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Use of Plant-Based Coagulants Sorghum Bicolor and Trifolium Repens as Future Alternatives for Textile Wastewater Treatment Based on Computational Model

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Abstract: Sorghum bicolor and Trifolium repens extracts as plant coagulants can be used for wastewater treatment instead of chemical coagulants. This study investigates the effects of pH and coagulant dose on COD and turbidity reduction by natural coagulants. In the optimum treatment conditions of 100 mg/L coagulant dose and pH 7, high turbidity (81.34%), and COD reduction (62%) (compared with alum and FeCl3) were obtained. The percentage of COD reduction is higher in Trifolium repens than the other plant coagulant, and the percentage of turbidity reduction is higher in Sorghum bicolor. FT-IR spectra were performed on the plant extracts to identify the existing factors in active extracts. Also, outlet COD and turbidity values can be predicted and calculated according to computational equation in similar treatment systems. It should be mentioned that the only applied solvent for extracting the natural coagulant seeds was distilled water, and none of the other solvents such as NaCl and NaOH were used. According to the results, the characteristics of the treated effluents by the plant extracts make them appropriate alternatives for textile wastewater treatment.

Keywords: plant-based coagulant, COD reduction, turbidity reduction, Sorghum bicolor, Trifolium repens, computational model

1. Introduction

Colored effluents destroy aquatic lives because of existing non-biodegradable compounds which lead to serious threats for human and environment (Wang et al., 2021). Dye and water are used excessively in different processes of the factory (Nigam et al., 1996). Biodegradation of dyes is a complex process because of their complicated molecular structure (Sarwan et al., 2012). Different chemical (Sanghi et al., 2006; Yin, 2010), physical (Gosavi & Sharma, 2014), and biological treatment methods (Popli & Patel, 2015) have been applied for textile wastewater (Gharbani & Mehrizad, 2022; Wang et al., 2021). Most of the chemical treatment methods are not economic and produce large volumes of toxic residual (Gautam & Saini, 2020). Moreover, using chemical coagulants have many disadvantages including high volume of sludge, bad effect on human health, increasing or decreasing pH of the wastewater after treatment (Sanghi et al., 2006; Yin, 2010). Plant coagulants do not have these problems; they are cost-effective and environment friendly (Gautam & Saini, 2020), but the acceptance of conventional coagulants is still more than natural coagulants (Balbinoti et al., 2023). The most important components of the natural coagulants are proteins and polysaccharides (Shamsnejati et al., 2015). Polysaccharides can perform polyelectrolyte's role when released in the environment with negative and positive charges. (Crouzier et al., 2010). Coagulation-flocculation mechanisms can occur by (a) compressing the solution layer (which presence of salts can destabilize the particles) (b) forming flocs by suspended particles (c) sorption of particulates with oppositely charged (d) adsorption and particles link bridge (Yin, 2010). *Moringa oleifera, nirmali* seeds, *tannin,* and cactus are common plant coagulants which have been applied for water and wastewater treatment in different research (Abidin et al., 2013; Yin, 2010). Sorghum bicolor is grown in Africa and the south of Asia; also, it has long existed in Iran (Dial, 2012; Motaleb, 2011). This grass is grown for food, feed, fiber, and fuel. Sorghum is one of the most important crops after rice.

50% of the *sorghum* endosperm is composed of protein (Shull et al., 1991). Nutritional values per 100 g of sorghum consist of 74.63 g carbohydrates and 11.30 g protein. (Dial, 2012; Motaleb, 2011). *Trifolium* is native to Europe and central Asia and is one of the most common types of clover. About 54 species of Trifolium have been identified in Iran. Per 100 g of *Trifolium* consists of 7.2 g protein. One of the outstanding points and novelty of the manuscript is that the optimization process during preparing the

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plant coagulants was carried out without adding any chemical substances or salt, and also, so far, there are no reports on applying the seeds extract of *Sorghum bicolor* and *Trifolium repens* as natural coagulants for wastewater treatment.

2. Research Methodology

2.1. Sorghum bicolor and Trifolium repens seeds

The Sorghum bicolor plant was collected from its farmland in Gilan province, and the seeds were separated. Also, the seeds of Trifolium repens were bought from a local market. The seeds were distinguished for protein and carbohydrate content, moisture, and ashes. The amount of protein in the seeds was obtained by Kjeldahl nitrogen analysis (protein = N(%) × 6.25) according to the Standard Methods (American Water Works Association and Water Environment Federation, 2005). Moisture content was determined by drying the seeds in oven (Memmert Germany) at 105ºC and using the weight of dried sample to calculate as follows (Horwitz, 1975):

$$\text{Moisture}\ (\%) = \frac{(B - A) \times 100}{W} \quad (1)$$

A = Weight of dried sample and crucible
B = Weight of wet sample and crucible
W = Weight of wet sample

The amount of ash was obtained at 550ºC for 3 h, and it was calculated as follows (Camacho et al., 2017; Horwitz, 1975):

$$\text{Ash}(\%) = \frac{(B - A) \times 100}{W} \quad (2)$$

A = Weight of crucible
B = Weight of ash and crucible
W = Weight of wet sample

Fat was obtained by the method of Bligh and Dyer (Camacho et al., 2017). The amount of fat and fiber was zero in both samples, and carbohydrate content was calculated as follows (Horwitz, 1975):

$$NFE = 100 - (moisture + ash + fat + protein) \quad (3)$$

$$Carbohydrate = NFE + Fiber \quad (4)$$

Also, the zeta potentials of the samples were determined. The tests were performed by using 0.01 molar potassium nitrate solution (KNO3) as electrolyte and 0. 1 molar NaOH and hydrochloric acid solutions as pH controlling factors. 12 beakers containing 50 mL of the solution (KNO3) were prepared, and after pH adjusting, 0.2 g of the plant seed integral powder was added to each beaker. Then, the samples were placed on the shaker (Model Rotamix, Behdad Co., Iran) for 24 h, and the final pH was measured (Gulicovski et al., 2008).

2.2. The seed extraction and experimental design

The stages of preparing plant extract according to the time period are shown in Table 1. Dry seeds were stored and packed in the plastic bags at room temperature until use for extraction. The seeds were cleaned, soaked in water, and shaken well through shaker (Model Rotamix, Behdad Co., Iran) for 5 h. The period of time for swelling the plant seeds in water to extract the mucilage can even be 1 h, it depends on the seeds' type (Beltrán-Heredia et al., 2009; Beltrán-Heredia et al., 2011; Razavi et al., 2009). Then, the seeds were separated from the mixture of water and plant extract via a 500-micron filter. The last stage was obtaining the solid extract to comparison with chemical coagulants which are in solid phase. So, the existed water in the extract was evaporated with a temperature of 40°C (Feng et al., 2011) by hot plate stirrer (Model HP 100, Korea), and eventually, the plant coagulants were obtained. Afterward, 5 doses of 50, 100, 200, 500, and 1000 mg/L of chemical and plant coagulants were prepared.

Table 1
Stages of preparing plant extract

Stages	Time period
Packed dry seed maintenance	Up to 2 weeks
Soaking and shaking the seeds in water	1–5 h
Separating the seeds from the plant extract	1/2 h
Solvent Removing	3–5 h

Table 2
Characteristics of the real wastewater

Parameter	pH	TDS[1] (mg/L)	EC[2] (mS)	Turbidity (NTU)	COD[3] (mg/L)
Value	7.02	1677	1.701	36.89	575

[1]TDS: Total Dissolved Solids [2]EC: Electrical Conductivity [3]COD: Chemical Oxygen Demand.

In this research, the coagulants were used to treat real wastewater, and the jar tests were conducted on the industrial samples collected from Gilan spinning and weaving factory. The samples were transferred to the laboratory in plastic containers, stored at 4 °C, and analyzed within two days. The wastewater consisted of dyes (Basic Yellow 28, Basic Blue 41, and Basic Red 46) and the other additives such as salt, acetic acid, dispersant, and basic softener. The characteristics of the real wastewater are brought in Table 2.

To obtain the optimum wastewater pH for achieving the best treatment results, at first, the specified doses of the coagulants were added to the wastewaters with pH 3, 7, and 10. 1.0 N NaOH or 1.0 N HCL and pH meter were applied for the solution pH adjustment (Model AL 15, Aqualytic). After preparing 500 mL of the real wastewater with the optimum pH in five 500 mL beakers, 5 doses of coagulants (50, 100, 200, 500, and 1000 mg/L) were added to five beakers, and the jar test was started (Model JTR 90, ZAG CHEMIE Co., Iran). The samples were stirred for 10 min at 120 rpm and for 30 min at 30 rpm; then, the wastewaters were settled in 30 min. The jar test processes were repeated for treating the wastewater samples by four coagulants separately; two types of plant coagulants and two types of chemical coagulants including Alum and FeCl3 (Merck, Germany). It means that same as the plant coagulants, the treatment processes were performed for 5 doses (50, 100, 200, 500, and 1000 mg/L) of chemical coagulants to compare the results of COD and turbidity reduction by plant and chemical coagulants. COD reduction was determined by closed reflux, colorimetric method (Model AL 125, Aqualytic

Co., Germany), according to the standard methods (Federation & Association, 2005), and turbidity reduction was determined by turbidity meter (HANNA G-104 Co, Portugal). The COD reduction percentage was calculated as follows:

$$\text{COD reduction}(\%) = \frac{(COD_0 - COD)}{COD_0} \times 100 \qquad (5)$$

COD_0 = The value before treatment COD = The value after treatment

2.3. Computational modeling and analysis of the data

Data of the plant coagulants were organized using Curve Expert Professional software (version 1.6.5) to achieve a standard equation for calculating and predicting outlet COD and turbidity values in similar treatment systems.

3. Results and Discussion

3.1. Chemical characterization of *Sorghum bicolor* and *Trifolium repens* seeds

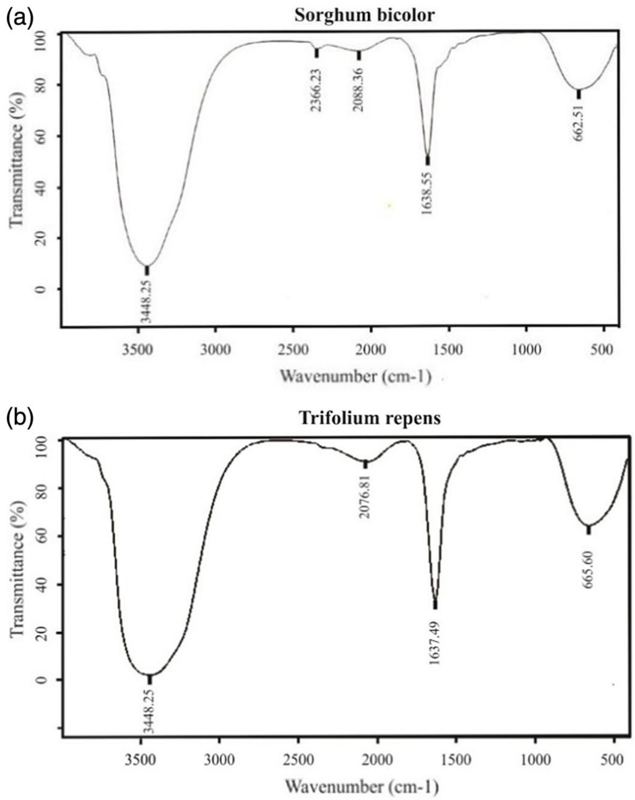
Table 3 presents the characterization of the *Sorghum bicolor* and *Trifolium repens* integral powder. According to the table, the amounts of protein and especially carbohydrates are more than the other constituent compounds in the seeds. These compounds play important roles in coagulation process. Also, there is not any oil in the integral powder of the seeds. The oil may disturb the treatment process as a by-product (Camacho et al., 2017). Moringa oleifera (MO) seeds with very low oil as a coagulant reached acceptable results of removing cyanobacteria from water body based on the research report (Camacho et al., 2017). The obtained plant powder contains both coagulating active agents and plant tissues (including oil, cellulose, etc.) (Yin, 2010).

To identify the existing factors in plant extracts which cause aggregating the particles in the wastewater and forming flocs, FT-IR spectra were performed on the plant extracts. Figure 1 shows FT-IR spectra of Sorghum bicolor and Trifolium repens extracts. According to Figure 1 (a (Sorghum bicolor)), regions 3448 and 1638 cm^{-1} are related to amide groups of protein structure (Yu, 2008); also, region 1638 represents the presence of steric and non-steric pectin compounds in the extract (Chylińska et al., 2016). Stretching vibration bands are due to C-H bonds of CH2 compounds which present in polysaccharides, in regions 2366 and 2088 cm^{-1} (Dong et al., 2016). Absorption band in region 662 cm^{-1} is considered as N = H functional group (Chylińska et al., 2016; Wang et al., 2016).

Table 3
Characteristics of Sorghum bicolor and Trifolium repens integral powder

Content (%)	Sorghum bicolor integral powder	Trifolium repens integral powder
Moisture	7.02	5.7
Ash	6.30 ± 0.08	3.46 ± 0.02
Protein	12 ± 2.3	8 ± 1.5
Fat/oils	0	0
Carbohydrates	74.68	82.84

Figure 1
FT-IR spectra of Sorghum bicolor (a) and Trifolium repens (b)



According to Figure 1(b (*Trifolium repens*)), in region 3448 cm^{-1}, stretching vibration bands of protein amide groups are observed (Yu, 2008). Region 2076 cm^{-1} is related to vibration of C-H bonds in the aliphatic compounds of polysaccharide (Chen et al., 2016; Guo et al., 2016). Sharp vibration band indicates the presence of amide bonds of the protein and also C = O glycoside bonds in the plant in region 1637 cm^{-1} (Yu, 2008). Absorption band of the region 665 cm^{-1} represents N = H bonds (Chen et al., 2016). Polysaccharides are a family of carbohydrates and essential components of plant cells. Their structure consists of sugar rings connected by glycosidic bonds. When they stay on near positive and negative charges like colloidal particles, polysaccharides behave as polyelectrolytes (Crouzier et al., 2010) which aggregate the oppositely charged particles besides each other and so the flocs would be formed, and it results in occurring coagulation. Hydroxyl and amino functional groups on polysaccharides and proteins (main compounds in natural coagulants) (Choy et al., 2015) have important role in coagulation ability. These compounds found in the plant cell wall and are available in the form of mucilage (Ang & Mohammad, 2020).

3.2. The effect of pH on coagulants function

By adding the extract of Sorghum bicolor to the samples with pH 3, 7, and 10 (via jar test device), the colloidal flocs formed only in the wastewater with pH 7, and the extract of Trifolium repens formed the colloidal flocs in both of the samples with pH 3 and 7, but the best result was achieved in pH 7. So, the optimum pH for wastewater was assigned pH 7 to perform the treatment operation. Zeta potential results explain these findings; the point of zero charge (pzc) is the

Figure 2
Zeta potential at different coagulants doses using Sorghum bicolor (a) and Trifolium repens (b) integral powder

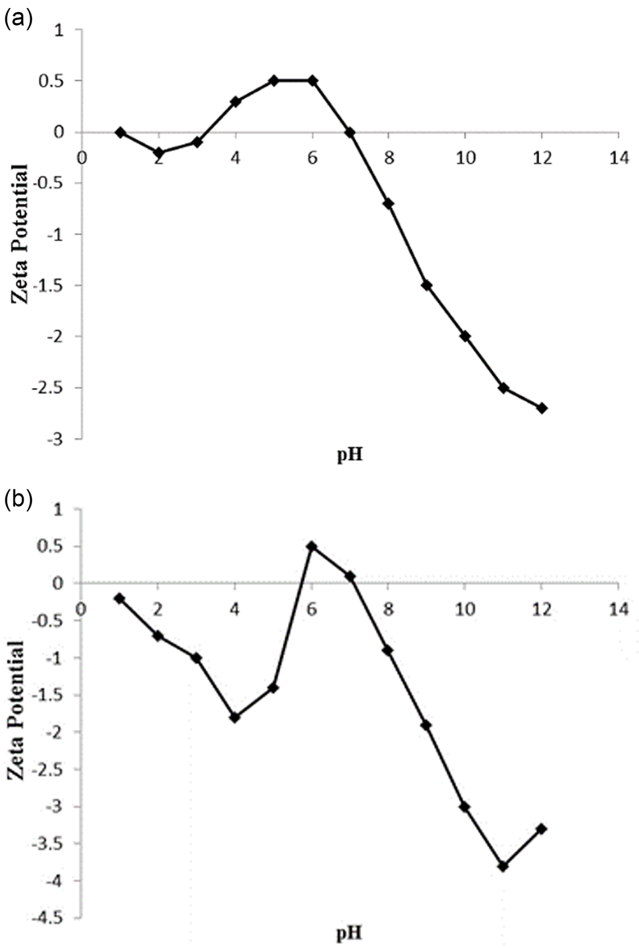
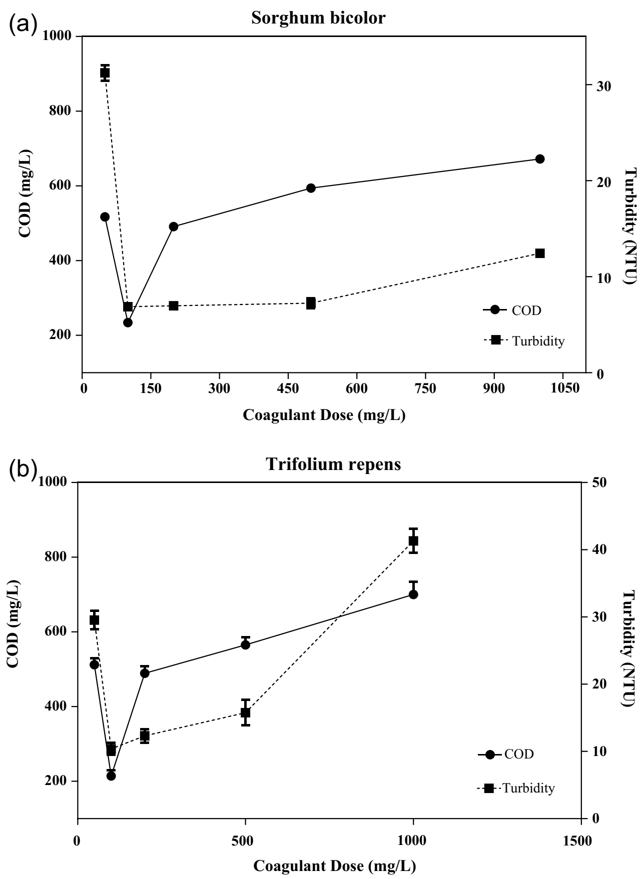


Figure 3
Effect of plant coagulants doses (a. Sorghum bicolor, b. Trifolium repens) on COD and turbidity reduction



pH at which negative and positive surface charges are equal that means zeta potential of the coagulant compound or surface is zero (Gulicovski et al., 2008). According to the results (Figure 2), at this point, pH is equal to 7 ± 0.1 in Sorghum bicolor (Figure 2(a)) and 7.2 ± 0.4 in Trifolium repens (Figure 2(b)).

Positive charges of the plant coagulants owing to their cationic proteins weaken the negative charge particles in the solution; therefore, at pHpzc (pH = 7) the destabilized particles can aggregate and lead to form floc and increase sedimentation. According to different researches, the efficiency of COD reduction depends on the wastewater pH directly. Shamsnejati used a plant-based coagulant to treat a synthetic textile wastewater and reported that the COD reduction depended on pH value, and the optimum pH was 6.5 (Shamsnejati et al., 2015). Also, Rasool and Zhao reported that for leachate treatment by a plant coagulant with ozonation process, after the treatment process, there was no need to adjust pH, because it was within standard limits that it would be economic; the optimum pH for high COD removal was 6–7 (Rasool et al., 2016; Zhao et al., 2009). In another similar research of using Plantago major L. for dye removal from wastewater, when pH of the solution increased from 5.5 to 6.5, COD amount decreased. In another word, when pH raised more than 6, the effect of plant coagulants could be observed (Chaibakhsh et al., 2014). Various findings indicate that maximum COD reduction occurs in pH equal to 6.5–7, because in neutral pH, the surface charge of the particles decreases and it leads to form wastewater colloidal materials (Freitas et al., 2015).

3.3. The effect of coagulant dose

Evaluating the effect of coagulant dose on treatment efficiency is one of the important purposes in this research. Figure 3(a, b) shows the effect of two types of plant coagulant doses on COD and turbidity reduction.

At 50 mg/L concentration of Sorghum bicolor extract, 10% of COD and 15% of turbidity were removed, and at 100 mg/L, the extract concentration, maximum COD (59%), and turbidity (81.34%) reduction were obtained. Also, when Trifolium repens extract was added to the sample at 100 mg/L concentration as a coagulant, minimum amount of COD and turbidity parameters were observed (COD reduction raised to 62%). So, the optimum coagulant dose for both of the extracts was 100 mg/L. By increasing coagulant doses of Sorghum bicolor and Trifolium repens from 200 to 1000 mg/L, COD and turbidity reduction decreased in wastewaters; it is due to increase in the wastewater organic load. In order to achieve the best treatment results and minimize the cost and the residual sludge, the optimum coagulant doses should be applied to the samples (Beltran-Heredia et al., 2009). When Moringa extract and PAC were used for water

Figure 4
Effect of Alum (a) and FeCl3 (b) on COD reduction

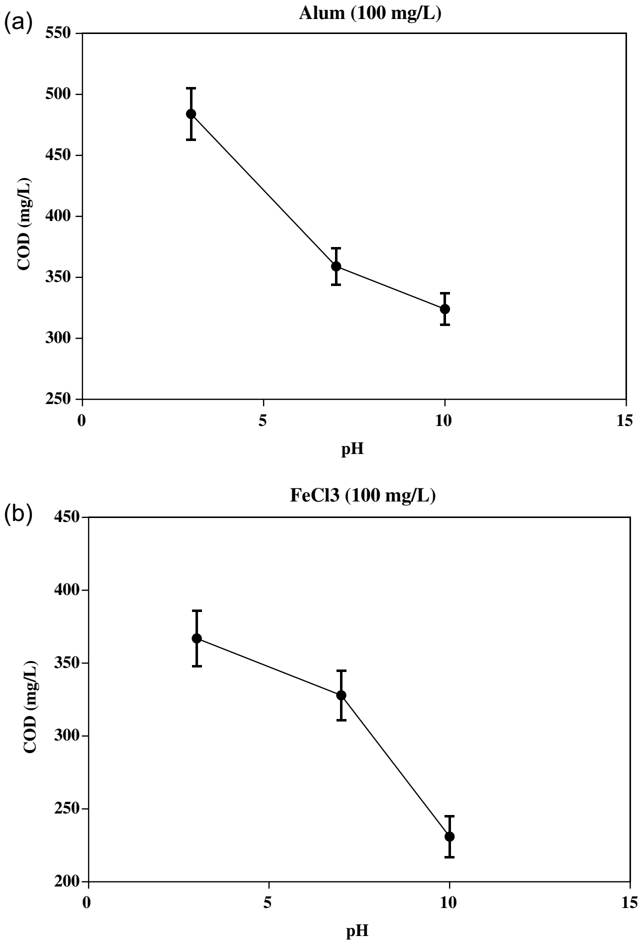
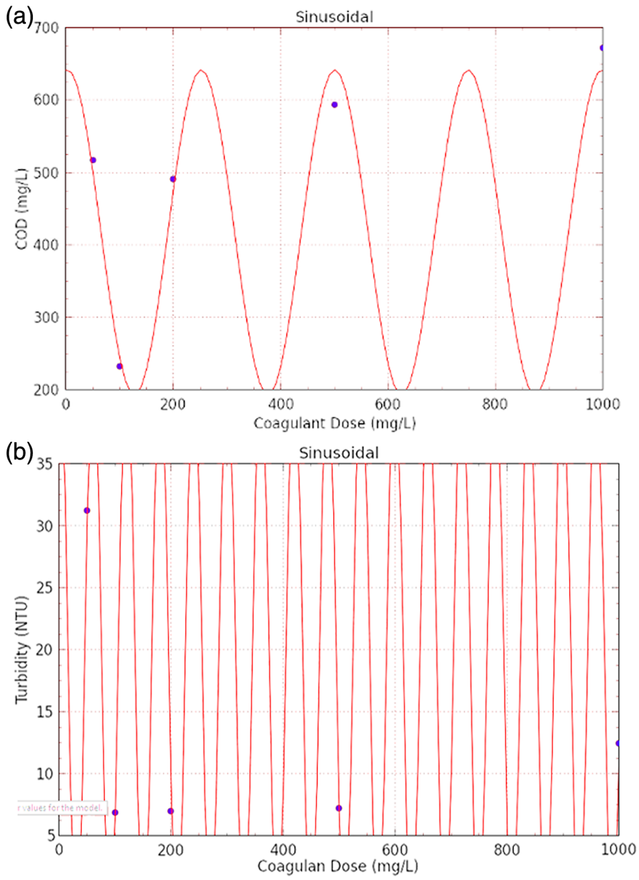


Figure 5
Effect of Sorghum bicolor's coagulant dose on COD (a) and turbidity (b) reduction



treatment, total organic carbon raised by increasing the extract concentration (Awad et al., 2013). Inorganic substances and biomolecules which are existing in natural coagulants structure lead to COD increasing (Antov et al., 2012; Ghebremichael et al., 2006). Applying plant coagulant more than optimum amount destroys flocs formation too by increases repulsive force between dye molecules in wastewater and coagulant (Shak & Wu, 2014).

3.4. Chemical coagulants

The treatment efficiency of optimum chemical coagulants doses was investigated on wastewater samples with pHs 3, 7, and 10. Figure 4(a, b) shows the effect of chemical coagulants' optimum doses on COD reduction based on the wastewater pH.

To compare the performance of plant coagulants with chemical ones, the similar treatment conditions were designed for both treatment methods. So, same as the plant coagulant, the optimum pH and chemical coagulant dose were assigned. According to the results, maximum COD reduction was obtained at 100 (43%) and 500 (46%) mg/L for Alum, when the alum dose was 100 mg/L and the pH value was 10 (Figure 5(a)). Maximum amount of COD reduction among three $FeCl_3$ coagulant' s concentrations was 59%, which was obtained at 100 mg/L of $FeCl_3$ and pH equal to 10 (Figure 5(b)). The optimum coagulant dose was selected according to the best treatment result, economic and environmental aspects (Rasool et al., 2016). Optimization of plant-based coagulant is very important to achieve the best results of applying this new approach for wastewater treatment as the appropriate alternative of conventional coagulants, and it is the main purpose of similar researches (Balbinoti et al., 2023). At lower plant coagulant dosage, the amount of organic compounds decreases in treated water (Ang & Mohammad, 2020).

3.5. Computational model fitting

The results for computational fitting are shown in Tables 4 and 5 and in Figures 5 and 6. The computational equations obtained from curve expert software can be used for calculating and predicting outlet COD and turbidity values in similar treatment systems where *Sorghum bicolor* and *Trifolium repens* extracts are used as coagulants for turbidity and COD reduction from textile wastewater with COD value near to 600 mg/L.

According to Tables 4 and 5, curve expert model reported 95% confidence for both coagulant plant; so, the uncertainty rate for the predicting models is 5% for both *Sorghum bicolor* and *Trifolium repens*; this uncertainty percentage is very low, and it shows an acceptable efficiency and accuracy of these equations.

Different researches use numerical modeling to describe their findings properly; The vulnerability of coastal aquifer was assigned in coastal and non- coastal regions by merging index and

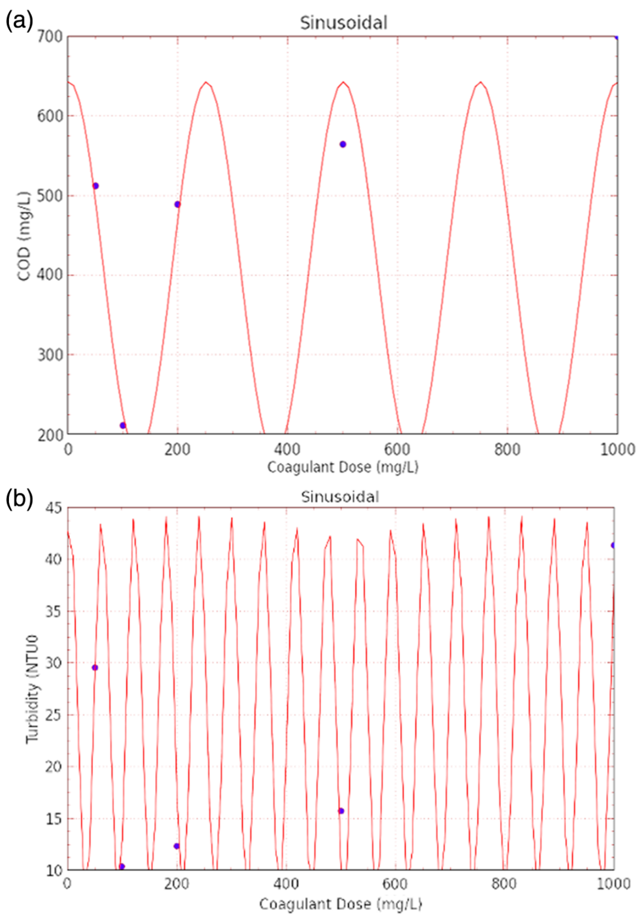
Table 4
COD and turbidity calculation using standard equation for Sorghum bicolor coagulant

		Value					95% Confidence			
Parameter	Equation	a	b	c	d	R^2	a	b	c	d
COD	$y = a + bcos(cx + d)$	417.80	224.57	0.025	224.57	0.96	−81.45 to 917.07	−403.71 to 852.87	−0.00 to 0.05	−5.51 to 5.38
		Std Err								
		39.29	49.44	0	0.42					
Turbidity		a	b	c	d	0.99	a	b	c	d
		19.94	24.30	0.10	−0.05		8.63 to 31.25	−0.58 to 49.19	0.103 to 0.106	0.70 to 0.59
		Std Err								
		0.89	1.95	0	0.05					

Table 5
COD and turbidity calculation using standard equation for Trifolium repens coagulant

		Value					95% Confidence			
Parameter	Equation	a	b	c	d	R^2	a	b	c	d
COD	$y = a + bcos(cx + d)$	408.76	234.78	0.02	-0.04	0.92	−460.55 to 1278.08	−767.62 to 1237.20	−0.03 to 0.08	−9.57 to 9.47
		Std Err								
		68.41	78.89	0	0.74					
Turbidity		a	b	c	d	0.90	a	b	c	d
		25.64	18.58	0.10	−0.40		−41.88 to 93.17	−133.69 to 170.86	0.089 to 0.123	−4.72 to 3.92
		Std Err								
		5.31	11.98	0	0.34					

Figure 6
Effect of Trifolium repens's coagulant dose on COD (a) and turbidity (b) reduction



numerical method (Javadi et al, 2022). In another research, an equation model was provided for investigating the usefulness of a number of mulches in more penetration of rainfalls for rising the underground water table (Banihabib et al., 2018). A Soil and Water Assessment Tool (SWAT) model was also used in southwest of Iran, Kohnak watershed (Sayyad et al., 2015). As well as applying DRAINMOD model, for predicting hydrology changes such as water quality and flow and water table depth (Golmohammadi et al., 2020).

4. Conclusion

Considerable turbidity and COD reduction make *Sorghum bicolor* and *Trifolium repens* as inexpensive and easily available coagulants for the treatment of dye-containing industrial wastewaters. The treatment processes were highly pH-dependent that more than 80% turbidity and 60% COD reduction were obtained at pH 7 and also, 100 mg/L coagulant dose. According to the FT-IR spectra of *Sorghum bicolor* and *Trifolium repens* extracts, the most important components of the plant cells are polysaccharides which played the main role in coagulation process. Despite the chemically treated wastewaters, treated effluents pHs by plant coagulants were in the standard range of discharge.

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Conflicts of Interest

The authors declare that they have no conflicts of interest to this work.

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