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Adsorption of Red Dye 40 from Wastewater via a Moringa Oleifera Coated Cotton Filter

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ABSTRACT

Clean drinking water affects millions of people worldwide. In this experiment, a sustainable method of removing contaminants from water's effectiveness is tested. *Moringa oleifera (MO)* seeds contain a cationic antimicrobial protein that can remove negatively charged particles from water. This positive protein can also "stick" to cotton, a negatively charged surface. MO trees grow commonly in areas where there are high levels of diarrheal diseases that come from drinking contaminated water. During production, 10-15% of food dyes leave the plant as effluents into the environment. These dyes can lead to toxic soil and can harm the wildlife that lives in the water it is released into. In this experiment, the viability of cotton coated in a MO serum to remove Red Dye 40 is tested. A cotton column model is used in this experiment to force contaminated water through the coated cotton. Initial results were inconsistent and did not show much of a trend. Through the course of the experiment, a pre-rinse of the cotton was added, the method for coating the cotton in MO serum was changed, and the flowrate of the pump was lowered. All of these were done to improve the consistency of the results to see if there is a trend relating the concentration of an initial dye solution and the amount of dye that can be removed from the water per gram of cotton used; the Qe. After these adjustments were made, the maximum Qe was experimentally found to be 1.3 mg dye/g cotton. A Langmuir isotherm is the model that best fits the data, as a monolayer is assumed. The positively charged dye only sticks to the cotton, it does not build on itself. The results overall show that this filter is a viable method of removal, and that it works best at higher concentrations of dye solution.

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Chapter 1
Introduction

1.1 Food Dyes and Water Treatment

Currently, 842,000 deaths a year can be attributed to waterborne infections according to the World Health Organization (WHO) [1]. Additionally, the United Nations (UN) reports that 2 billion people lack safe drinking water [2]. Figure 1 shows a map depicting water scarcity around the world [3].

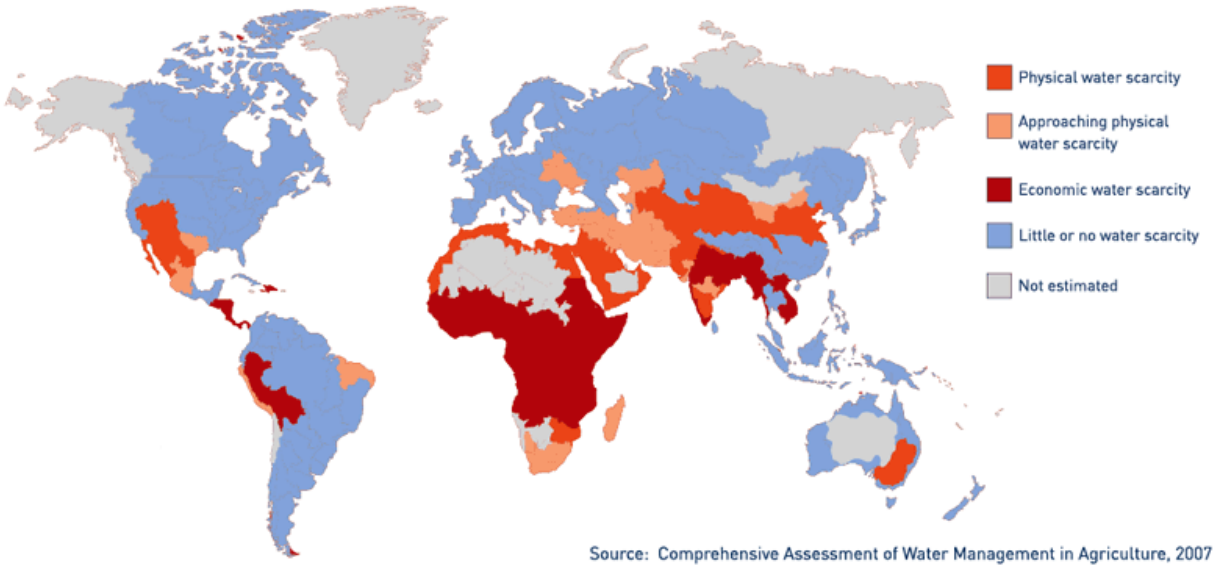

Figure 1 Map of Water Scarcity Worldwide

Contaminants in water come from many different sources. In developing countries, there are typically few regulations in place dictating what factories can output into the local water ways. In the production of food dyes, 10-15% of the dye leaves the factories as effluents into the environment [4]. These dyes can lead to toxic soil and can harm the wildlife that lives in the water it is released into. The effects of food dye on the developing brain are also a topic under heavy debate. The WHO deems dietary exposure to Red Dye 40 to not be a health concern. However, it may have links to aggression and mental disorders in children. Current research indicates no adverse behavioral effects due to the consumption of Red 40 by children, however some suggest that there may be a sensitivity to it in some children [5]. The main exporters of food dyes are India, Mexico, and Germany, of which India and Mexico suffer from clean water problems [6].

The consequence of contaminated water increases every year. As a result, there is a variety of different water treatment methods that have and are being developed. Point-of-use technologies

reduce diarrheal disease by 30-40% at a household level [7]. On a larger scale, methods include boiling, bio-sand filters, ceramic filters, and chemical disinfection, each presenting their own challenges [8]. These chemical methods can result in the formation of cytotoxic and carcinogenic biproducts [9]. Boiling water is an energy-intensive process that is not economically possible in developing countries [10]. An additional method is coagulation, which is highly effective and applicable to a wider range of water types. Aluminum, ferric salts, and polyaluminium chloride serve as coagulants; these chemicals generate large amounts of non-biodegradable sludge and affect the pH of the water [11]. This leaves a need for sustainable, affordable, and energy non-intensive water treatment methods, that utilize what is available in these areas of water vulnerability. The *Moringa oleifera* tree grows in the subtropic regions that often suffer from water issues. The seeds of the tree have antimicrobial properties that make it a possible solution to this problem.

1.2 *Moringa oleifera* Seeds

The *Moringa oleifera* (MO) tree grows in equatorial regions of the world, as can be seen in Figure 2, which happens to coincide with where there is a risk due to unsafe drinking water [8].

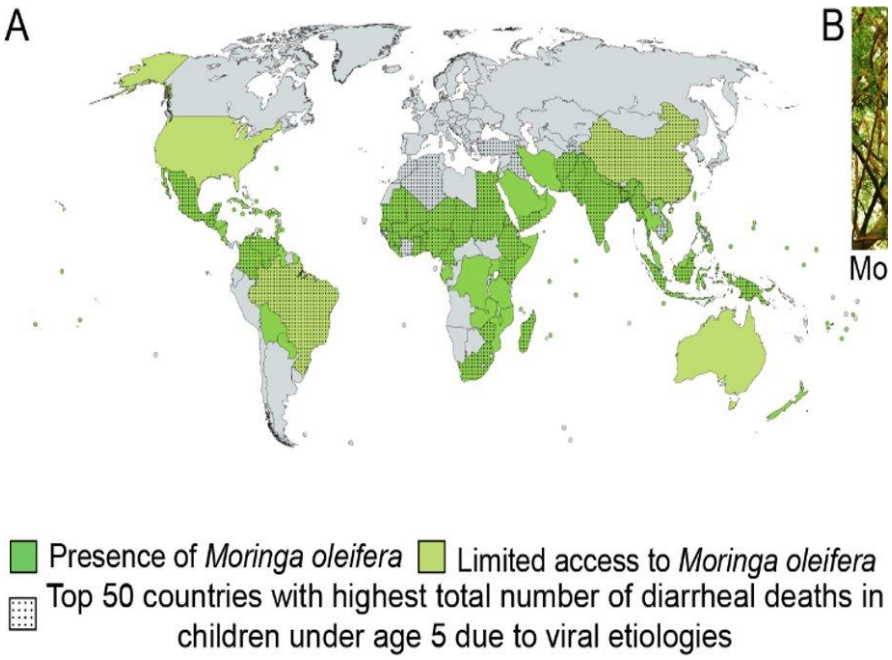

Figure 2 Locations *Moringa Oleifera* Trees Grow

MO seeds have traditionally been used as flocculants to clarify drinking water [12]. This is due to the cationic and antimicrobial proteins (MO cationic protein, MOCP) that the seeds contain. Negatively charged pathogens in water can form attractive electrostatic forces with the positively charged seeds [13]. The proteins are produced due to the helix-loop-helix motif that causes fusion of inner and outer cell membranes; the structure of these proteins can be seen in Figures 3 and 4 [14].

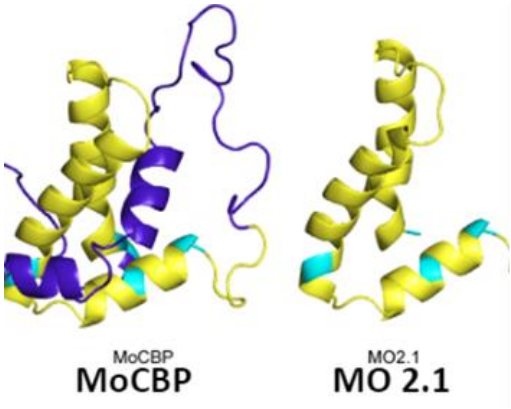

Figure 3 Helix-Loop-Helix structure of MO Seeds

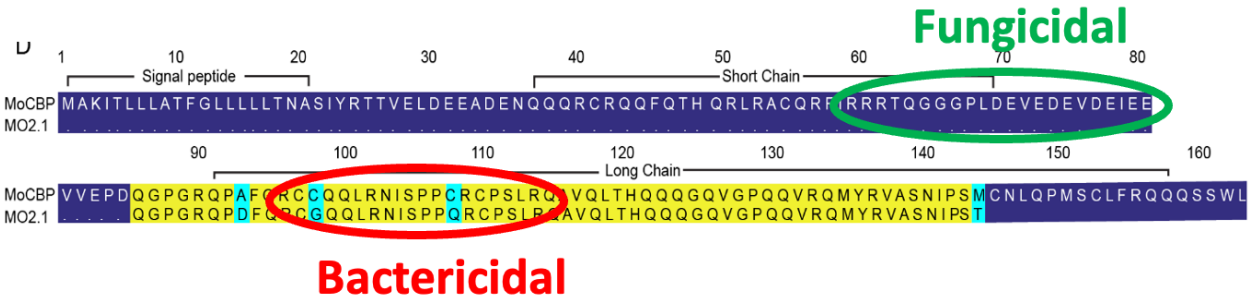

Figure 4 Fungicidal and Bactericidal coding of MO seeds

One concern with the use of MO seeds as drinking water treatment is a potential fouling of the treated water due to the organic matter released from the seeds [8]. The Stephanie Velegol lab has studied a solution to this: MOCP can be adsorbed through electrostatic attraction onto sand, forming a functionalized sand. This does not affect the capability of the antimicrobial and flocculating abilities of the protein, while it eliminates the organic matter [15]. The S. Velegol lab has also conducted experiments proving the viability of MO to remove viruses and *E. coli* from water. These are relatively large molecules, leading the group to consider whether this technique was applicable to remove small molecules as well. Success was seen from testing with humic acid, which can be found in water along with viruses and bacteria. As Red 40 is also seen in these waters, and is negatively charged, this experiment focuses on the viability of a MO filter to remove Red Dye 40 from water.

In this experiment, instead of isolating the MOCP protein, a MO serum is created by crushing the seed and mixing it with DI water. This serum is coated onto a cotton column to model the filter. For application of the column, parameter testing must be done to engineer it. The main challenges that come with engineering a column is determining how much more the moringa filter can remove from water than simple cotton, how to improve inconsistent results, and how to scale the column up for potential real implementation.

Chapter 2
Purpose and Hypothesis of Experimentation

2.1 Purpose

The purpose of this experiment is to assess the viability of a MO filter to remove Red Dye 40 from water. Based on the theory and charge of the dye molecules, the removal should work, however the effectiveness of the method needs to be observed. To do so, the ultimate goal is to determine the maximum amount of dye that can be removed from water/gram of cotton used.

2.2 Hypothesis

The first hypothesis (H1) states that increasing the concentration of the initial dye solution will increase the amount of dye adsorbed per gram of cotton according to a Langmuir Isotherm.

The second hypothesis (H2) is that pre-Stripping the cotton with a salt solution will create a more even surface for the dye and moringa to stick to and will lead to more consistent results.

The third hypothesis proposes that decreasing the flow rate will increase the amount of dye adsorbed per gram of cotton.

2.3 Langmuir Model

Approximation models are useful as they provide a way to predict the results of experiments. For non-linear data, linear models manipulate the data to be linear and equations of those lines are used to predict the trends of the data. All these approximation models exist under assumptions. Two of these approximations are the Freundlich and Langmuir Adsorption Isotherms. The Langmuir model assumes that a continuous monolayer of molecules covers a homogeneous solid surface, which is what occurs in this experiment [16].

The linear approximation is ruled by:

$$\frac{1}{Qe} = \frac{1}{Q_{max}K_{ad}}\left(\frac{1}{C_e}\right) + \frac{1}{Q_{max}} \quad (1)$$

Where Qe is the amount of dye adsorbed/gram of cotton, Ce is the concentration of the solution, and Kad is the adsorption coefficient [16]. From Equation 1, the maximum amount of dye/g of cotton can be found.

Chapter 3
Materials and Methods for Preparation of the Cotton Filter

3.1 Moringa Serum

The seeds used in this work were from PKM-2 Cape Coral 2019/2020. To prepare the MO serum, whole, unshelled MO seeds were crushed using a coffee grinder. 4 grams of crushed seeds were then weighed out and mixed with 200 mL of DI water to create a serum concentration of 20 mg/mL. This solution was stirred on a stir plate for 10 minutes and then the serum was separated from the seed pulp using vacuum filtration.

3.2 Cotton

The cotton in this experiment was cotton balls. 3.5 grams of this cotton was weighed out and placed into DI water. The cotton was lightly wrung out and pressed firmly down in a glass column to create a cotton filter. The column was attached to a pump set to 2 mL/min and 600 mmol NaCl was run through the column for 30 minutes, this was followed by 30 minutes of rinsing with DI water to ensure all salt was removed. At this point the control filters were ready. For the columns that were to be loaded with MO serum, the serum was run through the pump until none remained, ~50 min, followed by a 30-minute DI water rinse.

3.3 Red Dye 40 Solution

The contaminant in this experiment is Red Dye #40. To create the red dye solution, the appropriate volume of distilled water was measured and combined with the needed mass of dye powder to create the desired concentration of solution. 0.1xPBS was added to this solution to assist in better removal. To confirm the concentration of the made solution, a calibration curve was made with 100 mg/L dye solution using the UV-vis.

3.4 Running the Experiment

To measure the amount of dye that could be absorbed/gram of cotton in each column, the pump was set up to push the dye solution through the columns. The tubes attached to the column were observed and a timer was started when the dye visibly got to the column. An initial sample of the effluent was collected and then samples were subsequently taken at recorded time intervals. Throughout the course of the overall experiment being run, factors such as the flowrate and timing of collections were adjusted. Initially, the flowrate was set to 2 mL/min and samples were taken approximately every 5 minutes. When the concentration of the effluent began to approach the concentration of the starting solution, samples were taken more quickly to observe

the exact completion time. The flowrate was later adjusted to 0.5 mL/min, and the time between samples increased to every 15-30 minutes. Samples continued to be taken for 15 minutes after the concentration of the effluent reached that of the influent to ensure that the measured value stayed constant.

Experiments were also run to observe how the MO serum affected the longevity of the sticking of the dye. After coating the cotton in the dye, the same procedure was followed using 600 mmol NaCl instead of dye. Samples were collected until the concentration of the effluent stopped changing.

Chapter 4
Results and Discussion

4.1 Analysis Parameters

For each experiment run, a calibration curve of the initial dye solution was made using the UV-vis. All samples collected were put into the UV-vis where an absorbance reading was taken, which was used to find the concentration of dye for that sample. The amount of dye sorbed in that time slot, total amount of dye sorbed up to that point, and the concentration vs. initial concentration of solution were all looked at to analyze the data.

4.2 Adsorption Results

Stage 1 – Initial trials

In the beginning stages of the red dye research, the columns were coated with MO serum via a batch method, run with no pre-stripping of the cotton and at a flowrate of 2 mL/min. The results of these experiments were often inconsistent. Each experiment was run with one control and 2 MO coated columns. There were occasions where the final Qe of one of the coated columns was significantly higher than the other coated, as can be seen in Figure 5 which shows the Qe vs concentration for the initial stage of the research.

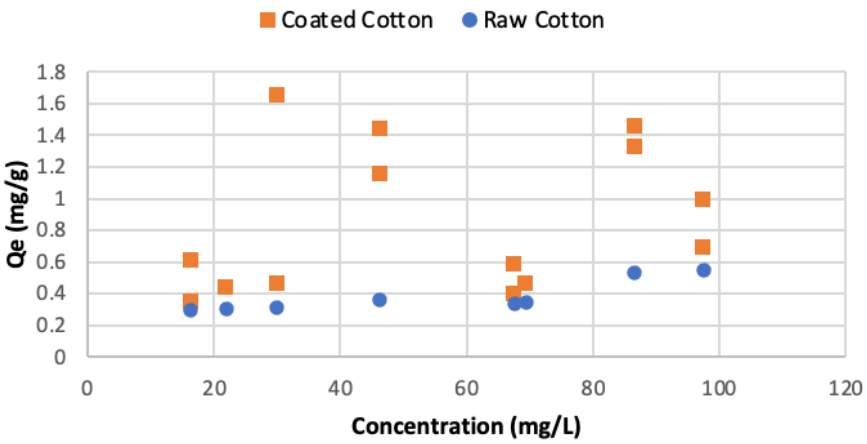

Figure 5 Qe vs. Concentration for Initial Trials

This stage of the research did show a very slight trend, where the Qe increased as the solution concentration increased. However, for just over half of the experiments, there was little to no difference between the results of the control and the tested columns. During these experiments it was also questioned whether the cotton could be reused. To test this, the columns were stripped with 600 mmol NaCl and then recoated with the red dye solution. This resulted in much more consistent results in the second round of dye adsorption that led to making the hypothesis that stripping the cotton with salt prior to running the experiments would result in more consistent results.

Stage 2 – Pre-rinsing the cotton to achieve more consistent results.

Adding in a pre-rinse step to the column preparation resulted in more consistent results, as predicted by the hypothesis. There was now a more clear definition between the results of the control and test columns, as can be seen in Figure 6 which shows the Qe vs Concentration for experiments run with a pre-rinse.

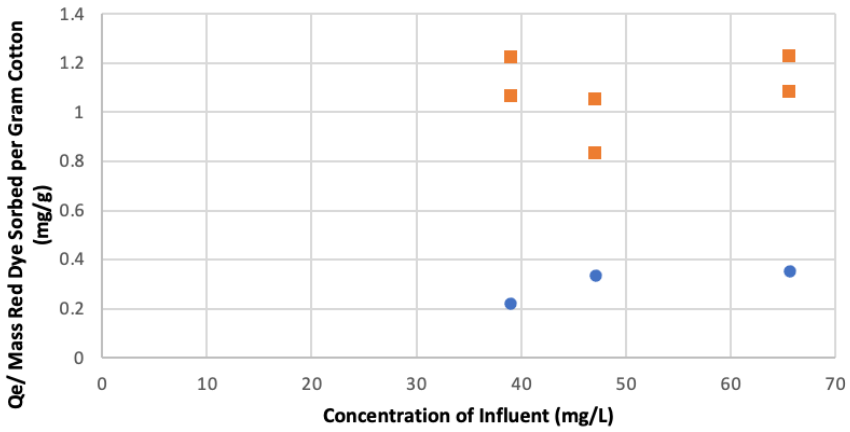

Figure 6 Qe vs. Concentration for Pre-Rinse trials

Despite this improvement, the data still did not follow the hypothesized trend of increasing as the concentration of the dye solution increased. Before declaring the hypothesis to be null, it was proposed that there may still be inconsistencies in the experiment, as theoretically the two coated columns should be identical in each experiment and there was now a consistent difference of ~0.2 mg/g. The main source of error seemed to be in the way that the columns were coated with MO serum. The batch method was not uniform. The cotton was placed in a beaker with a stir bar on a stir plate and soaked in the MO serum. In theory this should work, however the cotton would clump together, and the stir bar would get stuck at the bottom, not actually doing anything to help move the cotton around. The idea to coat the columns in MO the same way it is being coated in dye seemed reasonable. To make the experiment as uniform as possible, the preceding experiments were conducted with all rinsing and coating occurring in the column-pump set up.

Stage 3 – Changing the MO coating method to obtain more consistent results.

Following this change, the data became increasingly more consistent, and started to show a much clearer trend. Figure 7 shows the Qe vs. Concentration for this stage in the research.

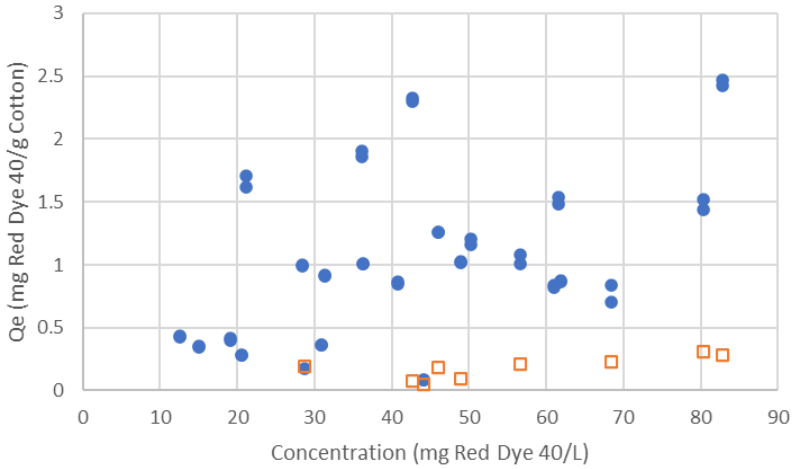

Figure 7 Qe vs. Concentration with changed MO coating method

In this graph, four experiments stand out as discrepancies, at approximately 20, 35, 45, and 85 mg/L dye solutions, the Qe of the test columns were ~1 mg/g higher than the trend set in the data. It is theorized that something went wrong in the preparation of these columns that allowed this to occur. For each of these errors, an additional trial was run that resulted in data that fit the trend of the graph.

Apart from these data points, the results show an increase in Qe as the initial concentration of dye solution increases, confirming H1. The same trend can loosely be seen in the data for the control, however the Qe values are so low that it is harder to discern than with the test data. The MO coated columns also seem to be approaching a plateau in the Qe as the concentration increases, suggesting that the data perhaps models a Langmuir isotherm.

One of the goals of this research was to see the maximum amount of dye the cotton could hold. The point at which the cotton could no longer adsorb any more dye was determined by when the effluent reached the same concentration as the incoming solution. However, when the cotton was pulled apart after the columns were run, it appeared as though the inside was not as saturated as it could have been as shown in Figure 8.

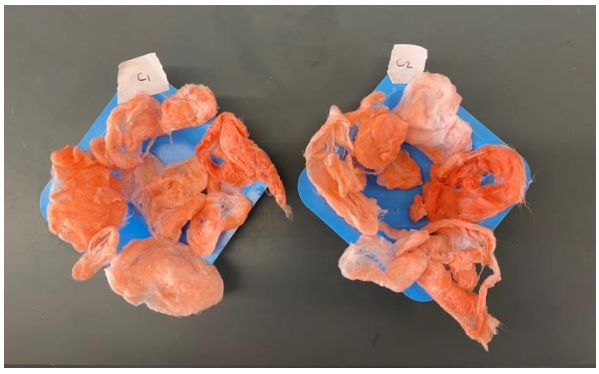

Figure 8 Cotton pulled apart showing uneven coating inside

Additionally, when the dye ran through the cotton, there was often “channeling”, where the dye would move down the column quicker in one spot than the rest of the column as can be seen in Figure 9. This could be occurring for a couple of reasons: the cotton not being evenly coated in the MO serum, or the glass column having scratches in it that provide this easier route for the dye to move through. A hypothesis was made that by decreasing the flowrate of the system, the Qe would increase. Doing so would give the dye more time to interact with the cotton and MO serum and allow it to find spaces on the protein that were being passed over at a higher flowrate.

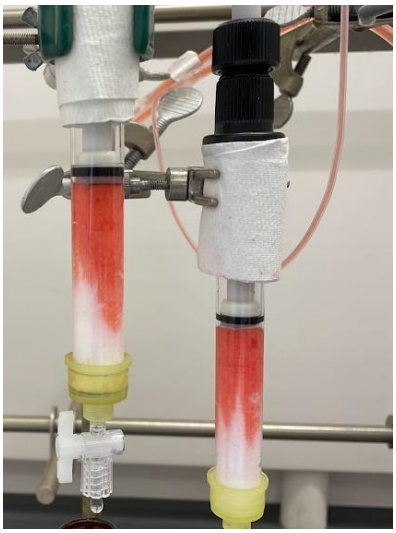

Figure 9 Columns showing channeling

Stage 4 – Does lowering the flowrate result in consistent data?

Running the experiment at a lower flowrate resulted in more consistent data that falls in nicely with the rest of the data from previous experiments. There was no real significant increase in the Qe however. The columns appeared to coat more evenly with the dye, yet there seems to be no advantage to using the lower flowrate. Most of the trials using this method were at higher concentrations, 50 - 110 mg/L dye solution. The Qe did not change much across these trials. There is a slight step up for the 110 mg/L run, however the others all stay around ~1.3 mg/g. Similar data was seen for the higher flowrate runs. With the Qe starting to plateau after 60 mg/L dye solution at 1.3-1.5 mg/g. This suggests that this range is the maximum adsorption of the cotton for red dye, and for these values lowering the flowrate will not change the Qe. It is possible that it would be advantageous to run the experiment at 0.5 mL/min for lower initial concentrations of dye solution, however not enough trials were run to make that conclusion.

4.3 Dye Breakthrough

Additional data of interest was the breakthrough time and volume for each method and trial. The breakthrough time was when the effluent had an absorbance >0, indicating that dye was starting to exit the column. Figure 10 shows the breakthrough time vs. concentration for all the trials where the cotton was coated with MO serum in the column. As expected, it took longer for the dye to breakthrough at a lower flowrate. Across each flowrate, the breakthrough time did not vary much with changing concentration.

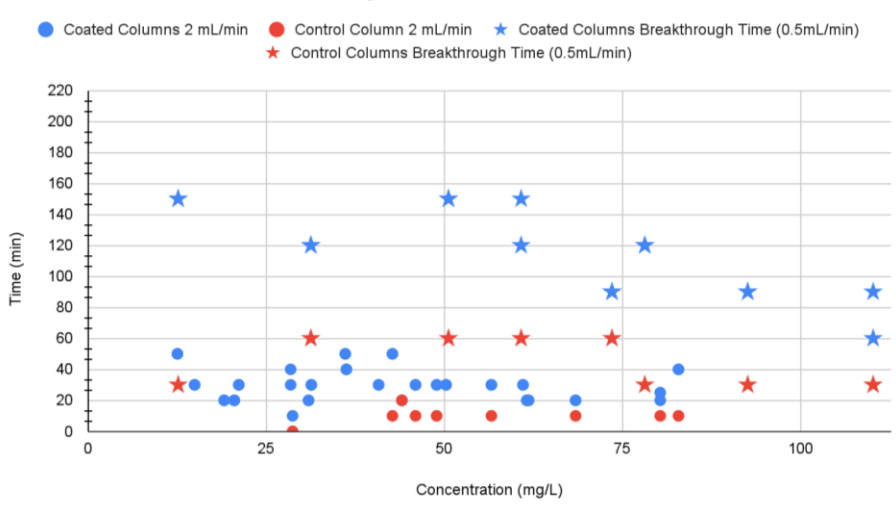

Figure 10 Breakthrough time vs. Concentration with new MO coating method

Since breakthrough time is influenced by the flowrate, the breakthrough volume vs. Qe was also analyzed in Figure 11. The breakthrough volume has a rough trend of increasing with increasing Qe, however it is not very clear. For the average plateau Qe of 1.3-1.5 mg/g, the breakthrough volume falls between 60-75 mL. For the lower flowrate, the breakthrough volume falls on the higher end of the range, with those plateau Qes resulting in a volume of 75 mL.

Figure 11 Breakthrough Volume vs. Qe

4.4 Linear Approximation

A linear approximation of the data can be made to predict the outcome of future experiments. Figure 12 shows the relationship between the inverses of the Qe concentration.

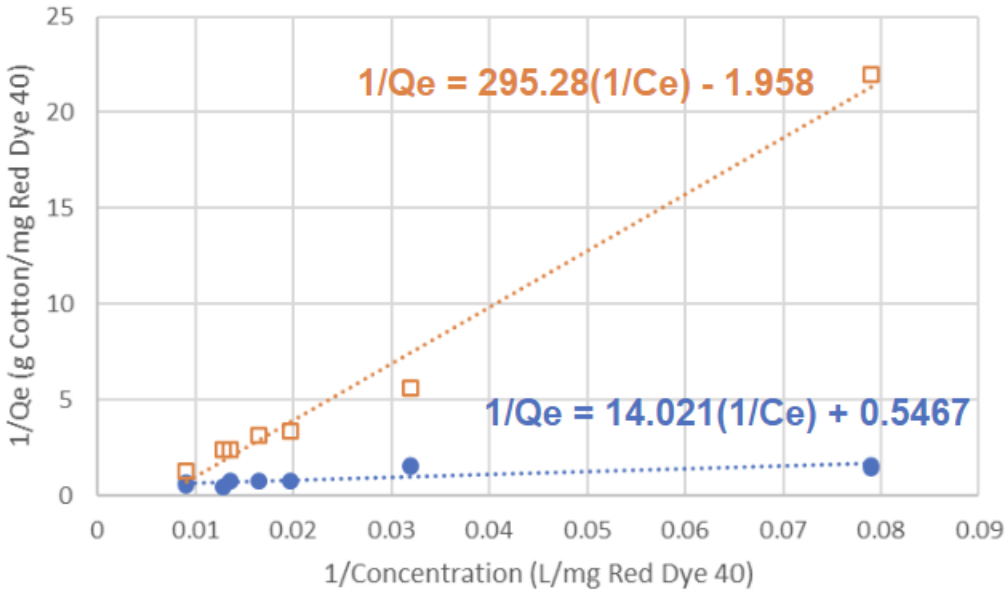

Figure 12 Linearized Data from Stage 4

From this graph and the equations of the Langmuir model, the theoretical Qe max can be found. For the control, this is found to be 0.55 mg/g while for the MO coated columns, it is found to be 1.96 mg/g. The max Qe would correspond to the value when the graph plateaus. Experimentally, this value was found to be closer to 1.3 mg/g for the MO coated columns, and around 0.5 mg/g

for the control columns. Figure 13 shows the Qe each experiment and its relation to the theoretical Qe_{max}.

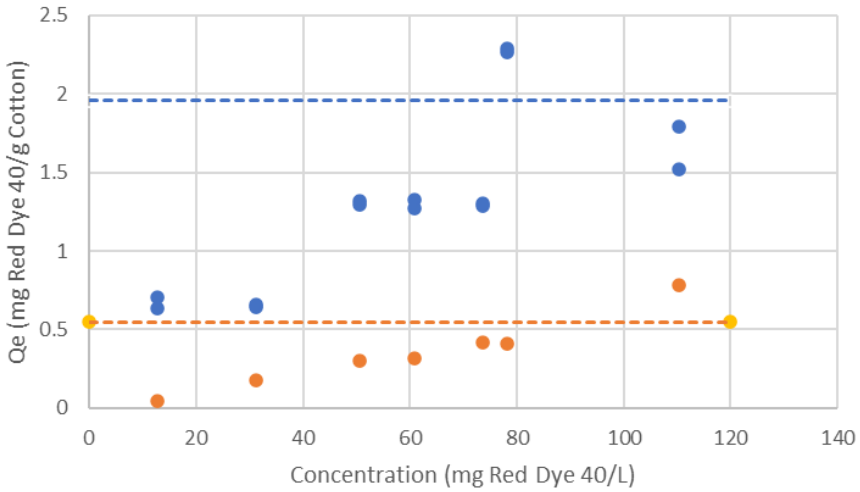

Figure 13 Stage 4 Qe vs. theoretical max Qe

Except for an outlier trial, the data does not cross this line. However, Figure 14 shows the data vs. the line predicted by the Langmuir model and it can be seen that it is not a great fit. The data is somewhat predicted at higher initial concentrations, yet at lower concentrations, it does not match the model. The general shape of the graph is accurate; however, the experimental values are lower than predicted by the Langmuir equations.

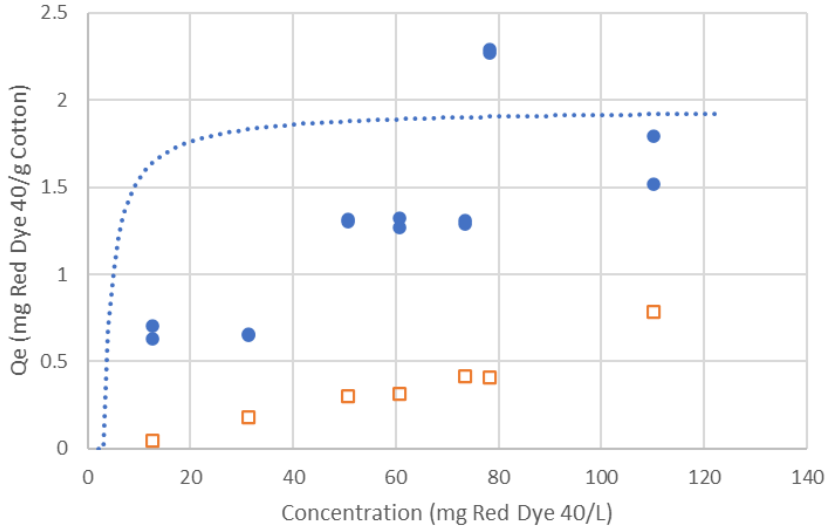

Figure 14 Stage 4 Data vs. Langmuir Model

As the shape of the graph is roughly correct, the Langmuir model is a somewhat reasonable prediction method. The discrepancy most likely comes from experimental error and the assumptions made in the Langmuir model. The Qe is calculated based on absorbance values

from the UV-vis. At lower concentrations of samples, the measured values are not as accurate due to the very low absorbance values. This inaccuracy would cause the Qe to be lower than theoretically predicted. In the Langmuir model it is assumed that there is monolayer adsorption. This does correctly model the experiment. Due to the charge of the dye molecules, they would not stick to other dye and therefore there is a monolayer. The other main assumptions of the model are that there is no interaction between the molecules on different sites and that each site can only hold one molecule. It is plausible that the lower than predicted Qe values are caused by an incomplete coverage of the dye. If not every site is filled by a dye molecule, then the resulting Qe would be lower. This could be caused by impurities remaining on the cotton, an uneven coverage of MO, or a too fast flowrate not giving the dye time to find open sites.

Chapter 5
Conclusions

5.1 Summary

Moringa oleifera is an effective method of removing Red Dye 40 from water. Its negative charge allows it to stick to the cotton filter and to the dye that is being removed. The adjusting of parameters confirmed the first two hypotheses. The data roughly models a Langmuir Isotherm, and variation from it can be attributed to the assumptions made of the model and experimental error. Pre-stripping the cotton with a salt solution as well as adjusting the method for coating the cotton with MO serum ensured that the cotton was evenly coated and lead to more consistent results. The third hypothesis was disproved. While decreasing the flowrate did improve the consistency of the results, ensuring that the dye had more time to evenly adsorb to the cotton, no significant increase in the Qe was seen. After making these adjustments to the method, the experimental Qe_{max} was found to be 1.3 mg dye/g cotton.

The Langmuir Isotherm is the best linear approximation method for this data due to the assumption that a monolayer forms. However, the model predicts the Qe to be higher than what is found experimentally. It best models the data at higher initial concentrations and the general shape matches the data. It theorizes that the Qe_{max} should be 1.96 mg/g, 0.66 mg/g higher than what is found experimentally.

5.2 Future Work

There are many directions that this experiment can branch out into. There is the option to continue running columns using the method from Stage 4 of parameter testing. More data points would provide more information about accurate prediction models, as well as seeing what the actual experimental Qe_{max} is.

Work in the lab has started on testing a scale-up model of the column. For this to be a viable removal method, it needs to be done on a much larger scale. It is not an exact scale-up however and requires its own set of parameter testing. Consistent packing methods, the right type of cotton to use, and the correct flow rate for the most effective removal all need to be tested and found.

Experiments have also been done to further test the capabilities of what MO serum can remove from water. The fragrance Eugenol was tested. This was unsuccessful as Eugenol is negatively charged. For MO to remove containments from water they must be positively charged. Tests have also been run to see its' effectiveness in removing fabric dye, specifically Red RIT dye. These tests are aimed at trying to determine if clothing/fabric could be coated in MO prior to dying to eliminate the bleeding of dye from clothes.

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