Drying And Extraction of Moringa Oleifera And Its Application In Wastewater Treatment

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Abstract. Conventional coagulants such as aluminum sulfate and ferric sulphate have proved to be harmful to human health as it may cause neurological diseases like Alzheimer and percentile dementia. These health concerns have led to the use of natural coagulants which are environmental friendly and not harmful to human health. In this study, Moringa Oleifera seeds are selected to study the preparation of the natural coagulant and further use it in dye wastewater treatment. The two important steps used in preparing the natural coagulants are drying of the raw materials and extraction of the protein content. Moringa Oleifera seeds were oven dried at 40°C, 50°C and 60°C and the drying data was recorded. The recorded data was then fitted into five thin layer kinetic models to obtain the most suitable drying temperature and its corresponding drying time. Using Microsoft Excel Solver 2011, values of (R²), (RMSE) and chi-square (X²) were calculated. Based on the highest value of (R²) and lowest value of (RMSE), Page kinetic model was selected. Moreover, for the selected Page kinetic model, values of (R²) and (X²) were also compared for all three temperatures and the temperature with the highest (R²) value and lowest chi-square value (X²) was selected as the most optimum drying temperature. The most optimum drying temperature for drying Moringa Oleifera seeds was obtained as 50°C as the seeds dried at this temperature holds the maximum protein content concentration which enhances its coagulation properties. Furthermore, the corresponding drying time of about 6.5 hours for seeds dried at 50°C was also taken as the most optimum drying time. In the next step, Moringa Oleifera seeds which have been dried at 50°C for 6.5 hours were used for protein extraction. Solid to solvent ratios of 0.5:1.00, 1:1.00, 2:1.00 and 5:100 (g of dried Moringa oleifera seeds: ml of distilled water) were used to extract the protein by using a domestic blender for 2 minutes. Using the Bradford method, the absorbance for each solid to solvent ratio solution was recorded and the protein content concentration was further calculated. The highest protein content concentration of 0.0017 ug/uL was obtained in the best solid to solvent ratio which is 5.0:100. Therefore, this solid to solvent ratio was used in the jar test. The jar test was based on three process parameters which includes coagulant dosage, pH and initial concentration of dye wastewater. The coagulant dosage of 300 mg/L, initial concentration of dye wastewater of 50 ppm and pH of 7 were selected as the most optimum process parameters for a maximum turbidity removal of 70.4% and a maximum color removal of 44.54% in the dye wastewater treatment with Moringa Oleifera seeds.
1. Introduction
Water pollution in the developing countries of the world, is considered as an alarming issue for the environmental depletion [1]. The wastewater discharged from the industrial, sewerage and agricultural sector is the main source that leads to this problem. Textile industries being one of the biggest contributors of industrial wastewater pollution are known to use synthetic and chemical dyes to dye and print the fabric cloth in various colors, however, a huge quantity of dye wastewater is produced during the ongoing dyeing and printing processes [2][3]. Dye wastewater produced through these processes contain many chemical constituents which if disposed in the lake or river can affect the marine life and cause aesthetic problems such as color change of the river water [3]. This dye wastewater can be treated by using proper treatment methods before its discharge [3]. The treatment processes are based on the characteristics of the dye wastewater as it can contain complex molecular structures which makes the dye wastewater treatment tough [4]. Some common techniques such as adsorption, electrochemical degradation, advance oxidation and also physico-chemical treatment are mainly used for removing the colored dyes from the wastewater. Among these removal processes, the most famous physico-chemical treatment includes the process of coagulation[3].

Coagulation is a very simple and efficient process in which the dissolved solid particles and negatively charged colloids are neutralized [3]. In this process of charge neutralization, all the tiny particles gather around forming a larger particle known as a floc. However, to remove the floc from the dye wastewater, sedimentation process is frequently used which helps in removing the color and reducing the turbidity to maximum. Commecial coagulation using chemical based coagulants such as ferric chloride, aluminum sulfate and ferric sulphate are very common today [3]. These chemical coagulants have various advantages and disadvantages, whereas, the most conventional aluminum sulfate contains many environmental and human health concerns such as neurological diseases like percentile dementia and Alzheimer’s disease [5]. Moreover, non-biodegradable sludge is also produced which can cause soil pollution, so a large amount of capital is required for this sludge treatment [5]. Due to these disadvantages and health concerns, researchers are mostly focusing more on natural coagulants to replace the conventional synthetic coagulants [6]. Natural coagulants can be animal based, microorganism based and plant based [7]. These natural coagulants are environmental friendly, non-toxic and also highly biodegradable [3]. They are cheaper than the conventional chemical coagulants as the most commonly used natural coagulants for wastewater treatment are easy to obtain locally [3]. These natural coagulants help in reducing turbidity in contaminated dye wastewater, and also helps in removing the suspended solids and other impurities efficiently. Furthermore, the sludge produced is also bio-degradable so it contributes in reducing the treatment cost [8]. Some common plant-based natural coagulants such as Moringa Oleifera (MO), Jatropha seeds, Hibiscus Sabdariffa and Nirmali seeds have been studied by the researchers over the last few years on a laboratory scale [3]. According to some researchers, there are some important steps involved in the preparation of these plant-based natural coagulants before being used for dye wastewater treatment. These steps include the preparation of the raw materials, its drying and also its protein extraction [3]. For the drying process, most of the researchers have dried the plant-based natural coagulant at different temperatures using a laboratory oven [9][10]. No optimization study has been conducted on the drying part yet, which is why this step is very important as this will influence the moisture content present in the coagulants which can further affect the protein concentration in plant-based natural coagulants [11]. Drying for preparation of natural coagulants involve the moisture transfer phenomena to reduce the moisture content in order to have a maximum protein content for coagulation to take place efficiently [12]. After oven drying, the next step involved in the preparation of natural coagulants is by blending a known amount of dried seeds with water to extract the protein. Previous researchers used several different solid to solvent ratios for this purpose. It is important to use a suitable quantity of coagulant seeds for efficient protein extraction [11]. However, not enough research is conducted on using natural coagulants for dye wastewater treatment [3].

For this study, Moringa Oleifera seeds are going to be used as natural coagulant to treat the dye wastewater. Moringa Oleifera plant has received much importance from the tropical regions in the world
and it is globally recognized for its nutritional and medical purposes [8]. This species belong to the family of single generic Moringaceae, the group *Moringa oleifera* which consists of 14 species having shrubs and trees [13]. The seeds of MO are rich in many bio-active compounds like lipids, carbohydrates, vitamins and proteins [14]. The proteins extracted from the seeds work mainly by setting up a coagulation mechanism which involves the charge neutralization method [13]. *Moringa Oleifera* seeds contain proteins which are soluble in water and have a greater tendency for minimizing the turbidity of the dye wastewater. The protein content acts as a natural cationic polyelectrolyte that can bind with a negatively charge colloid particle, present in the dye wastewater [3]. MO seeds also have a high moisture content and it is believed that this moisture content can influence the water-soluble protein content so it is necessary to remove the moisture content from the MO seeds, in order to obtain a maximum protein content so that its performance as a coagulant can be enhanced [10]. Therefore, after the oven drying of MO seeds for the removal of moisture content, the seeds dried at a temperature with maximum moisture content removal are used for protein extraction. Hence, the solid to solvent ratio with the highest protein content was further used for the jar test. The jar test for this coagulation experiment depends on three process parameters, which are coagulant dosage, pH and initial concentration of dye wastewater. Jar test was performed three times, each time a different process parameter was varied keeping the other two process parameters constant and results for turbidity removal and color removal were also recorded. By evaluating the results, the most optimum process parameters with maximum turbidity removal and maximum color removal efficiency were obtained.

2. Materials and Methods

2.1. Synthetic Dye wastewater preparation

The Congo red (CR) dye was purchased from Sigma Aldrich. 1g of Congo red dye (molecular weight of 696.6 g/mol) was dissolved completely in 1 liter of distilled water to form a stock solution having a concentration of 1000 ppm to be used in the jar test for dye wastewater treatment [3]. This solution was used as a stock solution for the coagulation experiment, however, by diluting this stock solution with different quantities of distilled water, different concentrations of dye wastewater were achieved. To alter the pH of the Congo red dye wastewater to the desired value, 1.0 M of NaOH solution or 1.0 M of HCL solution were also added [3]. Both NaOH and HCL solutions were from KSFE Malaysia.

2.2. Coagulant’s preparation

*Moringa Oleifera* pods were purchased from Sentul Market, a wholesale vegetable market located in Kuala Lumpur. Firstly, the MO seeds were extracted from its pods. The initial weight of the MO seeds was then recorded using a Metra precision weighing machine. Next, the MO seeds were placed in an oven drier to remove the moisture content. The seeds were oven dried till the final weight of the MO seeds gets constant. After drying, a household grinder machine was used to grind the dried MO seeds into powdered form.

2.3. Oven Drying of *Moringa Oleifera* seeds

Three samples of 5g of MO seeds were weighed using a Metra precision weighing scale. The seeds were then uniformly placed on a tray to be oven dried in an oven drying equipment present in the laboratory [15]. The experiment was conducted at three different temperatures such as 40ºC, 50ºC and 60ºC [9]. The oven drying process was monitored by weighing the samples every 30 minutes using the same weighing machine which has a range from 0.1g-5000g until the weight of the samples gets constant [16]. After oven drying, the final weight of the samples at 3 different temperatures were also recorded. This experiment was repeated to reconfirm the final weights achieved in the first experiment. Finally, the data was then used for further calculations.

To calculate the % of the removed moisture content in the sample, the equation below can be used [16].
Moisture content % = \frac{\text{weight of moisture (g)}}{\text{weight of sample (g)}} \times 100 \quad (1)

The drying data was then fitted in the five thin layer drying kinetic models shown below[17]:

| No. | Kinetic Models          | Equations               |
|-----|-------------------------|-------------------------|
| 1.  | Lewis Model             | \( RX = e^{-kt} \)      |
| 2.  | Page Model              | \( RX = e^{-kt^n} \)    |
| 3.  | Henderson and Pabis Model | \( RX = ae^{-kt} \)   |
| 4.  | Two-Term Model          | \( RX = ae^{-kt}t + be^{-kt}t \) |
| 5.  | Logarithm Model         | \( RX = ae^{-kt} + c \) |

Where[17]:

\( t = \) drying time (h)

\( k, k_0, k_1: \) drying constants (h\(^{-1}\))

\( a, b, c, n: \) model coefficients

Using Microsoft Excel SOLVER 2011, model coefficients, empirical constants, values for coefficient of determination (R\(^2\)), chi-square values and root mean square error (RMSE) were calculated [17][9]. Kinetic model selection was based on the magnitude of the coefficient of determination (R\(^2\)) [12]. The model with the greatest value for coefficient of determination (R\(^2\)) and smallest value of RMSE was selected as the best kinetic model for all three temperatures[17]. After selecting the best kinetic model, three graphs were plotted for that kinetic model at three different temperatures which are 40ºC, 50 ºC and 60 ºC. The graphs contained drying temperature (ºC) on y axis and drying time (h) on x axis [9]. Comparing the chi-square values (x\(^2\)) and (R\(^2\)) values for all three temperatures of the selected kinetic model, the optimum drying temperature was selected. The temperature with the highest (R\(^2\)) value and lowest chi-square values (x\(^2\)) was chosen as the optimum drying temperature and with its corresponding drying time [9].

2.4. Protein Extraction

Powdered MO seeds having four different amounts such as 0.5g, 1.0g, 2.0g and 5.0g were mixed with 100 mL of distilled water each and were blended in a household blender for two minutes. The mixtures were then filtered using Whatman 1001-125 Grade 1 qualitative filter papers in order to remove the seed residue impurities. Bradford method (1976) was then used to determine the protein content concentrations in order to select the best solid to solvent ratio of MO seeds in distilled water [11][18] [19]. 5ml of bradford reagent was also transferred into each filtrate sample and then a known amount of each sample was put inside the quartz cuvettes which was then placed in a UV-spectrophotometer (HALO RB-10-5110026), set to wavelength of 595nm. This wavelength was used to measure the protein absorbance value [19].
Absorbance will be calculated using the regression line equation below [19]:

\[
\text{Protein concentration (μg/μl)} = \frac{\text{Absorbance} \times \text{Dilution factor} \times \text{Volume of diluted protein used for the assay(μl)}}{\text{Slope (μg/μl)} \times \text{Volume of diluted protein used for the assay(μl)}}
\] (2)

2.5. Jar Test

MO seeds performance as a natural coagulant was evaluated using the jar test. Six beakers with different CR dye wastewater concentrations and MO seed solutions were placed at the Paddle Jar test apparatus for 2 minutes at the speed of 150rpm [20]. The speed was then reduced to 50rpm for 25 minutes for perfect mixing to happen. The paddler was then stopped, and the beakers were put to rest for 1 hour for the sedimentation process to occur and allow the floc to settle down. After that, the solutions were then filtered using the same Whatman 1001-125 Grade 1 filter papers into empty beakers.

UV-spectrophotometer (HALO RB-10-5110026), set to a wavelength of 500 nm, was further used to measure the absorbance values for the color removal in the mixtures, before and after the coagulation process.

Color removal efficiency will also be calculated using the following equation highlighted below [3].

\[
\text{Color removal %} = \frac{\text{absorbance value before treatment} - \text{absorbance value after treatment}}{\text{absorbance value before treatment}} \times 100
\] (3)

To calculate the turbidity removal, each untreated solution was first transferred to a turbidity sample bottle using a micro pipette and then each sample bottle was placed in the EUTECH Instrument TN-100 sample chamber to record the initial turbidity of all the six different concentrations. After recording the turbidity of untreated samples, each solution treated with MO seeds was also transferred to the sample bottle using the same micro pipette and then one by one each sample bottle was placed in the same sample chamber to get the final turbidity values of all six different concentrations. After getting the initial and final turbidity values, the following equation will be used to calculate the turbidity removal percentage [25].

\[
\text{Turbidity removal %} = \frac{(\text{RTc})-(\text{RT})}{\text{RTc}} \times 100
\] (4)

The jar test for this coagulation experiment depends on three process parameters, which were coagulant dosage, pH and initial concentration of dye wastewater. So the same jar test was performed three times, each time a different process parameter was varied keeping the other two process parameters constant and results for color removal and turbidity removal were recorded. By evaluating the results, the most optimum process parameters with maximum turbidity removal and maximum color removal efficiency were obtained.

3. Results and Discussion

For the coagulation process, in which CR dye wastewater was to be treated with MO seeds, oven drying was performed for the best optimum drying temperature followed by protein extraction process using Bradford method.

3.1. Selection of the optimum drying temperature and optimum drying time

Table 3.1. shows the drying characteristics of MO seeds samples when oven dried at 40°C, 50°C and 60°C.
Table 3.1. Drying Characteristics

| Samples at °C | Initial Weight (g) | Final Weight (g) | Drying time (hrs) | Moisture content removal % |
|---------------|--------------------|------------------|-------------------|---------------------------|
| 40°C          | 5.0271             | 2.1807           | 8                 | 56.62%                    |
| 50°C          | 5.0655             | 1.6324           | 6.5               | 67.8%                     |
| 60°C          | 5.0293             | 1.9747           | 3.5               | 60.73 %                   |

Based on Table 3.1, it was seen that for 60°C the moisture content removal was 60.73 % which was less than the moisture content removal at 50°C, this means that at a high temperature, the heat energy is too high which causes the outer surface of MO seeds to dry out quickly than the inner core. With this, the rigidity of the outer surface of MO seeds increases, restricting the moisture content to be removed from the seeds. This phenomena is known as “case hardening”[9]. Moreover, at 40°C, the moisture content removal % was also found to be 56.62% which was also less than the moisture content removal % at 50°C, this means that the heat energy supplied to the MO seeds at 40°C was not powerful enough to remove the moisture content rapidly[9]. This could also lead to the spoilage of proteins inside the seeds due to some deteriorative reactions [9]. Therefore, maximum moisture content removal % was found as 67.8 % for 50°C. Figure 3.1 shows the moisture content ratio versus drying time for temperature of 40°C, 50°C and 60°C.

![Figure 3.1 Drying curves for all temperatures (Moisture content Vs Drying time)](image)

All the drying kinetic data was then fitted into 5 thin layer drying kinetic models, and values for coefficient of determination ($R^2$), chi-square values and root mean square error (RMSE) were calculated.
Table 3.2. Drying kinetic models and its corresponding results

| Drying Kinetic Models | Parameters at 40 °C | Parameters at 50 °C | Parameters at 60 °C |
|-----------------------|---------------------|---------------------|---------------------|
| Lewis Model = \(\exp(-kt)\) | \(K = 0.8987\) | \(K = 0.8987\) | \(K = 1.6753\) |
| \(\text{RMSE} = 0.03348\) | \(\text{RMSE} = 0.06068\) | \(\text{RMSE} = 0.04350\) | \(\text{RMSE} = 0.05345\) |
| \(r^2 = 0.9913\) | \(r^2 = 0.4791\) | \(r^2 = 0.9881\) | \(r^2 = 0.9881\) |
| \(\text{Chi squared value} = 11.6247\) | \(\text{Chi squared value} = 10.2373\) | \(\text{Chi squared value} = 5.3789\) | \(\text{Chi squared value} = 5.3789\) |
| Page Model = \(\exp(-kt^n)\) | \(K = 0.8987\) | \(K = 0.8987\) | \(K = 1.6753\) |
| \(n = 0.807616\) | \(n = 0.735978\) | \(n = 0.89174\) | \(n = 0.89174\) |
| \(\text{RMSE} = 0.02039\) | \(\text{RMSE} = 0.02551\) | \(\text{RMSE} = 0.03449\) | \(\text{RMSE} = 0.03449\) |
| \(r^2 = 0.9932\) | \(r^2 = 0.9928\) | \(r^2 = 0.9881\) | \(r^2 = 0.9881\) |
| \(\text{Chi squared value} = 11.6247\) | \(\text{Chi squared value} = 10.2373\) | \(\text{Chi squared value} = 5.3789\) | \(\text{Chi squared value} = 5.3789\) |
| Henderson and Pabis Model = \(a\exp(-kt)\) | \(K = 0.9017\) | \(K = 1.1951\) | \(K = 1.6753\) |
| \(a = 1.003074\) | \(a = 0.735978\) | \(a = 0.89174\) | \(a = 0.89174\) |
| \(\text{RMSE} = 0.03371\) | \(\text{RMSE} = 0.02551\) | \(\text{RMSE} = 0.03449\) | \(\text{RMSE} = 0.03449\) |
| \(r^2 = 0.8832\) | \(r^2 = 0.9928\) | \(r^2 = 0.9881\) | \(r^2 = 0.9881\) |
| \(\text{Chi squared value} = 11.6247\) | \(\text{Chi squared value} = 10.2373\) | \(\text{Chi squared value} = 5.3789\) | \(\text{Chi squared value} = 5.3789\) |
| Modified Page Model = \(\exp(kt^n)\) | \(K = 0.8987\) | \(K = 1.1825\) | \(K = 1\) |
| \(n = 1\) | \(n = 1\) | \(n = 1\) | \(n = 1\) |
| \(\text{RMSE} = 0.03348\) | \(\text{RMSE} = 0.05471\) | \(\text{RMSE} = 0.01142\) | \(\text{RMSE} = 0.01142\) |
| \(r^2 = 0.9913\) | \(r^2 = 0.9743\) | \(r^2 = 0.9881\) | \(r^2 = 0.9881\) |
| \(\text{Chi squared value} = 11.6247\) | \(\text{Chi squared value} = 10.2373\) | \(\text{Chi squared value} = 5.3789\) | \(\text{Chi squared value} = 5.3789\) |
| Two Term model = \(A\exp(-k_1t) + B\exp(-k_2t)\) | \(K1 = 0.8987\) | \(K1 = 1.1825\) | \(K1 = 53687\) |
| \(K2 = 53687092\) | \(K2 = 53687092\) | \(K2 = 1.675322\) | \(K2 = 1.675322\) |
| \(a = 0.38046566\) | \(a = 0.26574698\) | \(a = 0\) | \(a = 0\) |
| \(b = 0.61953434\) | \(b = 0.73425302\) | \(b = 0.98577286\) | \(b = 0.98577286\) |
| \(\text{RMSE} = 0.11255\) | \(\text{RMSE} = 0.11768\) | \(\text{RMSE} = 0.01142\) | \(\text{RMSE} = 0.01142\) |
| \(r^2 = 0.8899\) | \(r^2 = 0.9743\) | \(r^2 = 0.9881\) | \(r^2 = 0.9881\) |
| \(\text{Chi squared value} = 11.6247\) | \(\text{Chi squared value} = 10.2373\) | \(\text{Chi squared value} = 5.3789\) | \(\text{Chi squared value} = 5.3789\) |

Based on the above values, it can be concluded that the highest value for \(R^2\) among all the kinetic models can be found in the Page model. Moreover, RMSE values for the page model were also the smallest among all the other drying kinetic models. Therefore, Page model was selected as the most
optimum drying kinetic model. Looking at the above values for Page model, first of all the values of $R^2$, it can be seen that $R^2$ value for 60°C was smaller than the $R^2$ values for the other two temperatures, and since our interest was to obtain the temperature with the highest $R^2$ value, 60°C was rejected. Moving on, since the $R^2$ values of 40°C and 50°C were close, as $R^2$ for 50°C was 0.993 and for 40°C was 0.9932, $\chi^2$ values were compared for 40°C and 50°C. The $\chi^2$ values were compared and as per previous researches the best optimum drying temperature should have the highest $R^2$ and lowest $\chi^2$ values [9], so by comparing the $\chi^2$ values for 40°C and 50°C, it can be seen that 50°C had a lower $\chi^2$ value of 10.24 which was smaller than $\chi^2$ value of 40°C which was 11.6. Hence, 50°C was selected as the most optimum drying temperature for the drying *Moringa Oleifera* seeds in an oven drier. Moreover, the corresponding drying time for the most optimum drying temperature of 50°C took 6.5 hours for the final weight of MO seeds to get constant, hence this time was as selected as the most optimum drying time. These results are also similar to a previous research conducted on the drying kinetics of MO seeds [9].

3.2. Determination of best solid to solvent ratio for extraction

Based on the above Figure 3.2, it can be concluded that the maximum protein content concentration was achieved as $1.70 \times 10^{-3}$ ug/uL for the solid to solvent ratio of 5g *Moringa Oleifera* seeds mixed with 100 ml of distilled water.

![Figure 3.2 Protein Concentration](image)

Therefore, these results highlight that a higher weightage of MO seeds in the MO seed solution will have more water soluble proteins which can further enhance its coagulation properties and help to perform an effective coagulation experiment.

3.3. Jar Test Experiment

The jar test was based on three process parameters which were coagulant dosage, pH and initial concentration of dye wastewater. Each parameter was varied keeping the other two parameters constant.

3.3.1 Effects of initial concentrations of CR dye wastewater on turbidity removal and color removal
In this experiment the constant parameter was pH of CR dye wastewater and coagulant dosage of MO seeds (mg/L), while the variable parameter was initial concentration of Congo red dye wastewater (ppm).

Based on Figure 3.3, it can be concluded that 50 ppm of CR dye wastewater at pH 7 when treated with 150 mg/L MO seed solution was able to achieve the highest color removal efficiency of 22.54% which was the greatest among all other color removal efficiency percentages. Moreover, for 50 ppm concentration, the initial turbidity was recorded as 0.76 NTU and the final turbidity was recorded as 0.46 NTU. Thus, it was also able to achieve the highest turbidity removal percentage of 41.03% which was also the greatest among all other turbidity removal percentages.

Figure 3.3. Turbidity removal and color removal against the initial concentration of CR dye wastewater

The initial concentration of CR dye wastewater is known to affect the color removal and turbidity removal on treatment with MO seeds in the coagulation process. The turbidity removal percentages and color removal percentages can be calculated using equation (3) and (4). From the above figure, it is seen that with increasing initial concentration of CR dye wastewater, the turbidity removal and color removal percentages decrease and the maximum turbidity removal of 41.03% and maximum color removal of 24.54% were only achieved at the lowest initial concentration taken for the jar test which was 50 ppm. Moreover, the pH was fixed to 7 for all the initial CR dye wastewater solutions but on treatment with MO seeds, the pH was seen to reduce a little as shown in Table 3.3.

Table 3.3. Before and after values for concentration and pH of CR dye wastewater

| Initial concentration of wastewater (ppm) | pH of CR dye wastewater before treatment | pH of CR dye wastewater after treatment |
|------------------------------------------|-----------------------------------------|----------------------------------------|
| 50                                       | 7                                       | 6.75                                   |
| 100                                      | 7                                       | 6.91                                   |
| 150                                      | 7                                       | 6.96                                   |
| 200                                      | 7                                       | 6.80                                   |
It also highlights that for 50 ppm CR dye wastewater solution, the pH dropped maximum to 6.75 than all other concentrations for CR dye wastewater. This trend concludes that MO seeds does not affect the pH of the solutions. This trend is very similar to those of previous studies and it is known that with increasing initial concentration keeping the pH of CR dye wastewater and the MO coagulant dosage constant, the coagulation process becomes less effective [21].

3.3.2. Effects of coagulant dosage on turbidity removal and color removal

In this experiment, the constant parameter was pH of CR dye wastewater and initial concentration of CR dye wastewater, and the variable parameter was coagulant dosage of MO seeds. Based on Figure 3.4, it can be concluded that when 300 mg/L of MO seeds solution was added to 50 ppm of CR dye wastewater at pH 7, the highest color removal efficiency of 49.7 % was achieved which was the greatest among all other color removal efficiency percentages. Moreover, at same process parameters, the initial turbidity was recorded as 0.29 NTU and the final turbidity was recorded as 0.08 NTU, hence, a maximum turbidity removal % of 72.4 % was also achieved which was the greatest among all other turbidity removal percentages. The calculations for color removal efficiency and turbidity removal were performed using equation 3 and equation 4 to see which MO coagulant dosage was the most optimum.

![Figure 3.4. Turbidity removal and color removal against the coagulant dosage.](image-url)

Coagulant dosage is a very important process parameter that is necessary to be studied as it is known to directly affect the coagulation mechanism, hence, affecting the turbidity removal and color removal percentages of CR dye wastewater on treatment with MO seeds [22]. It is believed that a higher coagulant dosage will allow a greater reduction in final turbidity and final absorbance values which will further help in maximizing the color removal percentage and turbidity removal percentages. By analyzing the above collected data, varying coagulant dosage and keeping the pH and initial concentration of dye wastewater constant, a maximum turbidity removal of 72.4 % was achieved.
followed by a high color removal efficiency percentage of 49.7 % which was also achieved on using 300 mg/L of MO seeds for CR dye wastewater treatment. The results match the previous studies as MO seeds are rich in many bio-active compounds like lipids, carbohydrates, vitamins and proteins [14]. The proteins extracted from the MO seeds operate mainly by setting up a coagulation mechanism which involves the charge neutralization method [13]. MO seeds contain proteins which are soluble in water and have a greater tendency for minimizing the turbidity of the dye wastewater. These proteins acts as natural cationic polyelectrolytes that can bind with negatively charge colloid particles, present in the dye wastewater and this way it acts as an effective natural coagulant to be used for the treatment of CR dye wastewater, hence, more coagulant dosage will allow more proteins to bind with negatively charged particles present in dye wastewater minimizing its turbidity and also increasing the color removal efficiency [3]. Moreover, the pH was fixed at pH 7 for all the 50 ppm CR dye wastewater solutions but on treatment with MO seeds, the pH of all 50 ppm solutions was seen to reduce a little. It also highlights that for 300 mg/L coagulant dosage of MO seeds, the pH dropped to 6.39. This concludes that MO seeds doesn’t really affect the pH of the solutions.

Table 3.4. Results for different MO coagulant dosage solutions before and after treatment

| MO seeds Coagulant dosages (mg/L) | pH of CR dye wastewater before treatment | pH of CR dye wastewater after treatment |
|-----------------------------------|-----------------------------------------|----------------------------------------|
| 50                                | 7                                       | 6.27                                   |
| 100                               | 7                                       | 6.51                                   |
| 150                               | 7                                       | 6.61                                   |
| 200                               | 7                                       | 6.61                                   |
| 250                               | 7                                       | 6.52                                   |
| 300                               | 7                                       | 6.39                                   |

3.3.3. Effects of pH on turbidity removal and color removal
In this experiment, the constant parameter was coagulant dosage of MO seeds and initial concentration of Congo red dye wastewater (ppm) and the variable parameter was pH of CR dye wastewater solution. Based on Figure 3.5 it can be concluded that 50 ppm of CR dye wastewater at pH 7 when treated with 300 mg/L MO seed solution was able to achieve the highest color removal efficiency of 44.53 % which was the greatest among all other color removal efficiency percentages.

Moreover, at this pH of 7 with the same process parameters, the initial turbidity was recorded as 0.27 NTU and the final turbidity was recorded as 0.08NTU, hence, a maximum turbidity removal of 70.4% was also achieved which was also the greatest among all other turbidity removal percentages. Hence, the coagulant dosage of 300 mg/L was selected as the most optimum Moringa Oleifera seeds coagulant dosage in the coagulation process of CR dye wastewater solutions as it gives the highest turbidity removal and color removal efficiency percentages. The calculation for color removal efficiency and turbidity removal were performed using equation 3 and equation 4 to see which pH values of the CR dye wastewater solution was the most optimum.

Another important process parameter which is to be studied is the pH of the CR dye wastewater. It is known that in the process of coagulation, pH is seen to affect the surface charges of the coagulants and also affects the stability of suspension [23].
By analyzing the data in Table 3.5 for before and after coagulation results for pH, it can be concluded that solutions which were initially at pH 3 and 4, after treatment with MO seeds their pH slightly increased but for the solutions having initial pH of 6, 7, 8 and 10 when treated with MO seeds, their final pH slightly decreased.

Moreover, when analyzing the results in Figure 3.5, where the variable process parameter was pH and initial concentration of dye wastewater was kept constant at 50 ppm and coagulant dosage was kept constant at 300 mg/L, it was seen that at acidic pH of 3 and 4, the turbidity removal percentages were 29.1 % and 43.5 %, however, the color removal percentages were 4.5 % and 1.22 %. These results obtained were not effective at all which is why alkaline pH of 8 and 10 were also analyzed and the color removal percentages obtained at these pH were 9.1 % and 12.7 %. Furthermore, the turbidity removal percentages obtained for these pH values were calculated as 30 % and 23.91 %. Therefore, the pH of 6 and 7 were analyzed and at pH 6, the turbidity removal and color removal percentages were high as compared to the acidic and alkaline pH values but the maximum color removal percentage of 44.53% and maximum turbidity removal percentage of 70.4 % were obtained at pH 7. Hence, 7 was taken as the most optimum pH for the coagulation process of CR dye wastewater with MO seeds, and these results
also match the results from previous studies [23]. This concludes that a neutral pH of CR dye wastewater can allow an efficient coagulation process to take place on treatment with Moringa Oleifera seeds, as the amino acids present in the seeds consists of positively charged particles which tends to act as cationic polyelectrolyte[21]. When the CR dye particles which were initially negatively charged were brought to pH 7, they were still able to perform a natural coagulation process by allowing the cationic polyelectrolytes to bind on the CR dye surface via hydrogen or an ionic bonding. This eventually neutralized the surface charges explaining the particle destabilization phenomena by charge neutralization method [21].

4. Conclusion
Several research studies on natural coagulants for treatment of CR dye wastewater were evaluated and it was proved that the plant-based natural coagulants are much more effective than conventional coagulants. Among the plant-based natural coagulants, Moringa Oleifera seeds show a greater coagulation efficiency when compared with other plant based natural coagulants[24]. In this study, MO seeds were subjected to hot air drying at 40°C, 50°C and 60°C for 8 hours, 6.5 hours and 3.5 hours. Evaluating the drying results, the temperature of 50°C allowed the maximum removal of moisture content from MO seeds of about 67.8 %. The drying kinetic data were then fitted in the kinetic models and page model was selected as the best kinetic model as it had the highest $R^2$ value and lowest RMSE value. Then by comparing the $R^2$ and $X^2$ values for Page model, temperature of 50°C was selected as the most optimum drying temperature with its corresponding time of 6.5 hours as the most optimum drying time. The seeds dried at the optimum temperature were then subjected to the protein content experiment and by using the Bradford method, the best solid to solvent ratio was selected. 5g of MO seeds in 100 ml of distilled water was selected as the best solid to solvent ratio for the coagulation experiment because of the high protein content present in this solid to solvent ratio which can enhance the coagulation mechanism in treatment of CR dye wastewater. Moreover, jar test experiment was performed which revolved around three basic process parameters that are coagulant dosage of MO seeds, pH and initial concentration of dye wastewater. For each jar test experiment, a single process parameter was varied keeping the other two constant. Values for turbidity and absorbance for CR dye wastewater solutions were first measured before treatment with MO seeds and then after treatment with MO seeds. These values were used to calculate the color removal efficiency and turbidity removal percentages. It can be concluded that the most optimum initial concentration of dye wastewater was obtained as 50 ppm, the most optimum pH was at 7 and the most optimum coagulant dosage of MO seeds for the coagulation experiment was proved to be 300 mg/L as these optimum process parameters allowed the maximum turbidity removal of 70.4 % and a maximum color removal of 44.54%. from CR dye wastewater and performed an efficient and environmental friendly coagulation experiment, whereas, using chemical coagulants would have raised a lot of environmental and human health concerns.

5. References

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