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Investigation on *Moringa oleifera* extracts function to reduce microbial load in water treatment

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ABSTRACT

Reduction of raw water microbial load during water treatment process has important advantages such as decreasing algal and microbial growth in the water treatment plant, control of bacterial toxins leaching in water, and decreasing the amount of disinfectant usage. Application of ferric and other metallic coagulants for water coagulation has some disadvantages, therefore the natural coagulants may be compatible with these. In this study, the efficiency of natural coagulant extracted from *Moringa oleifera* seed was compared with $FeCl_3$ as a metallic coagulant in reduction of microbial load during water treatment process. Both, *M. oleifera* extract and $FeCl_3$ were applied in dosage of 1.5 up to 4 mg/L and then, microbial load, total and fecal coliforms and *Clostridium perfringens* were measured before and after coagulants application. The results did not show any significant differences between *M. oleifera* extract and $FeCl_3$ efficiency in reduction of fecal and total coliforms. But, *M. oleifera* extract was more efficient than $FeCl_3$ in removal of *C. perfringens*' spores significantly. It may be due to adsorption of spores on polypeptide structure of *M. oleifera* extract. Furthermore, there isn't any risk of disinfection by-products' formation such as trihalomethanes by well purification of *M. oleifera* extract before usage.

Keywords: Moringa oleifera; *Clostridium perfringens*; Water treatment; Microbial load

1. Introduction

Decreasing of microbial load during water treatment processes is one of the main goals of treatment processes [1]. Lesser microbial load can control biofilm growth in treatment units and moving devices such as pumps and protect them from microbial fouling and damage [2]. It can also control leaching of microbial or algal toxins, taste, and odor-producing substances [3]. Another advantage of microbial load reduction is the control of entrance of microbial load to sand filters. In addition, lower microbial load can decrease the amount of post disinfectant application and biofilm growth in distribution systems [4–6].

The first common step for this issue occurs in water reservoirs [6]. Several investigations mentioned that the storage of water for 24–48 h in reservoirs could decrease 90% of microbial load [7]. However, this does not happen in every water resources, especially in catchment from rivers. Another method for microbial load control is prechlorination of raw water. Prechlorination of raw water containing humic acids can form disinfection by-products (DBPs) such as *trihalomethanes* (THMs), in which some of them are potentially carcinogen and one of them (dibromo, chloromethane) is certain carcinogen [8–10]. Therefore, application of prechlorination is being limited more and more in water treatment plants. Lime application for softening of water is another effective process on microbial load by increasing pH over 10 and inactivation of microorganisms, but softening unit is not essential process in every water treatment plant [11–13]. Besides these particular processes, conventional treatment processes including

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coagulation, flocculation, and clarification can reduce microbial load effectively depending on the coagulant type, coagulation condition, quality of flocculation, and efficiency of clarification [14,15]. The main mechanism for microbial load reduction in conventional treatment processes is sedimentation of microorganisms in the clarifiers. There are no inactivation mechanisms in conventional treatment process and the removal efficiency is directly related to solid separation in clarifiers [16]. In conventional treatment process, coagulation is prior to flocculation and clarification. Thus, application of suitable coagulant and preparation of optimum condition for coagulation is necessary for appropriate function of flocculation and clarification processes. Several chemical coagulants are applied for water treatment around the world such as $FeCl_3$, $Fe_2(SO_4)_3$, and $Al_2(SO_4)_3$. All of them have some advantages and disadvantages, but the main important characteristic of them is impression from some critical variables including pH, turbidity, alkalinity, and total organic carbon (TOC) content [6]. Any change in these critical variables can influence on microbial load reduction efficiency [17]. Another technology for microbial load reduction is application of porous ceramics as a filter [18]. Porous ceramics are highly efficient, but are expensive and also need to clarify water for prevention of fouling on ceramics [19–21]. Therefore, finding and application of new coagulants such as natural coagulants may have some advantages comparing with traditional chemical coagulants. Recently, application of agro wastes is being studied in pollutants removal from water and wastewater [22–25]. Of course there are advanced processes in removal of humic substances which might add other compounds to finished water [26–30].

Moringa oleifera is a tropical plant with seeds that contain a natural coagulant and the seeds could be used for coagulation and turbidity removal historically [31]. Extracted active agent from the seeds can be used for removal of turbidity, and hardness from many colloidal systems. In addition, effective removal of antibiotics, humic compounds, and pesticides has been reported by adsorption of them on *M. oleifera* flocs [32–36]. Besides, several studies focused on microbial inactivation capability of *M. oleifera* extract. Doughari et al. and one other researcher reported bacterial inactivation properties for *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus* by *M. oleifera* extract [37–39]. Therefore, it seems *M. oleifera* extract has bactericidal and coagulation characteristics simultaneously.

The aim of this study is to investigate on the *M. oleifera* extract efficiency as coagulant for decreasing microbial load of raw water compared with common metal coagulants.

2. Material and methods

2.1. Sampling

For this study, the natural raw water was captured in 30 L volume in polyethylene containers from *Suleqan* River in North West of Tehran province (37.773255 N, 51.265265 E), Iran in summer of 2017. The samples were transferred to the laboratory during 5 h, in 2°C.

2.2. Measurement of microbial load

The three-pathogen microorganisms were measured in raw water. After mixing, total and fecal coliforms and *Clostridium perfringens* were determined by standard method and results were reported as colony formation unit per liter (CFU/L) [40].

Briefly, for total and fecal coliforms measurement in presumptive step, 15 tubes containing Lauryl tryptose broth were inoculated with 10, 1, and 0.1 mL of water sample and were incubated in 35°C in 24 h. In the confirmatory step, the tubes including brilliant green lactose broth were inoculated by positive tubes of presumptive step and were incubated at 37°C for 24 h. At last in completed step, plates containing Eosin Methylene Blue agar were streaked with positive tube from confirmatory step and were incubated at 44.5°C ± 0.2°C for 24 h. The number of total and fecal coliforms was calculated by using MPN formula, introduced in standard method.

For measurement of *C. perfringens*, the standard method was used. Briefly, the water samples were pretreated by heating in 80°C for 10 min. A series of water samples including 10, 1, and 0.1 mL were added to the 15 tubes, containing Iron Milk culture media and Litmus indicator and were incubated in 37°C for 5 d. The *C. perfringens* numbers were reported according to number of positive tubes as CFU/L.

2.3. Preparation of $FeCl_3$ solution

For comparison, *M. oleifera* extract with common metal coagulants, $FeCl_3$ was used as metal coagulant. $FeCl_3$ stock solution was prepared by dissolving 100 mg of $FeCl_3$ in 100 mL distillated water to prepare 1 g/L $FeCl_3$ solution. A jar test was done to estimate the optimum required concentration of $FeCl_3$. According to this test, we select a range of $FeCl_3$ concentration that the turbidity removal per each milligram of $FeCl_3$ was more than 5%. According to the results of jar test, optimum range of $FeCl_3$ was from 1.5 up to 4 mg/L. This range was divided to three concentrations of $FeCl_3$ for the test as 1.5, 2.5, and 4 mg/L. Subsequently, each of 1.5, 2.5, and 4 mL of stock solution was added to water samples for coagulation.

2.4. Preparation of *M. oleifera* extract

M. oleifera seeds were grinded after barking. 1,000 mg of *M. oleifera* powder was added to a 1 L of 1 M NaCl solution. The solution was mixed for 20 min. Then, the mixture was passed through a 0.45 μm fiberglass filter and stored for usage [41].

2.5. Application of both coagulant solution

After that, six samples in 1 L volume were prepared from main sample. $FeCl_3$ solution was added to three of samples in 1.5, 2.5, and 4 mL/L. *M. oleifera* extract was added to the three remaining samples in 1.5, 2.5, and 4 mL/L. The samples were then mixed in a test tube by 100 revolutions per minute (RPM), during 30 s. Then, the samples were mixed during 30 min by decreasing trend in RPM as gradient mode according to following pattern: 0–10 min = 40 RPM, 10–20 min = 20 RPM, and 20–30 min = 10 RPM. Finally, the samples were kept in ideal situation for 1 h for sedimentation of formed flocs after which the microbial load of samples was examined by standard methods.

2.6. Statistical analysis

For statistical analysis of achieved data, the statistical distribution of data for both coagulants efficiency was analyzed by one sample Kolmogorov–Smirnov test ($\alpha = 0.05$). The analysis has shown that the statistical distribution of data does not follow normal distribution. Therefore, nonparametric statistics test must be used for data analysis. In order to compare different coagulants efficiency in terms of concentration and bacteria type, the *Kruskal–Wallis* test was used. The mean of removal efficiencies was compared using *Mann–Whitney U* test. All of the tests were done by significant level equal to 0.05 ($\alpha = 0.05$).

3. Results

Fig. 1 shows the results of jar test for determination of suitable range of $FeCl_3$.

As shown in Fig. 1, increasing in turbidity removal efficiency in $FeCl_3$ concentration more than 4 mg/L is less than 5%. Therefore, the range of $FeCl_3$ for the test was chosen less than 4 mg/L. Also, equal *M. oleifera* extract dosage was chose for comparison of both coagulants.

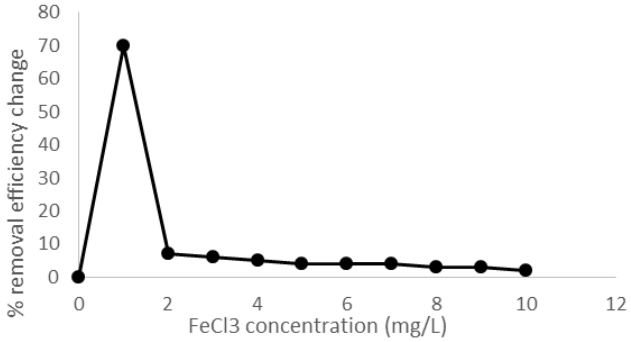

Fig. 1. Trends of change in turbidity removal efficiency per each milligram increase in $FeCl_3$ dosage as coagulant.

Table 1 summarizes both coagulants efficiency for reduction of various kinds of microorganism.

The *Kruskal–Wallis* test on removal efficiency of total and fecal coliforms between applied different concentrations of $FeCl_3$ was shown significant difference between them (P-value < 0.05). Similar results were observed about application of different concentrations of *M. oleifera* extract (P-value < 0.05). Both of coagulants were shown significant differences in removal efficiency of *C. perfringens* just between 1.5 and 4 mg/L coagulant concentration. In addition, the Kruskal–Wallis test on $FeCl_3$ efficiency was illustrated significant lower efficiency in removal of *C. perfringens* compared with other types of bacteria. Also, in application of *M. oleifera* extract this statistical analysis showed significant differences in removal efficiency of *M. oleifera* extract between coliforms and *C. perfringens* (P-value < 0.05). In addition, comparison of both coagulant with *Mann–Whitney U* test showed no significant differences between them in removal efficiency of total and fecal coliforms, but their efficiency in removal of *C. perfringens* was significantly different.

4. Discussion

Previous studies on coagulants efficiency in removal of bacteria reported a wide range of performance according to coagulation's variations such as pH [42,43]. As shown in Table 1, there isn't any significant differences between *M. oleifera* extract and $FeCl_3$ efficiency in removal of total and fecal coliforms. However, *M. oleifera* has shown significant difference in removal of *C. perfringens* comparing with $FeCl_3$. *M. oleifera* extract has bactericidal properties, but the bacterial

Table 1
The efficiency of both applied coagulant for removal of microbial load

Bacteria	Coagulants and dosage (mL/L)		*N*	Mean of % removal	S.D.	*P*-value
Total coliform	$FeCl_3$	1.5	3	78.2	3.79	0.218
		2.5	3	84.55	4.46	
		4	3	93.95	4.95	
	MO	1.5	3	83.78	5.31	
		2.5	3	92.45	3.9	
		4	3	97.4	6.94	
Fecal coliform	$FeCl_3$	1.5	3	90.8		0.126
		2.5	3	92.6		
		4	3	97.5	4.53	
	MO	1.5	3	83.76		
		2.5	3	90.98		
		4	3	98.2	7.28	
Clostridium perfringens	$FeCl_3$	1.5	3	62.35		<0.05
		2.5	3	65.6		
		4	3	70.2	14.12	
	MO	1.5	3	77.18		
		2.5	3	81.64		
		4	3	88.62	9.05	

spores are highly resistant against disinfectants and antibiotics [44]. Thus, it is unlikely that *M. oleifera* extract to be able to inactivate them. Therefore, the main reason for significant removal of *C. perfringens* can be surface interaction between spore external surface and the bulk of flocculation formatted by *M. oleifera* extract in water. The *C. perfringens* endospores have a *peptidoglycan* coat. On the other hand active agent in *M. oleifera* extract is a polypeptide, so, it seems that endospores have tendency to adsorption with *M. oleifera* extract. In this study we couldn't distinguish between destroyed spores and adsorbed spores. But, future studies can specifically focus on this matter. For this matter in vitro studies can be useful.

The most important issue in application of any chemicals for drinking water treatment is human toxicity. Several studies reported some toxic features for the compounds in *M. oleifera* extracted coagulant such as lectin [18,45–47]. De Oliveira et al. [47] mentioned that coagulant *M. oleifera* lectin (cMoL) is a strong fungicide against moth flour. In coagulation of raw water during water treatment, the formatted floc because of interaction between colloidal material and coagulant agent will be precipitate and can be easily removed from the water. Although it may remain as coagulant residual in the finished water. Therefore, measurement of lectin residual in the water is necessary before the judgment about toxicity of cMoL in the water. Besides, determination and definition of human health risk induced by lectin needs to be focused on toxicological and epidemiological studies. Another main challenge in use of organic coagulants like cMoL for water treatment is increasing of total organic material (TOC) in waters. This is an important issue due to probability of chlorine reaction with them and formation of DBPs such as THMs. In application of *M. oleifera* for this purpose, some studies have reported that this extract can increase TOC content of water, unless the extract production be consist of well purification step [23]. Therefore, there is not any risk of DBPs formation because of this extract usage. Furthermore, several studies reported well performance of this extract in removal and decreasing of raw water TOC content [48].

5. Conclusion

M. oleifera extract is a compatible coagulant with metallic coagulants for decreasing microbial load. In addition, this extract has better application in removal of bacterial spores such as *C. perfringens*. Though, it is not clear that the well performance of this extract is due to spores' distraction or adsorption of spores. Although by well purification of *M. oleifera* extract, any increasing in water TOC content doesn't happens, however this extract can remove humic acids from raw water and decrease the potential of DBPs formation.

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