization, industrialization, and urbanization. Currently, the most prevalent water-quality problem is eutrophication which is widely recognized to be serious, primarily human-caused environmental issue; the end result of the eutrophication process is the formation of harmful algal blooms in the water bodies (Yatigammana et al., 2011). Such ongoing eutrophication, which lead to dense harmful algal blooms (HAB) and floating scums in freshwater bodies such as lakes, reservoirs and rivers that serve as drinking water sources has become a worldwide ecological problem (Wurtsbaugh et al., 2019). These blooms may cause high turbidity, anoxia, fish kills, bad smell, and serious environmental and human health problems because several harmful algal blooms can produce a variety of very potent toxins (Wurtsbaugh et al., 2019). Harmful algal bloom (HAB) formation is a major environmental concern in nutrient - rich, warm, and still freshwater bodies in many countries around the world including the United States, Asia, Europe, North and South America, China, Africa and Sri Lanka (Hallegraeff et al., 2021 ; Lanka, 2022).

While there are concerns about HABs in inland surface water bodies in Sri Lanka, the Beira Lake is one of the most threatened lakes with HABs as a consequence of Eutrophication which is the biggest problem that has been identified over many years (Idroos et al., 2017). When considering the Harmful Algal Blooms in the Beira Lake, the main types of phytoplankton found in the Beira Lake, which is a polluted artificial waterbody in Sri Lanka, are blue-green and green algae. The dominant blue-green algae, including *Microcystis aeruginosa*, *Spirulina* sp., *Merismopedia* sp., *Pediastrum* sp., and *Ankistrodesmus* sp., are responsible for the frequent harmful algal blooms in the lake (Kulasooriya, 2017). Blue-green algae greatly outnumber green algae in all basins of the lake, and make up more than 99% of the plankton community (Lanka, 2022).

When harvesting the HABs, coagulation and flocculation are important processes that aim to remove suspended particles by destabilization and floating scums produced due to the eutrophication process (Yu et al., 2017). In recent years, researchers have been exploring the use of natural coagulants for harvesting HABs, due to their safety for human health, biodegradability, environmentally-friendly nature, and ability to effectively flocculate various types of colloidal suspensions over a wider dose range (Alshahri, 2021; Abouzied & Hassan, 2021). Among them, Chitosan, the second-most abundant natural biopolymer, is mainly derived from the shells of shrimp and other crustaceans that are discarded as wastes of the seafood industry which result in a positive surface charge. Charge neutralization is the key mechanism leading to flocculation(Yin et al., 2021).

Magnetic nanoparticles have received great attention in water treatment because of their unique physical and chemical properties due to their small size and high specific surface area (Santos et al., 2016). Fe - based nanoparticles effectively cause the deactivation of harmful algal blooms (cyanobacteria) in a fully-established bloom (Tress, 2019). These nanoparticles have been found to be effective at deactivating cyanobacteria by disrupting their normal cellular function, while causing minimal harm to desirable aquatic species (Tress, 2019). This makes them a potentially useful tool for addressing the problem of harmful algal blooms in water bodies.

This study aimed to develop a highly efficient and environmentally friendly approach for eliminating harmful algal blooms from surface water. The proposed solution involved the use of a magnetic coagulant that was developed using chitosan and iron oxide nanoparticles which were considered to be efficient and effective in addressing the issue of harmful algal blooms in a sustainable manner.

2. METHODOLOGY

2.1 Study Area

Water samples were collected from five locations in Southwest Beira Lake, in Sri Lanka (Latitude: 6° 55' 3.9504" N, Longitude: 79° 51' 15.48" E). Triplicate surface water samples were collected at 10cm depth from the surface into sterile polypropylene bottles. Water samples were kept in an ice box and transported to the laboratory within 24 hours and kept at 4 °C until they were used for analysis. In each sampling, the location and the GPS points were recorded.

2.2 Laboratory Studies

2.2.1 Magnetic nanoparticle synthesis and characterization

The coprecipitation method was used for the synthesis of Fe_3O_4 nanoparticles (Kandpal et al., 2014). First, a solution of 0.1M of $FeCl_3$ (100.00 mL) and a solution of 0.1M of $FeCl_2$ (II) (50.00 mL) was degassed in an inert environment for about 30 minutes. A solution of NH_4OH (40 mL of NH_4OH + 60 mL of distilled water) was added drop-wise into this mixture over a period of about 30 minutes under N_2 until the pH of the medium approached 12. The resulting black slurry was separated using a magnet and washed using distilled water until the pH reached neutral. Then the product was dried in a vacuum desiccator.
The resulting iron oxide powder was characterized by an X-ray diffractometry (XRD) measurement. The reflection X-ray powder diffraction data were collected from 10° to 90° in 2θ.

2.2.2 Preparation of Chitosan solution and magnetic coagulant with chitosan solution

Chitosan powder in quantities of 0.5, 1.0, and 2.0 g of chitosan powder were dissolved in 0.1M of 100 mL of HCl solution for 30 minutes to prepare the chitosan solutions. A qualitative membrane was used to filter the mixtures under vacuum, producing solutions containing 0.5, 1, and 2% (w/v) chitosan.
The iron oxide dispersions were made by combining various concentrations of Fe_3O_4 (5 mg, 10 mg, and 20 mg) in 20mL chitosan solution for 5 minutes while using an ultrasonic mixer. The different concentrations of chitosan and Fe_3O_4 were combined.

2.2.3 Jar test assays

C/F assays were carried out in a six-paddle stirrer Jar test using 500 mL of raw water by adding the Chitosan and prepared magnetic coagulant with chitosan. The operational conditions such as the rapid mixing rate, coagulation time, slow mixing rate, flocculation time and settling time were adjusted (**Operational condition for jar test assay:** Rapid mixing rate -100 rpm**,** coagulation time - 3.0 min, slow mixing rate - 15 rpm**,** coagulation time 0 – 15 min**,** settling time - 30 min)
After the raw water mixture, the final concentrations of chitosan were 100 mg (initial 0.5 %), 200 mg (initial 1 %), and 400 mg (initial 2 %). For Fe_3O_4, the final concentrations were 5 mg, 10 mg and 20 mg.

2.2.4 Enumeration of HABs

Algal cell density (cells/mL) was analyzed after and before adding the prepared magnetic coagulant. Immediately after collection, a 100 mL portion of the water sample was fixed with acidified Lugol's solution at a final concentration of 1% and the fixed sample was concentrated by natural sedimentation. HABs were enumerated for each species using a Sedgwick rafter counting chamber under a microscope at a magnification of x 400 at least three times. Each species of HABs was identified (Manage, 2019).

2.2.5 Statistical Analysis

ANOVA was used to analyze data using the Minitab Statistical software package version 20.4. the General Linear Model, Tukey pairwise comparison was conducted using Minitab to test each parameter measured on the prepared coagulant.

3. RESULTS AND DISCUSSION

3.1 Effect of coagulant combinations of Fe_3O_4 and chitosan on algal cell density

Table 1: The *Microcystis* spp, *Spirulina* spp, cell density removal percentages of coagulant combination of Fe_3O_4, and chitosan

Fe_3O_4 Concentration (mg)	**Chitosan Concentration (mg)**	***Microcystis spp* removal %**	***Spirulina spp* removal %**
Without Fe_3O_4	0	8.33±0.07 ^{m}	0.1±0.1 ^{l}
	100	18.56±0.148 ^{k}	12.8±0.15 ^{j}
	200	32.34±0.04 ^{g}	20.35±0.05 ^{h}
	400	50.34±0.15 ^{d}	45.59±0.08 ^{c}
With 5 mg of Fe_3O_4	0	11.53±0.4 ^{l}	4.42±0.02 ^{k}
	100	28.46±0.05 ^{h}	16.63±0.015 i
	200	42.03±0.04 ^{e}	25.14±0.12 ^{g}
	400	60.3±0.1 ^{b}	50.46±0.153 ^{b}
With 10 mg of Fe_3O_4	0	22.23±0.25 ^{j}	25.63±0.280 ^{f}
	100	40.27±0.34 ^{f}	37.46±0.15 ^{d}
	200	53.75±0.029 ^{c}	46.5±0.1 ^{c}
	400	87.75±0.22 ^{a}	86.51±0.10 ^{a}
With 20 mg of Fe_3O_4	0	26.7±0.1 ^{i}	29.64±0.15 ^{e}
	100	87.82±0.106 ^{a}	86.4±0.1 ^{a}
	200	87.8±0.13 ^{a}	86.6±0.1 ^{a}
	400	87.82±0.1 ^{a}	86.6±0.1^{a}

(a-m values in the same column with different superscripts are significantly different at $P<0.05$)

Effect of on algal cell density reduction

In this study, seven species of HABs were identified including *Microcystis* spp, *Spirulina* spp, *Anabaena* spp, *Ankistrodesmus* spp, *Staurastrum* spp, *Scendesmus* spp and *Pediastrum* spp in the collected water sample from the Beira Lake. But the reduction efficiencies of *Microcystis* spp. and *Spirulina* spp. were specifically analyzed due to their prevalence in the collected samples. Table 01, shows that the concentration combinations of both Fe_3O_4 and chitosan caused a significant reduction in cell density of both *Microcystis* spp and *Spirulina* spp ($P < 0.05$) compared to the chitosan concentration combinations. The maximum removal efficiency (87.75±0.2%, and 86.51±0.10% respectively) was achieved for both species at the concentration combination of 10 mg of Fe_3O_4 and 400 mg of chitosan (Table 01) than the chitosan concentration combinations.

According to Figures 01 and 02, chitosan without Fe_3O_4 did not show a significant reduction of both these species. It was observed that the reduction efficiency of *Microcystis* spp and *Spirulina* spp was increased with the combination of both Fe_3O_4 and chitosan. According to the results shown in Table 01, there is no significant effect in the reduction of *Microcystis* spp and *spirulina* spp after the concentration of 10 mg of Fe_3O_4 and 400 mg of chitosan. Therefore, it was observed that, the concentration of 10 mg of Fe_3O_4 and 400 mg of chitosan is best for the reduction of cell density of both *Microcystis* spp and *Spirulina* spp.

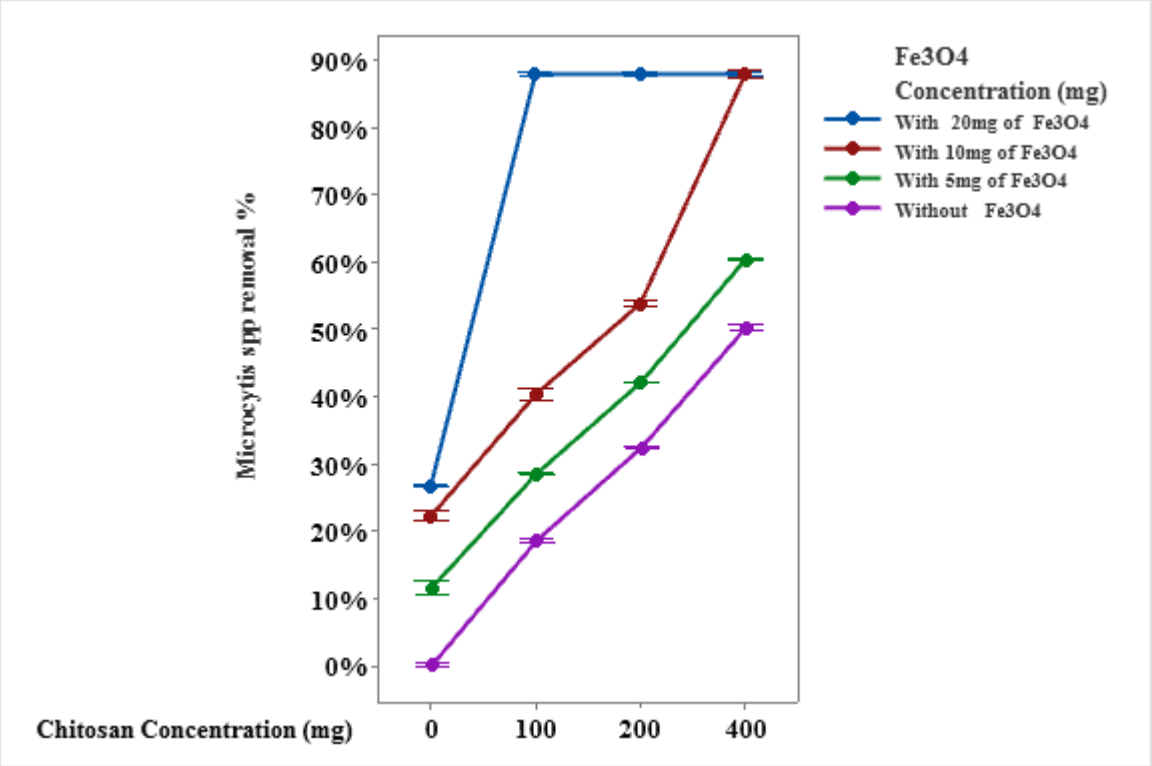

Figure 01: Removal percentages of *Microcystis* spp cell density over the treatment time with coagulant combinations of Fe_3O_4 and Chitosan

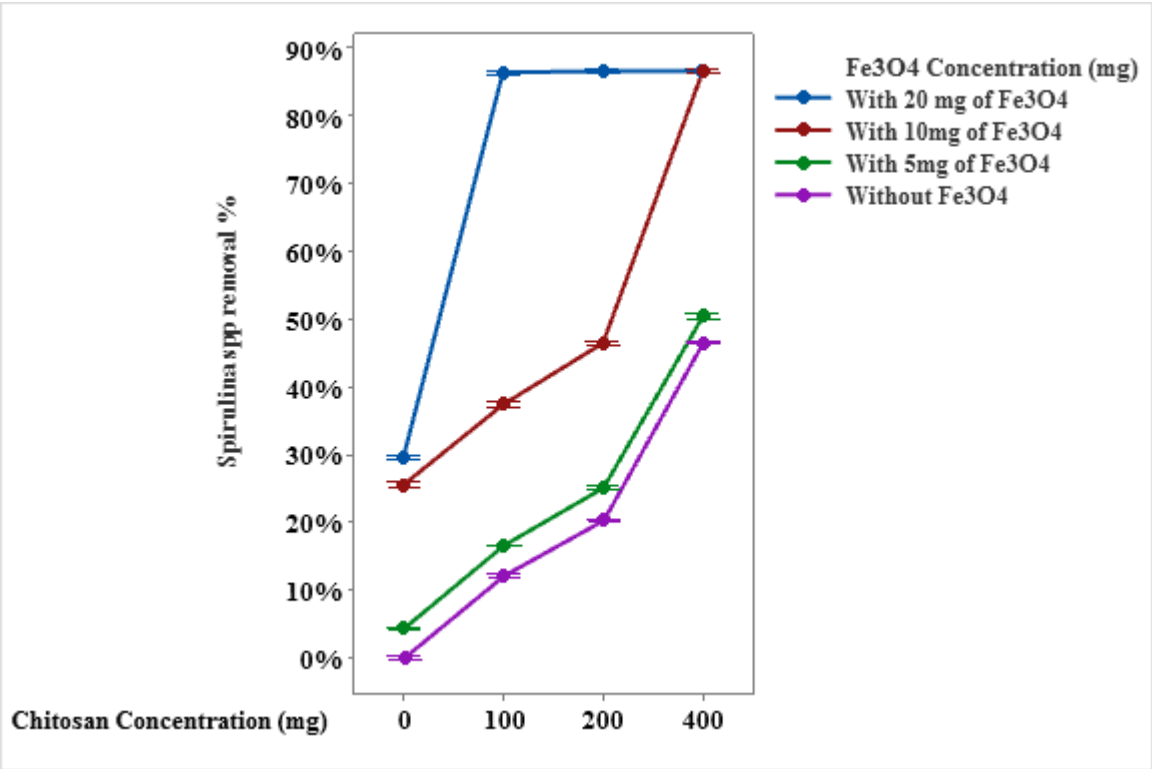

Figure 02: Removal percentages of *Spirulina* spp cell density over the treatment time with coagulant combinations of Fe_3O_4 and Chitosan

CONCLUSIONS/RECOMMENDATIONS

The harvesting of harmful algae in surface waters with a combined concentration of iron oxide nanoparticles and chitosan showed a better reduction of *Microcystis* spp, and *Spirulina* spp cells when compared with the chitosan coagulant. The magnetic iron oxide nanoparticle functionalized with chitosan coagulant significantly reduced 87% of *Microcystis* spp cell density, and 86% of *Spirulina* spp cell density when compared with the chitosan coagulant. It was found that the magnetic coagulant; which is the coagulation - flocculation treatment using magnetic iron oxide nanoparticles functionalized with chitosan coagulant was the best, cost-effective, robust, and environmentally friendly method to reducing the cell density of harmful algal blooms in the surface waters.

Further investigation is needed to determine whether the iron oxide nanoparticles were functionalized with chitosan.

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ACKNOWLEDGMENTS

The authors of this research paper wish to extend their heartfelt thanks and deepest appreciation to all those who generously contributed their knowledge, expertise, and support, which were instrumental in the successful completion of this work.