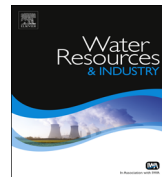




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Treatment of water turbidity and bacteria by using a coagulant extracted from *Plantago ovata*

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ARTICLE INFO

Article history:

Received 16 April 2014

Received in revised form

4 June 2014

Accepted 11 July 2014

Keywords:

Biocoagulant

Coagulation

Humic acid

Plantago ovata

Water turbidity

ABSTRACT

A biocoagulant was successfully extracted from *Plantago ovata* by using an FeCl_3 -induced crude extract (FCE). The potential of FCE to act as a natural coagulant was tested for clarification using the turbid water of a river. Experimental tests were performed to evaluate the effects of turbidity concentration, coagulant quantity, water pH, and humic acid concentration on the coagulation of water turbidity by FCE. The maximum turbidity removal was occurred at water pH < 8. At the optimum dosage of FCE, only 0.8 mg/L of dissolved organic carbon was released to the treated water. An increase in the humic acid led to the promotion of the water turbidity removal. Results demonstrated that the FCE removed more than 95.6% of all initial turbidity concentrations (50–300 NTU). High bacteriological quality was achieved in the treated water. FCE as an eco-friendly biocoagulant was revealed to be a very efficient coagulant for removing turbidity from waters.

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1. Introduction

Often, the waters from surface water resources have a high level of turbidity and need to be treated with flocculation/coagulation to remove the turbidity. Many flocculants and coagulants are widely used in conventional water treatment processes. These materials can be classified into inorganic coagulants (e.g., aluminum and ferric salts) and synthetic organic polymers (e.g., polyacryl amide

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derivatives and polyethylene imine). All of them are very efficient at turbidity removal from water [1]. The cost of achieving the desired level of water quality depends primarily on the cost and the availability of the coagulation agents. Aluminum salts are most widely used together with synthetic organic polymers in water treatments. These coagulants are often expensive, and in many developing countries, they have to be imported.

Moreover, the high sensitivity of inorganic coagulants to the water pH and the possibility of secondary contamination of drinking water with traces of toxic synthetic polymeric coagulants or residual iron and aluminum ions are the main challenges of flocculation–coagulation water treatment processes [2]. Further, many researchers have related Alzheimer's disease to the residual aluminum ions in the treated waters [3]. Moreover, as reported by Anastasakis et al. [4], the sludge formed in water treatment plants during flocculation–coagulation with synthetic polymers has a limited potential for recycling because of the non-biodegradability of synthetic polymers. To make the flocculation–coagulation process more attractive, novel low-cost coagulants with higher coagulation capability are required. This has led a growing research interest in the production of natural and food grade coagulants from renewable and relatively cost-effective precursors. It can contribute to achieving sustainable water treatment technologies. Natural coagulants, mainly polysaccharides and proteins, are considered eco-friendly in comparison with inorganic and organic coagulants because of their biodegradability [5,6].

Recently, the use of some natural polymers for the treatment of various types of water and wastewater has been reported. Table 1 summarizes some of the published literature on turbidity removal by natural coagulants. As shown, the most common coagulants are plain or chemically modified and exhibit relatively low turbidity removal of between 50% and 90%. Moreover, most of these natural coagulants changed dissolved organic carbon (DOC), which can cause a concern in the chlorination process [15]. Because of the biomaterials' relatively low efficiency of turbidity removal, the production and extraction of these biomaterials as coagulants is very expensive, making them impractical for full-scale applications.

Regarding the difference in coagulation capacity of various coagulants as well as cheapness, variety and ease of access, the use of regional coagulants is studied to evaluate their applicability. In this study, we have attempted to use *Plantago ovata* (Fig. 1a and b) as an abundantly available, low-cost, and renewable precursor for producing a coagulant for the removal of water turbidity. We have considered *P. ovata* because it is a self and fast grown and common plant that grown mainly in throughout Iran especially the surrounding area of Bushehr city and is easily prepared and used. *P. ovata* is a small, one-year-old plant without or with a very short stem covered with soft fibers, from

Table 1
Summary of recently published literature on coagulating efficiency by natural coagulants.

Coagulant	Optimum dose (mg/L)	Turbidity removal (%)	DOC change	References
<i>Hibiscus esculentus</i> seedpods	5	93–97.3	190% increase	[4]
<i>Malva sylvestris</i> mucilage	12	96.3–97.4	80% increase	[4]
Hercofloc	9	50	–	[7]
Nalco 610	10	62	–	[7]
Purifloc C-31	50	50	34% reduction (10% increase at lower doses)	[8]
Grafted <i>Plantago psyllium</i> mucilage	1.6	–	–	[9]
<i>M. oleifera</i> seeds	100	–	64% reduction	[10]
<i>M. oleifera</i> seeds	50	90	> 50% increase	[11]
<i>M. oleifera</i> seeds	6000	–	52% reduction	[12]
1-Step purification of <i>M. oleifera</i>	2	97	32% increase ^a	[13]
2-Step purification of <i>M. oleifera</i>	2	98.5	17% increase ^a	[13]
Chestnut and acorn	0.5 mL/L	70–80	1 mL/L	[14]

^a The data was derived from Table 1 and Fig. 2 of Ref. [13].

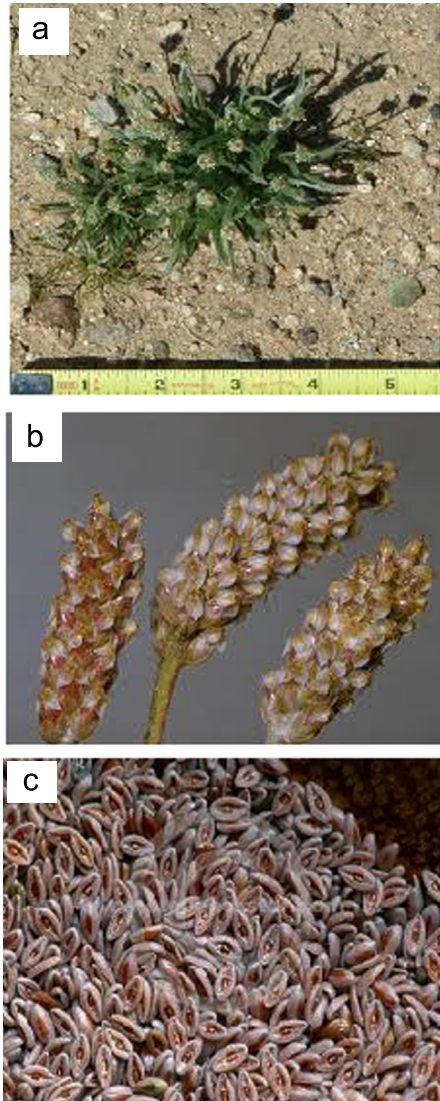


Fig. 1. (a) and (b) *P. ovata* plant and (c) *P. ovate* seeds.

Plantaginaceae family; its height ranges from 7 to 30 cm [16]. *P. ovata* seeds (Fig. 1b) contain mucilage, protein, fixed oil, cellulose, and starch [17]. Interestingly, as is clear from Table 1, the efficiency of turbidity removal by a natural coagulant is significantly influenced by the characteristics and the origin of the coagulant. Therefore, we have developed an extraction method aimed at improving the material's coagulation capability and therefore reducing the rate of coagulant consumption to make water turbidity removal more cost effective.

Several chemicals have been used in the process of coagulant extraction, and the best extraction has been obtained with the presence of KCl or NaNO₃ [18,19] and NaCl [13]. However, the main challenge related to this method of coagulant extraction is the material's relatively low efficiency for turbidity removal and the DOC formation during the treatment process. These salts, which have a one-valence metal, are deposited in the internal structure and the pores of the coagulant and may be useful for turbidity removal. In this research, an innovative method is used. We have used FeCl₃ during

the purification of the extracted coagulant with the assumption that the Fe^{3+} ions get trapped in the structure of the extracted bio-coagulant from *P. ovata*. Therefore, according to the Hardy–Schulze rule [20], Fe^{3+} can improve the coagulation potential of the coagulant as compared to K^+ and Na^+ . Literature survey by the author in most of the peer reviewed journals indicated that turbidity and bacteria removal by FeCl_3 -induced crude extract (FCE) as a coagulant has not been investigated and this is the first such study undertaken by the author.

Therefore, this paper evaluates the coagulation potential of FCE obtained from *P. ovata* seeds for the turbidity removal from water. The effect of the operational parameters including FCE dose and initial water turbidity and the water characteristics (water pH and humic acid) is studied to better understand the coagulation process using FCE. The influence of FCE dosage on bacteriological characteristics of spiked water is also assessed.

2. Materials and methods

2.1. Materials

Dry seeds of *P. ovata* were purchased from a local Herbal market and stored at room temperature. Analytical-grade ammonium acetate, sodium hydroxide, hydrochloric acid, ferric chloride, and calcium chloride were supplied by Aldrich Co. Ethanol (98%), *CM-Sepharose*, and kaolin were purchased from Merck Co. Further, humic acid powder with molecular weight ranging from 2 to 50 kDa, extracted from brown coal using NaOH (Aldrich Chemical Co., Inc., USA), was applied.

2.2. Extraction and purification processes of coagulant from *P. ovata*

Extraction of the crude coagulant from *P. ovata* seeds was carried out as follows:

- The seeds were soaked in water for 1 day.
- The obtained gelatinous material was filtered and dried at 70 °C in an oven and then milled in a domestic blender (VARING).
- The obtained powder was defatted by mixing it with ethanol 98% using a magnetic stirrer for 60 min. Then, the supernatant was separated by centrifugation (3500 rpm, 40 min), and the settled powder was dried overnight in an oven (at 60 °C).
- The crude coagulant was extracted from the oil-free powder using 10-mM ammonium acetate in 5% w/w. The mixture was stirred for 50 min using a magnetic stirrer, and the supernatant, namely the crude extract, was separated by centrifugation (3500 rpm, 40 min).

The purification of the coagulant protein from the crude extract was performed as follows:

- *CM-Sepharose* ion exchange was equilibrated using a 10-mM ammonium acetate solution.
- Equilibrated *CM-Sepharose* ion exchange was added to the crude extract in 10% (v/v) proportion and mixed using a blade stirrer for 50 min.
- Finally, the absorbed coagulant protein was eluted by different concentrations of a FeCl_3 solution.

The purification procedure was carried out directly with 0.025-M FeCl_3 first and then with 0.05-M FeCl_3 elution according to the basis of the ion-exchange processes [13]. In the first elution, proteins that presumably did not lead to a higher coagulation performance and simply added DOC in the treated water were removed. Consequently, the second elution produced a more purified coagulant as it contained only the active coagulant proteins. The elution stages were repeated three times to recover as much coagulant as possible. Further, we noted that after the each purification of extraction by FeCl_3 , the resultant coagulant was rinsed many times with double distilled water until Fe ions not detectable in rinsed water.

2.3. Water sample preparation

Raw surface water was obtained from Karun River in Khuzestan province, Iran. The average specifications of Karun River water are presented in Table 2. The raw water was spiked with kaolin as a model suspension to provide the desired turbidity as follows: a stock solution of kaolin (10 g/L) was prepared with fresh river water and was stirred for 24 h. Then, a certain volume was taken and diluted with river water until the designated level of turbidity was achieved. The resulting suspension was found to be colloidal and used as a stock solution for the preparation of turbid water samples.

2.4. Coagulation procedure

Coagulation tests were carried out using a standard jar test apparatus (Phipps and Bird, Model 300). The jar test is the most widely applied method for evaluating and optimizing the coagulation–flocculation process based on standard methods [21]. The prepared turbid water (300 mL) having different initial turbidities was filled into beakers (1 L), and the standard procedure for the jar test was followed at a constant room temperature of 24 °C. The standard procedure involved 1 min of rapid mixing (120 rpm) followed by 10 min of slow mixing (45 rpm) for flocculation. Then, the treated water was allowed to settle for 15 min, and the supernatant sample was withdrawn by a syringe from approximately 2 cm below the liquid level for analysis. The initial pH of the river turbid water was adjusted to a desired value with 1-M NaOH or 1-M HCl, and a further correction of the pH was not carried out during the experiments. The same coagulation test was conducted with no coagulant as a blank. The impact of water pH, initial turbidity concentration, coagulant quantity, and humic acid concentration as variable parameters was evaluated as different conditions in this research. Table 3 presents the details of the experimental runs and conditions. All the experiments were conducted in duplicate (the accuracy is considered to be $\pm 4\%$) to ensure the reproducibility of the results; the mean of these two measurements are presented herein. The turbidity removal efficiency (TRE) was calculated using Eq. (1) as follows:

$$\text{TRE (\%)} = [(T_0 - T)/T_0] 100 \quad (1)$$

where T_0 and T represent the initial and final turbidities (NTU) of water, respectively.

2.5. Analytical methods

The physical and chemical parameters of the river water were determined using standard methods [22]. Turbidity was measured using a WTW TURB555IR turbidimeter (Germany) and was expressed in nephelometric turbidity units (NTU). Samples were filtered through a Whatman filter paper having a pore size of 0.45 μm . Further, the DOC was determined using the APHA [22] method (3510-B).

Table 2
Average raw water characterization of Karun River.

Characteristic	Unit	value
Turbidity	NTU	76
Suspended solids	mg/L	10
pH	–	7.01
Conductivity	$\mu\text{S}/\text{cm}$	328.8
TOC	mg/L	8.6
DOC	mg/L	4.02
Hardness	mg/L CaCO_3	290
Nitrate	mg/L	27.8
Nitrite	mg/L	0.07
Total coliforms	CFU/100 mL	537
Fecal coliforms	CFU/100 mL	369
Fecal Streptococcus	CFU/100 mL	131

Table 3
Experimental runs and conditions for turbidity removal by FCE.

Run	Experiment	Conditions			
		pH	Turbidity (NTU)	Coagulant (mg/L)	Temperature (°C)
1	Synergistic effects of FeCl ₃ and crude extract	As per original	100	FCE: 0.25 crude extract: 0.25 FeCl ₃ : 0.25	24
2	Effects of FCE dose	As per original	100	0.25–5	24
3	Effects of initial water turbidity and sludge producing	As per original	50–300	0.25	24
4	Effects of water pH	2–12	100	0.25	24
5	Effects of humic acid	As per original	100,300	0.25	24
6	Microorganisms monitoring during treatment	As per original	As per original	0.25–5	24

The pH of the zero point charge (pH_{ZPC}) for FCE was determined. For this purpose, 50 mL of a 0.01-M NaCl solution was placed in a 100-mL Erlenmeyer flask. Then, the pH was adjusted to successive initial values between 2 and 12, by using either NaOH or HCl, and 4 g/L of FCE was added to the solution. After a contact time of 24 h, the final pH was measured and plotted against the initial pH. The pH at which the curve crossed the line pH (final)=pH (initial) was taken as the pH_{ZPC} of the considered FCE. The pH_{ZPC} for the FCE used in this study was determined to be 7.9. The zeta potential was measured with Zeta-Meter 3.0+ (Zeta-Meter 3.0+, ZM3-001, USA). The settling sludge volume was determined according to the following equation [23]:

$$\text{Sludge volume} = (\text{Settled sludge volume, mL}) / (\text{Sample volume, L}) \quad (2)$$

The digital camera was used to take photos and record our observation of water turbidity removal by FCE. A scanning electron microscopy (SEM) image of the sludge produced after the coagulation/flocculation treatment was obtained using a scanning electron microscope (JSM-6390LV; Make: Jeol, Japan). Fourier transform infrared (FTIR) spectra were recorded using a Nicolet FTIR instrument (Model: Magna 550) with KBr phase in the range 400–4000 cm⁻¹ against KBr as the reference material. An electron dispersive spectroscopy (EDAX) analysis was performed to determine the elemental composition of the crude extract coagulant, fresh FCE, and FCE sludge by using the FE-SEM (QUANTA 200 FEG).

The bacteriological quality of raw and treated water was analyzed for fecal and total coliforms bacteria using selective media, i.e., m-FC with rosolic acid for fecal and m-Endo for total coliforms (Millipore). KF-Broth was applied for analyzing fecal streptococcus (Whatman). A membrane filtration technique was applied as well [22].

3. Results and discussion

3.1. Synergistic effects of FeCl₃ and crude extract on turbidity removal

In order to provide supplementary evidence for the proposed nature of synergistic effects involved in the turbidity removal by FCE, an experiment was conducted to remove turbidity by using FeCl₃, crude extract, and FCE. For this, a dosage of 0.25 mg/L of coagulants was added to the sample water with initial turbidity of 100 NTU. We selected this dosage basis on the optimal dosage of FCE for water turbidity removal (see next section). As can be observed from Fig. 2(a), the turbidity removal by FeCl₃, crude extract, and FCE at pH 7.01 was attained at 26%, 36%, and 99%, respectively. However, the coagulant of FeCl₃ is an efficient coagulant in waterworks; here, the low turbidity removal for FeCl₃ can be accounted for its low percentage of turbidity removal. For better understanding the synergistic

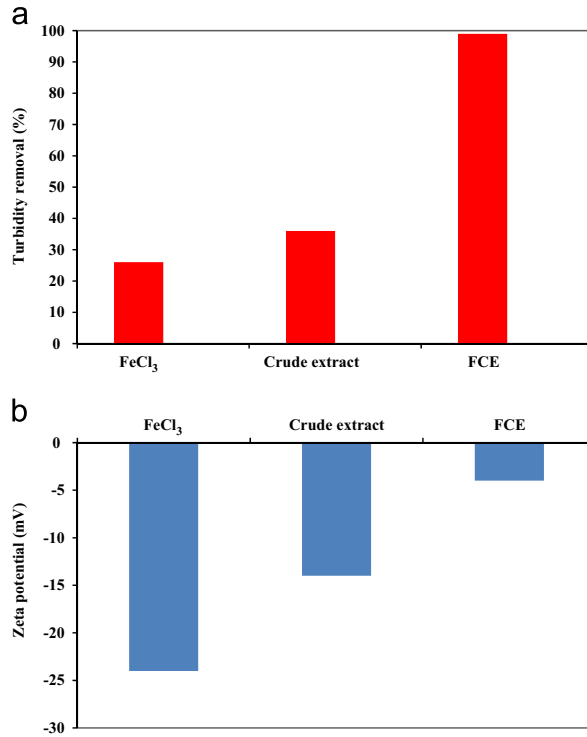


Fig. 2. (a) Effects of FeCl₃, *P. ovate* crude extract, and FCE on water turbidity removal (b) zeta potential of water samples coagulated with FeCl₃, *P. ovate* crude extract, and FCE.

effect of FeCl₃ and crude extract the zeta potential was measured after coagulation and results depicted in Fig. 2(b). The zeta potential can serve as an excellent tool for the quick check of coagulation performance. If the turbidity particles have a charge, these particles will move in the field with a speed and direction which is easily related to their zeta potential [24]. The substantial increase in the absolute zeta potential after coagulation indicates the occurrence of charge neutralization. In other words, the increment in zeta potential shows the presence of coagulation.

Zeta potential of the raw water samples was measured and the initial zeta potential values varied from -26 to -31 mV. The results are in agreement with the previous study which stated that practically all aqueous colloids are electronegative, with the general range of zeta potential between -14 and -30 mV [24]. As it can be seen from Fig. 2(b), the zeta potential values of water samples coagulated with FeCl₃ and crude extract were -24 and -14 mV, respectively. Whilst, for water sample coagulated with FCE, the value of zeta potential was -4 mV. From the results it can be seen that water samples coagulated with FCE showed higher increment in zeta potential. The increase of zeta potential indicated the higher frequency of charge neutralization and better coagulation efficiency of FCE.

3.2. Visual observations of coagulation process and effects of FCE dose

When the coagulation experiments were started, the first dramatic observations indicative of coagulation process were the visual observations. As soon as the low FCE value, 0.25 mg/L, was dosed, the formation of large flocs was observed. Fig. 3(a–d) shows the photographs of FCE-coagulated 250 NTU turbid water sample before and after settlement. The flocs looked strong and heavy and they settled rather quickly as soon as the mixing was terminated.

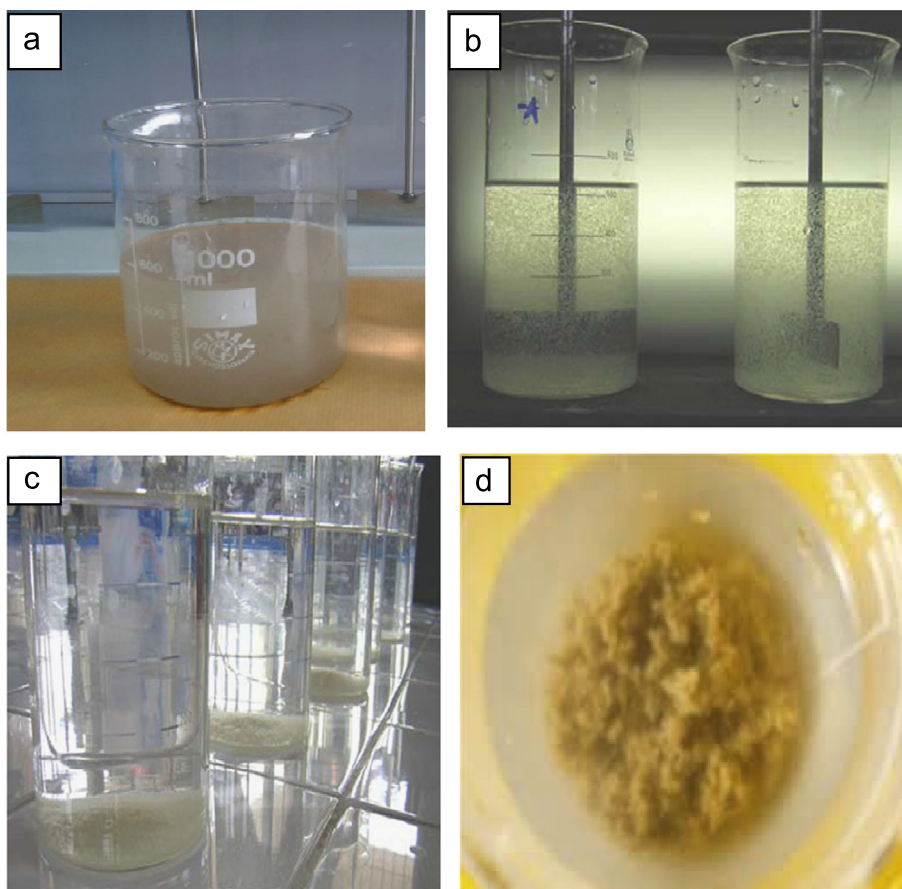


Fig. 3. (a) Water with initial turbidity of 250 NTU and 1 mg/L humic acids (b) floc formation during coagulation by FCE, (c) settled flocs after 15 min settling, and (d) bulky flocs formation.

The coagulation process is generally a surface phenomenon; therefore, coagulation performance can be significantly affected by the surface charge due to the mass of the coagulant. Thus, from an economic point of view, the optimization of the coagulant dosage and the best-required mass of the coagulant for the scale-up and design of large-scale equipment is necessary. Hence, the influence of FCE quantity on turbidity removal was considered at an optimum pH of 7 (Table 3); the obtained results are plotted in Fig. 4 from the perspective of the turbidity removal efficiency. As can be inferred from Fig. 4, for the two lowest FCE quantities, i.e., 0.25–0.5 mg/L, the final turbidity was low, less than 5 NTU, which is the standard for drinking water. Further, the results of the effect of the coagulant dose on the turbidity removal exhibited the following trend: First, the turbidity removal efficiency decreased from 99% to 26% as the coagulant mass increased from 0.25 to 1 mg/L. Then, the turbidity removal efficiency increased with an increase in the FCE dose from 1 to 2 mg/L. Finally, the turbidity removal efficiency decreased when the FCE dose was more than 2 mg/L (Fig. 4). This phenomenon could be attributed to a couple of reasons. On one hand, at a lower coagulant concentration, its long chain adsorbed on the surface of one colloid particle was adsorbed onto the surfaces of the others, and thus two or more particles aggregated by bridging flocculation. However, when the coagulant concentration was increased to a certain value (here, 1 mg/L), the adsorbed aggregation completely covered the particle surface and prevented the particles from flocculating. On the other hand, under experimental conditions and $\text{pH} = 7 < \text{pH}_{\text{zpc}} = 7.9$, FCE was positively charged as the functional groups bonded with hydrogen ions (see *Effects of water pH*). Therefore, when the FCE dosage was increased to

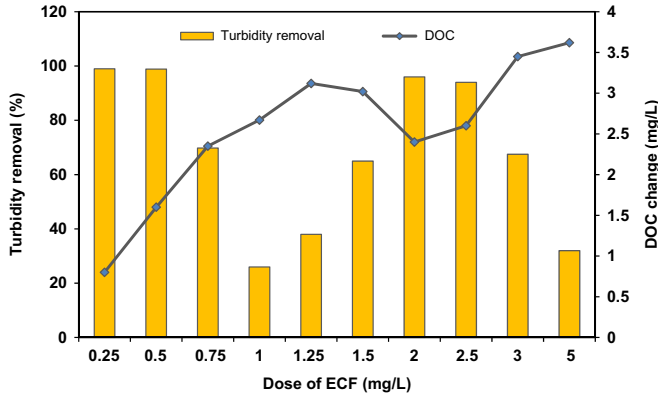


Fig. 4. Effects of FCE dose on water turbidity and DOC change in treated water.

a given value (2 mg/L), the positively charged FCE adsorbed on the surface of the negatively charged colloid particles by charge neutrality coagulation, resulting in a better coagulation effect. These results conform to a previous study [25]. In another research [15], it has been stated that the coagulation efficiency depends considerably on the content of the bivalent cations in water. As the water hardness was relatively high (Table 2; 290 mg/L of CaCO_3), another reason for the low efficiency of the relatively high FCE doses could be the water characteristics. As stated by Devrimci et al. [3], calcium ion can form complexes with certain ionogenic groups on the coagulant and on the particle surfaces. After the coagulant dosage was increased further (> 2 mg/L), it was observed that the presence of the excessive coagulant made the suspended particles positively charged and thus, caused mutual repulsion. In this case, a further increase in the coagulant dosage resulted in the re-dispersal of flocs and the rise in turbidity. These results supported the results of our previous study [26].

The use of natural coagulants might increase the organic load in water, which might result in increased microbial activity [11,15]. Further, the organic matter might consume additional chlorine in the water treatment plant and act as a precursor of toxic byproducts during the disinfection process. Thus, in another effort, we monitored the DOC changes during the experiment. As can be seen from Fig. 4, the DOC change during the turbidity removal in the case of all the considered FCE doses was less than 3.6 mg/L. However, the DOC change was only 0.8 mg/L for the optimum FCE dose of 0.25 mg/L, implying that the background DOC 19.9% was increased. The DOC induced to water as compared to other coagulants (see Table 1) used for turbidity removal was very low. Overall, it could be concluded that lower doses of the investigated natural coagulant were better than higher ones. This is very critical not only for process economy but also for lower organic matter load in treated water because it is known that a high organic load can cause microbial growth. However, the obtained result is valid only under the considered conditions. For each natural coagulant and natural water, the optimum dose and the other optimum factors that influence the coagulation process have to be found.

3.3. Effects of initial water turbidity

The turbidity may have different concentrations in different water resources, and different turbidity concentrations may have an effect on the FCE coagulation. Therefore, the influences of 50–300 NTU of water turbidity on the performance of FCE for removing turbidity from water under the conditions specified in Table 3 were considered. The results of the effect of the initial turbidity concentration on the turbidity removal by FCE are shown in Fig. 5. In all reactions, different concentrations caused changes in the driving force. Hence, an increase in the turbidity's initial concentration up to 250 NTU resulted in a reduction in its removal percentage due to the ratio of turbidity to the constant mass of FCE. A similar result was reported by Šciban et al. [14]. The increase in the turbidity removal for the turbidity's initial concentration more than 250 NTU might be attributed to other mechanisms such as sweeping flocculation rather than the neutralization of the

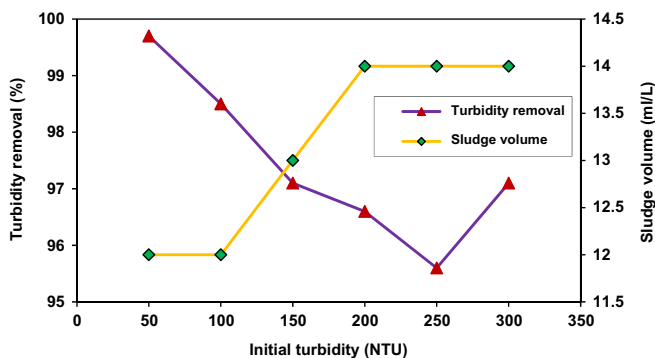


Fig. 5. Effects of initial water turbidity on sludge producing and turbidity removal.

surface charge of colloids. However, the percentage turbidity removal for all the initial turbidity concentrations was significant (> 95.6%). The ability of the coagulant to work in different turbidity ranges reflected its versatility to treat various waters.

We also considered the sludge volume during the turbidity removal of the turbid water by using FCE. As shown in Fig. 5, the sludge volume for all turbidity concentrations was under 14 ml/L, and for the usual water turbidity concentration (< 100 NTU), it was 12 ml/L. The sludge produced by FCE was less than that produced by chitosan (30 ml/L) and aluminum salt (> 50 ml/L) [27]. The relatively low sludge volume led to a decrease in the general costs of the treatment. The use of FCE may be a promising method for treating turbid raw water.

3.4. Effects of water pH

Since the pH of the water affects the surface charge of the coagulants as well as the degree of the stabilization of the suspension [28], the effect of the water pH on the removal of turbidity by FCE was studied in the pH range of 2–12 under the conditions given in Table 3. The results of the pH effect on the turbidity removal are depicted in Fig. 6. This figure clearly shows that the removal of turbidity was considerably influenced by the water pH; as the water pH increased from 2 to 6, the efficiency of the turbidity removal increased from 97% to 99.6%. This phenomenon can be explained by the fact that the pH of the water influences the surface charge of the FCE particles in the solution. The pH_{zpc} for the FCE particles was determined to be 7.9. Therefore, at values less than pH_{zpc} , the surface of FCE was positive. Because the surface charge of kaolin clay was negative and electrostatic attraction phenomenon can be taken place more easily as follows (Eq. (3)), the turbidity removal at water pH of less than 8 was improved.



However, the turbidity removal efficiency was maximized for a water pH of 6, but we chose pH 7.01, the original water pH, for further experiments on account of the increase within the recommended range of pH for drinking water, which was from 6.5 to 8.5 [29]. The turbidity removal for water pH 7 was 98.9%, a little less than turbidity removal efficiency that was attained at water pH 6, 99.6%. Because the surface water resources are typically neutral, attaining maximum coagulation efficiency at around neutral pH is advantageous because the pH of water does not need to be adjusted for efficient coagulation. Furthermore, the final pH of the solution at the end of each experiment was similar to the initial value (Fig. 6). It is critical to control the pH at an optimal value to attain maximum performance. However, the results indicated that the adjustment of water pH during coagulation by FCE was unnecessary, which reduced the overall cost of treatment. This result is disagreement with the observation that higher pH values are optimal for other natural coagulants, e.g., from *M. oleifera* [15], from *P. juliflora* and *C. latifaria* [5], and from *C. angustifolia* [30] or common bean [14], but in line with other

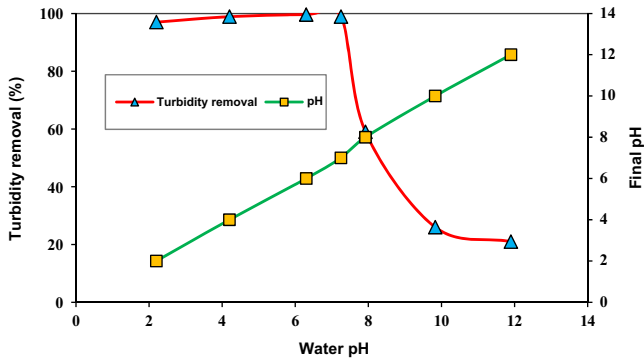


Fig. 6. Effect of initial water pH on the turbidity removal by FCE.

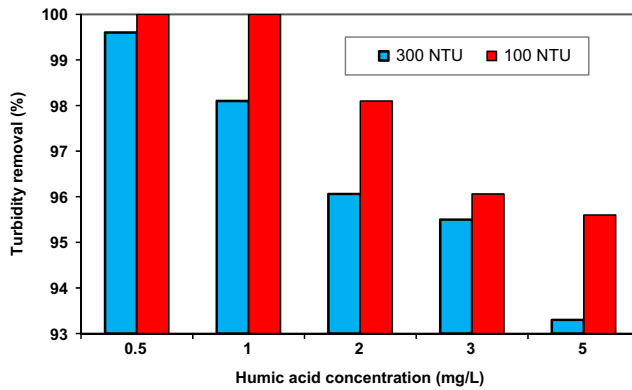


Fig. 7. Effects of humic acid concentration on the water turbidity removal by FCE.

research (e.g., [23]). These contradictory reports in the literature could be explained by the differences in the experimental conditions and the type of coagulant.

3.5. Effects of humic acid concentration

Surface water resources have various concentrations of natural organic matter. The most important component of natural organic matter that may affect the water treatment process is humic acid. Humic acids are the main organic compounds affecting the coagulation processes, and their removal has attracted extensive research interest because of the potential for the formation of trichloromethane in the disinfection process [31]. Therefore, the influence of 0.5–5 mg/L of humic acid on the performance of FCE for removing turbidity from water under the conditions given in Table 3 was evaluated. The results of the effect of humic acid on turbidity removal are shown in Fig. 7. The presence of a small amount of humic acid (0.5 and 1 mg/L) moderately enhanced the removal of water turbidity in the case of both initial turbidity concentrations (100 and 300 NTU). This phenomenon could be explained by the skeletal role of humic acid in floc formation and thus increase the coagulation efficiency by the bridge-aggregation mechanism. Therefore, the water turbidity with an initial concentration of 100 NTU was completely removed at low humic acid concentrations of 0.5 and 1 mg/L. The presence of a relatively high amount of humic substances (here, > 1 mg/L) yields bulky flocs (Fig. 3d), whose settleability is poor, leading to high supernatant turbidity [32]. As reported in the literature [33,34], the mechanisms to explain the significant coagulation of turbid water in the presence of a low concentration of humic substances include charge neutralization, precipitation,

bridge-aggregation, adsorption, and sweep-flocculation. Under different conditions, the different mechanisms or their combination may be dominant. Because of the high positive charge and high molecular concentration of humic acid, charge neutralization and bridge-aggregation may play a more important role.

3.6. Microorganisms monitoring during treatment

Since disinfection aspects have been thoroughly considered in the existing literature [31], microorganism removal was monitored in treated water by FCE under the conditions specified in Table 3, Run 5. Fig. 8 illustrates how the three studied species are removed by the action of FCE. As depicted, microorganism populations are observed before and after the coagulation and flocculation treatment. This removal is decreased with an increase in the FCE dose. Thus, it is clear that upon the removal of turbidity (i.e., organic suspended matter), microorganisms undergo a loss of their natural supporting structure; hence, high removal is achieved. Above all, in cases of streptococcus and fecal coliforms, an almost total removal is achieved.

3.7. SEM, FTIR, and EDAX

From the SEM micrographs (Fig. 9), it is clear that the flocs produced after the water treatment with FCE are hard and sturdy. The honeycomb-like structure, with small colloidal particles entrapped in it, might indicate the contribution of the sweep flocculation mechanism for the removal of colloidal

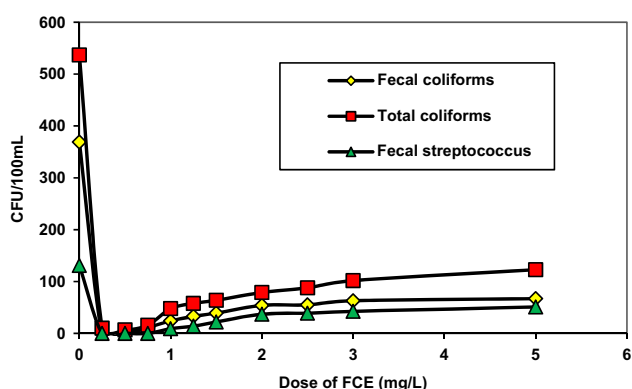


Fig. 8. Microorganisms reduction in treated water by FCE.

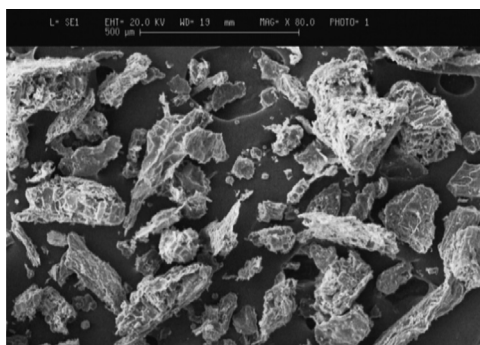


Fig. 9. The SEM image of FCE floc.

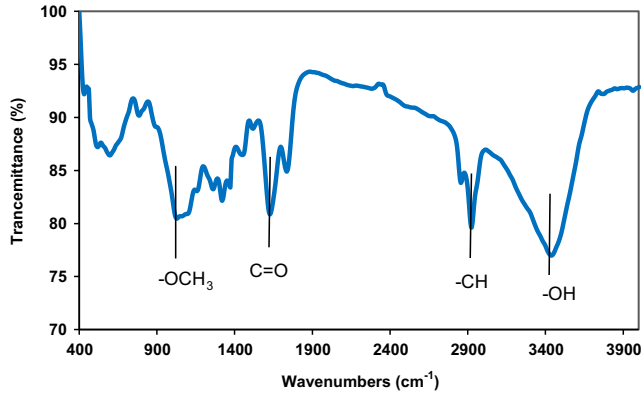


Fig. 10. FTIR spectra of fresh FCE.

Table 4

The EDAX analysis of the crude extract, fresh FCE, and FCE sludge.

Element (wt%)	Value		
	Crude extract	Fresh FCE	FCE sludge
C	22.10	24.15	58.07
N	0.72	0.82	1.91
O	0.61	0.63	0.761
S	2.22	2.98	3.85
Al	0.16	0.170	0.132
P	0.77	0.52	0.61
Fe	0.64	4.77	4.01
Na	3.01	5.11	6.22
Cl	0.22	2.132	5.80

particles in the water mixture. The FTIR spectra of FCE shown in Fig. 10 indicates several main peaks at 3410, 2920, 1604, and 1000 cm^{-1} on the spectra which corresponds to $-\text{OH}$ groups, $-\text{CH}$ groups, $\text{C}=\text{O}$ bands, and $-\text{OCH}_3$ groups [35]. These active groups on the surface of FCE may be involved in turbidity removal from textile water. The chemical composition of the sludge and fresh FCE is given in Table 4. Among various elements, carbon was found to be dominant in the sludge. Regarding to polysaccharide $[\text{C}_n(\text{H}_2\text{O})_m]$, fatty acids $[\text{CH}_2-(\text{CH}_2)_n-\text{COOH}]$, and protein general formula $[\text{RCH}(\text{NH}_2)\text{COOH}]$, the obtained coagulant could be neither polysaccharide nor fatty acid as it contained N. Also, regarding to mentioned formula, the polysaccharides contain high amount of oxygen rather than proteins and fatty acids, so the coagulant structure could not be made from polysaccharide. Further, we know that some amino acids such as *met*- and *cys*- amino acids contain S, thus presence of S in the structure of coagulant might be another reason for proteinuos of the coagulant. However, low values of P may be due to phospholipids acids in the extracted coagulant. Some proteins namely metallo-protein contain metals such as Fe, however we modified the crude extract with FeCl_3 . Na and other such metal is naturally presented in plant seeds. Therefore, we can conclude that the extracted coagulant was majority protein and minority phospholipids acids.

4. Conclusions

This study showed that the water turbidity removal using the recently developed biocoagulant, FCE, was influenced by various operational variables and water characteristics. The FCE has a high degree of significance for use as a coagulant in water treatment. The optimum dose of FCE for

coagulation was 0.25 mg/L, which is very low, and the DOC change of the treated water was negligible. Wide range of water turbidities could be effectively removed by using FCE to under the standard of drinking water turbidity, 5 NTU. Maximum water turbidity removal was observed at water pH < 8. Water characteristics such as the concentration of humic acid had a significant effect on water turbidity. According to EDAX results, the FCE was of proteinuos coagulant. The FCE improved the bacteriological quality of the treated water. The finding demonstrated the conclusion that the FCE can be effectively used in water treatment plant.

However, the application in pilot and full-scale systems and a cost analysis are necessary steps for the eventual application of FCE.

Conflict of interest

There are no conflicts of interest.

Acknowledgments

This investigation has been supported by the Bushehr University of Medical Sciences under BUMS14H037 project.

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