Moringa Oleifera seeds and Chitosan as alternatives to conventional coagulation agents

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Abstract. This article discusses the possibilities of using natural polymers in the water treatment process to replace conventional chemical-based reagents. Mainly part of article presented the results of a laboratory-scale research focused on the use of alternative coagulants in water treatment. Attention was particularly paid to natural cation coagulants derived from Moringa oleifera seeds and chitin. These two coagulant agents were used in laboratory research for treating real raw water. Several series of laboratory jar tests were performed to identify the effectiveness of the selected coagulants in removing turbidity from water.

1. Introduction

The introduction of a coagulation process is often required for treating water from surface sources to decrease turbidity and color, as well as the volume of pathogenic organisms. The process may also be optimized for the removal of natural organic matter (NOM) and heavy metals [6]. Various coagulant agents are used for the process and the selection of a suitable coagulant depends upon the type of substance removed, as well as the overall water treatment process [8]. Coagulation may be combined with other physical, chemical or biological processes.

Coagulants are primarily divided into the organic and inorganic. Inorganic coagulants are metal salts, or possibly in a hydrolyzed polymer form. Organic coagulants are available in many types of substances and create long chains. Coagulants may further be divided based on their charge into cation and anion and, according to their origin, divided into synthetic or natural.

Salts of aluminum and iron appear to be most commercially successful. However, in recent years, polymer coagulants are also used [25], most often as auxiliary agents used to increase the efficiency of water treatment on the one hand and decrease costs on the other.

Various coagulants display differing efficiency in destabilizing individual pollutants [18]. Therefore, the properties of the raw water must be known in order to establish the type and volume of the necessary coagulant. The degree of concentration also affects the potency of their coagulation.

Choice of coagulants primarily depends upon the composition of the water treated, including its pH, temperature, oxidation-reduction potential, volume of substances suspended in the water, etc. The choice of an optimal destabilization agent must always be made based on laboratory tests, ideally by testing under partial operating conditions. The size of floccules is the major physical parameter affecting the treatment process. Another important property is the resistance of these floccules to decomposition into smaller parts. Smaller parts decrease the effectiveness of the subsequent separation, as they settle more slowly than larger particles of similar density [18].
2. Inorganic coagulants, their properties and use

2.1. Metal coagulants
Metal salts are probably the most frequently used coagulants in practical water treatment. These are most often trivalent compounds of aluminum and iron [8]. The effectiveness of their coagulation is affected by dosage, the method of homogenization, pH and the properties of the substance removed [29]. Water temperature is also an essential factor for their function [9], particularly in the case of aluminum-based coagulants. Satisfactory coagulation effectiveness will not be achieved at low temperatures during the colder part of the year, even by increased doses of the agent.

Inorganic coagulants are typically dosed in higher concentrations, resulting in the production of a larger volume of sediment. A certain amount of the metal cation of the respective salt remains in such treated water. Metal coagulant residues alone pose certain health and/or environmental risks.

The disadvantages of inorganic salts, along with their sensitivity to seasonal changes in the physical properties of raw water (pH and temperature) as well as their minimal effectiveness in removing fine particles, brought a demand for different types of coagulants. Effectiveness is improved by polymerization of inorganic coagulants and their use in water treatment [29].

Examples of metal coagulants used in water treatment are iron(III) sulfate, aluminum sulfate, aluminum chloride, iron(III) chloride, as well as titanium disulfide and zirconyl chloride octahydrate [11].

2.1.1. Pre-hydrolyzed metal coagulants
To minimize the disadvantages of inorganic coagulants, they may be hydrolyzed, creating an inorganic polymer with improved coagulation properties. But generally, we could say that they are much more effective than the monomers of salts from which they are produced. Among their most frequently mentioned benefit is a higher effectiveness at lower dosage. Because a smaller amount of the coagulant is used during the water treatment, a far lower volume of sludge is produced [12], nor do they leave as many residual cations in the treated water. Unlike their monomers, they function within large ranges of pH and are less sensitive to temperature [21]. These groups of coagulants include the frequently used polyaluminium chloride (PAC), as well as polymers of iron(III) sulfate, polyaluminium silicate-chloride and polyaluminium silicate sulfate.

3. Organic polymers as coagulation agents
Natural or synthetic macromolecular water-soluble substances are primarily used as destabilizing or auxiliary aggregation agents (flocculants). The use of polymers enables faster floccule sedimentation (the formation of floccules with stronger, denser structure) and improved filterability of the formed suspension. Further, polymer flocculants decrease the content of organic substances, including turbidity as well as residual coagulant, thus improving the ultimate quality of the treated water. The use of these substances also enables a decrease in dosage of inorganic coagulants.

Unlike metal coagulants, organic polymers function within a larger pH range, while having no effect on the pH of the treated water. Their use produces formations that are large, compact, thick and display good sedimentation properties. Unlike metal coagulants, organic polymers leave no metal cations in the treated water. High effectiveness is achieved even at relatively small doses of the coagulant agent, reducing the production of sludge [21]. The addition of an appropriate polymer to an inorganic coagulant may significantly improve its effectiveness in removing impurities, because it forms larger thicker floccules with better sedimentation properties [4].

Organic polymers are also effective in removing small-size particles. They have a double effect on coagulation – aside from charge neutralization, they also have a bridging effect [7]. Bridging is specifically most notable in long molecules with large molecular weight. During the reaction, the polymer is bound to the polluting particle in several places and the loops or loose ends of the polymer float in space, ready to bind to another particle. Among other factors, the effectiveness of coagulation is determined by the dosage of the agent. Should the dose be too low, a certain ratio of impurities remain
in the water. To the contrary, should the dose be too high, the pollutants are dispersed again and the polymers subsequently plug the filters [3].

Organic polymers are currently used primarily as auxiliary coagulation and flocculation agents [15], although they may also be used as primary coagulants. Wider use of organic polymers is prevented by their higher cost. Currently, they are also a center of focus regarding possible health risks [30].

Organic polymers may primarily be divided into either 

**synthetic or natural.** Depending upon their overall charge they further divide into cation, anion, and charge neutral. Synthetic organic polymers (such as cation polyacrylamides) are usually very efficient coagulants, but unlike natural polymers, they tend to be more costly, are not biodegradable and show certain dangerous properties (toxicity, carcinogenic properties). Natural organic polymers are somewhat less effective, but are biodegradable, not toxic and do not produce any secondary pollution [21].

4. Natural polymers

Over time, there has been an effort to replace classic coagulants with natural non-toxic and environmentally-friendly substances. The reason may be the high cost or limited availability of commonly used coagulants (aluminum sulfate, iron(III) sulfate, synthetic polymers etc.), the requirement of highly effective doses of these agents, production of an notable volume of sludge [12] and the undesirable toxicity of the residual coagulants that remain in the treated water.

The use of seeds of certain plants (for example *Lens esculenta, Tamarindus indica, Cyamoppsis psoraloides*) presents a cost-effective, practical and suitable solution for water treatment in developing countries and beyond. A certain preparation is necessary in order to use these seeds, including drying, grinding and dilution with water.

4.1. Cation polyelectrolytes

Strong cation polyelectrolytes have a positive charge regardless of the pH of the solution. To the contrary, weak cation polyelectrolytes have a positive charge only in an acidic environment, as covered [4]. Natural coagulants were used long before chemical salts. But in the end they were pushed to the background, as the use of salts appeared to be an easier and better solution to treating drinking water. In this regard, with the increasing necessity to treat water in developing countries, natural polymers are returning to the center of attention [23]. Natural polymers are easily available, cost-effective and easy to handle.

Among the common natural cation polymers are the **polysaccharides.** The main advantage of polysaccharides is that they are biodegradable, although they are the least effective and must be used in high concentrations. A combination of synthetic polymers onto polysaccharides forms a branched polymer with improved effectiveness [4].

The most frequently used natural cation polymer is **chitosan.** Chitosan is not water-soluble and must therefore be dissolved in a solution of a carboxylic acid, such as acetic acid [22].

Among other natural cation polymers are both starch and tannins [23]. Tannins include many secondary metabolites of plant origins. They are found in bark, leaves or seeds of plants. A very promising cation polymer among the tannins is an extract obtained from the seeds of the *Moringa oleifera* tree, containing a cation protein. The main advantage of this extract is that it is not toxic for either humans or animals, while serving as a rather effective coagulant. Further, it does not cause problems with corrosion and produces less sludge. Compared with aluminum or iron salt, the extract shows a lesser effect in removing turbidity. Treatment of water with this extract increases the content of organic substances (particularly orthophosphates and nitrates) in the treated water. These residue substances may worsen the organoleptic properties of water, such as color, taste and odor [4, 16, 19].

Other options for water treatment use extracts from the seeds of the European horse-chestnut (*Aesculus hippocastanum*), English oak (*Quercus robur*), Australian oak (*Quercus cerris*), Northern red oak (*Quercus rubra*) and sweet chestnut (*Castanea sativa*). The concentration of the obtained cation proteins are approximately ten-times less than in the case of *Moringa oleifera*, yet, despite this, good coagulation properties were confirmed [5, 24].
Organic cation polymers may be used both as auxiliary coagulation-floculation agents and as primary coagulants. Coagulation properties of cation polymers used as primary coagulants may additionally be improved by the addition of insoluble solid substances such as clay or metal oxides (iron, aluminum and manganese oxides). For example, chitosan alone has a low efficiency in removing turbidity and color, but when mixed with bentonite, its effectiveness improves synergically. Solid particles function as sorbents for NOM and, simultaneously, as a core for condensing a NOM-polymer complex.

4.1.1. Moringa oleifera tree seeds

*Moringa oleifera* is an oil-producing tree originally from Northern India (Himalayas), currently spread across tropical regions worldwide. The plant is notable for its high resistance to drought. *Moringa* seeds are composed of approximately 31% protein, 18% saccharides and 37% fats. Powder from ground seeds contains soluble proteins capable of floculating turbidity contained in water. The solubility of these proteins is increased with the content of salts in water. Sodium chloride (NaCl) is thus sometimes used during laboratory preparations of the coagulation agent. The coagulation effect of the extract prepared with the use of salt shows greater effectiveness than agents prepared using only tap or distilled water. Studies prove that, in the case of *Moringa oleifera*, adsorption, charge neutralization and formation of polymer bridges between particles are the predominant mechanisms for removing turbidity [13, 26].

The processes of preparing a coagulation agent from *Moringa oleifera* seeds may differ. Nonetheless, the polyelectrolyte used for water treatment is in most cases obtained by grinding dried seeds from the tree and mixing them with water. The proteins with predominantly positive charge on their surface are thus transferred into the water solution. A typical dose for preparation ranges from 10 to 50 grams of ground seeds per liter of water. The suspension of seeds with water is shaken intensively to allow the development of molecules, followed by filtration. The dosage of coagulation agent ranges from 75-200 mg·l⁻¹ depending on the properties (particularly turbidity) of treated water [16].

In case cooling of the suspension is possible, its effect is extended by up to one week. Otherwise it is necessary to prepare a fresh suspension daily. The effect of *Moringa oleifera* extract in removing turbidity from water decreases by extending the storage period of the prepared coagulation agent [26].

4.1.2. Chitosan

Chitosan is a substance insoluble in most organic solvents. However, it dissolves in both organic and inorganic acids (such as acetic acid, formic acid, etc.). This polysaccharide is produced through an alkaline deacetylation of chitin, which is found in the outer shells of crustaceans, insects and gastropods, as well as within the cellular walls of yeast and certain fungi. Chitin and chitosan may be produced from the waste products of processing marine animals [28]. Chitosan produced for the purposes of water treatment is typically prepared using acetic acid where it is a subject of hydrolysis.

Chitosan is a cation polyelectrolyte used for the reduction of water turbidity [20], sorption of metal ions, as well as removal of organic pollution. Destabilization primarily takes place through the mechanism of charge neutralization. Chitosan is a biodegradable substance and its use in water treatment is therefore more environmentally friendly than commonly used coagulants. The frequent use of chitosan is increasing in recent years. This substance is used for example in Norway, where it was selected as a coagulant in several water treatment facilities, used either exclusively or in a combination with metal-based coagulants [20].

Chitosan has many advantages in comparison with traditional coagulants (floculants). Among them are effectiveness in low doses, success in the reduction of chemical oxygen demand (COD), the ability to remove metal ions, the formation of large (voluminous) flocules with higher sedimentation rates, biological degradability, major effect in the removal of algae and suspended particles, anti-bacterial effects and zero formation of secondary pollution [1]. Chitosan is capable of binding both fats and metals, including arsenic, molybdenum, cadmium, chromium, lead and cobalt [14].

Many studies have been conducted regarding the use of chitosan as a single coagulant or in combination with other agents in removing turbidity [2, 20].
Chitosan, as a positively charged polymer with long chains, is able to coagulate negatively charged and finely dispersed colloidal particles through adsorption, charge neutralization, hydrophobic effect or through the formation of polymer bridges. Considerable savings in terms of the volume of agents used may be achieved by using such natural coagulants in combination with the more classic forms, resulting in additional cost reductions for sludge removal, as it is produced in smaller quantities [1].

A coagulation jar test was [20] performed using raw water from the Evans Lake in California, comparing the effects of chitosan, aluminum sulfate and iron(III) chloride. Upon performing the coagulation, sedimentation and filtration using filtration paper, the resulting values of turbidity reduction were 98.8% (aluminum sulfate), 96.9% (chitosan) and 98.9% (iron(III) chloride). The removal of coliform bacteria was most effective with iron(III) chloride and least effective using chitosan (upon optimal doses of coagulants and pH values). Chitosan was the most effective of all agents used in removing color and also caused only minor water softening (by 22%). In the case of chitosan, excessive doses caused re-stabilization of the dispersion. Optimum pH must be kept in mind when treating raw water into drinking water.

5. Methods

Our laboratory research focused on establishing the effect of selected natural polymer coagulants originating from the seeds of the Moringa oleifera tree and chitosan. The effectiveness of both these agents was compared to a conventional coagulant – aluminum sulfate (50% solution).

5.1. Raw water

Raw surface water sampled from the Svratka River was used in the laboratory test. The research used a volume of approximately 30 liters. At the time of testing, the raw water had a temperature of 18.2° C, turbidity 8.92 (NTU) and a pH value of 7.85. The pH value was adjusted using sulfuric acid to a value of 7.5. This value was stipulated with regard to previous tests with aluminum sulfate.

Our research was performed with a standard jar test using a blending column with a paddle blender. Turbidity was used as the indicator of effectiveness. Aside from turbidity, the parameters of water temperature and pH were also monitored. Process parameters were identically maintained for all three agents with the particular goal of verifying whether other conditions of an actual application process would require adjustment upon changing the coagulant.

Fast blending was first performed for a duration of 2 minutes at the rate of 150 rpm, followed by slow blending at 40 rpm for 20 minutes. Subsequently, a 60-minute undisturbed sedimentation completed the process.

5.2. Moringa oleifera seeds

A coagulant was obtained by processing 9 pieces of Moringa oleifera seeds and used in a series of tests. The white rounded cores under the peel were pulverized in a mortar and pestle to powder form. The amount obtained was transferred to a porcelain bowl and weighed. The weight of the agent amounted to 1.262 g.

A coagulant was prepared by mixing the powder from the seeds with 306 ml of drinking water (the additional 6 ml were added due to loss of water in the subsequent filtration by being absorbed by the cloth). The suspension was intensively shaken for the duration of 5 minutes and subsequentley filtered through a clean cotton cloth to separate larger particles. A dose ranging from 2.5 to 42.5 ml of the solution was added to the individual vessels, representing 20.6 to 350.9 mg of powder.

5.3. Chitosan

The effect of chitosan in removing turbidity was examined last. The agent was used in the form of 25 g of white powder – chitosan originating from shrimp shells. Chitosan was dissolved in acetic acid (99%), that was subsequently mixed with 32.6 ml of drinking water and used in the form of a solution at the concentration CH₃COOH 1 mol·dm⁻³. Subsequently, 0.1 g of chitosan was added to the acid solution. This formed a liquid of a rather thick consistency that was further diluted with drinking water to a 100
ml volume. This coagulant (100 mg of chitosan in 100 ml of the agent) was dosed to the vessels in an optimization jar test.

6. Results
Our research focused on the comparison of effectiveness in reducing turbidity after applying three coagulants used in water treatment. The selected coagulants were aluminum sulfate commonly used as a conventional coagulant, as well as chitosan and Moringa oleifera as alternatives. An optimization jar test was performed with the tested aspect being turbidity. The research was carried out in the laboratory format as a series of jar tests.

6.1. Aluminum sulfate
Aluminum sulfate was selected as representative of conventional agents used in water treatment. The purpose of its use in this research study is primarily as a comparison with the other two agents that are not as frequently used and practically never in the Czech Republic.

The authors of this article have extensive previous experience with this coagulant in relation to raw water used in this research. As is apparent from previous jar tests, the optimum pH for turbidity removal is 7.5, the rate for the first phase of mixing is 150 rpm (for the duration of 2 minutes) and the rate for the second phase of coagulation is 40 rpm (for 20 minutes). The same values were also used for the comparison tests with the other agents. The results are apparent from Table 1 and Figure 1.

| Sample | Dosage | Turbidity | Turbidity removal |
|--------|--------|-----------|-------------------|
|        | Aluminum sulfate | Al | @ 60 min | [ml] | [mg] | [mg] | [NTU] | [%] |
| 1      | 0.050  | 65.5      | 2.6     | 3.56  | 60.09 |
| 2      | 0.075  | 98.3      | 3.9     | 2.13  | 76.12 |
| 3      | 0.100  | 131.0     | 5.2     | 0.94  | 89.46 |
| 4      | 0.115  | 150.7     | 6.0     | 0.46  | 94.84 |
| 5      | 0.130  | 170.3     | 6.8     | 0.65  | 92.71 |
| 6      | 0.175  | 229.3     | 9.2     | 0.85  | 90.47 |
| 7      | 0.220  | 288.2     | 11.5    | 1.00  | 88.79 |
| 8      | 0.265  | 347.2     | 13.9    | 1.26  | 85.87 |

The lowest measured value of turbidity after sixty minutes of sedimentation was 0.46 NTU in case of 0.115 ml dose of aluminum sulfate. The decrease of NTU by 8.46 shows removal of turbidity at 94.84 %. The worst result was recorded at doses of 0.050 ml – the value of the turbidity 3.56 ZF and turbidity removal at only 60.09 %.
A large number of discernable floccules formed when using aluminum sulfate, even in the early stage of fast blending. The most aggregates were formed in the water to which most of the agent was dosed. Upon 60 minutes of sedimentation the floccules settled at the bottom, where they formed sludge of a light brown color. The optimal dose of aluminum sulfate 150.7 mg·l\(^{-1}\) managed to reduce the turbidity by 94.84 % to a value of 0.46 NTU.

6.2. *Moringa oleifera*

The coagulant obtained from *Moringa oleifera* seeds by the method described above was used to treat water of identical quality as above and also within a laboratory-scale test. The same parameters were set for the coagulation test. The formation of small light-colored floccules in the treated water was recorded as early as the slow blending stage.

The amount of these small observed clusters grew with increased amounts of the coagulation agent. However, the floccules remained suspended in the volume of the vessels throughout the entire phase and were only partly sedimented. Only a portion of the floccules settled on the bottom, along with sediment. Despite this, lower levels of turbidity were measured than prior to water treatment. Results are shown in Figure 2.
As is apparent from the above results, the most effective dose of *Moringa oleifera* for turbidity removal was 164.6 mg. The initial turbidity value was reduced to 1.60 NTU, representing the removal of 82.04% of turbidity. The lowest value of turbidity removal was measured at the dose of 20.6 mg of *Moringa oleifera*. Here, the turbidity value only decreased by 0.01 NTU.

When using *Moringa oleifera*, the flocules formed during coagulation were of pale color. They were visible to the naked eye only in the second phase of blending. The sedimentation of the aggregates took place very slowly. Even after one hour of sedimentation, there were no apparent changes. The optimum dose was 164.6 mg·l⁻¹, which is the largest in comparison with both aluminum sulfate and chitosan. Better results of turbidity removal could apparently be achieved by prolonging the sedimentation period or using filtration.

6.3. Chitosan

The next coagulation tests were using chitosan as an agent – also under the same conditions. The formation of flocules in raw water was visible from approximately the middle of the slow blending phase. The small aggregates had a light brown color and settled more quickly than the aggregates using *Moringa oleifera*. Although most flocules settled after 60 minutes of sedimentation, a portion of the flocules remained suspended. As regards turbidity removal, the resulting values are shown in Figure 3.
In the case of chitosan, the optimal dose was low—the mentioned 0.8 mg·l$^{-1}$. At this dose, the remaining turbidity measured had a value of 1.73 ZF, representing 80.66% less than measured in raw water. In the jar test, the effect of chitosan was only visible during the second half of slow blending. Very tiny light brown flocules were formed in the vessels and their amount was in direct proportion to the coagulant dose. The aggregates showed much better sedimentation properties than in the case of Moringa oleifera.

7. Conclusions and Discussion
The laboratory experiments described evaluated the removal of turbidity from a water environment using a coagulation process with three different coagulation agents. As is apparent from Figures 1-3, the course of coagulation and flocculation during the optimization jar test, as well as the dependency of the agent dosage on turbidity removal, were different for each agent.

The most effective coagulant in terms of turbidity removal under the given conditions is without doubt aluminum sulfate. But in case of chitosan, the much lower optimum dosage compared to the other two agent is noteworthy (Table 2).

| Coagulant      | Optimal Dosage [mg] | Turbidity [NTU] @ 60 min | Turbidity removal [%] |
|----------------|---------------------|--------------------------|-----------------------|
| Aluminum sulfate | 150.7               | 0.46                     | 94.84                 |
| Moringa oleifera    | 164.6               | 1.60                     | 82.04                 |
| Chitosan           | 0.8                 | 1.73                     | 80.66                 |

The best results in removing turbidity from raw water were achieved by aluminum sulfate. However, the use of aluminum sulfate in water treatments from secondary products had related health risks. The results of the experiment using Moringa oleifera are less impressive, although removing turbidity by 82% indicates a very good overall effectiveness of this agent. Better results could be obtained through different preparation of the coagulant (the use of NaCl, distilled water). In the case of chitosan, a slightly
better result was expected. However, the reduction of turbidity by 80.66% indicates very good properties of this polyelectrolyte in removing turbidity from raw water. Chitosan efficiency may be supported by using it in combination with traditional coagulants.

Studies researching this version prove that the application of certain amounts of substances with natural origins reduces the necessary optimal amounts of commonly used coagulants. Water treatment using a combination of both substances is more environmentally friendly. Lesser amounts of harmful residual products are formed during the course of such process (residual Fe and Al) and the amounts of necessary agent are reduced, resulting in savings as the volume of the formed sludge is reduced.

The authors of this study are aware that the results in case of Moringa oleifera seeds and chitosan might even be better, as the jar test conditions were optimized for aluminum sulfate. In the case of a possible replacement of an existing coagulant (aluminum sulfate) by an alternative in a real-application situation, it would be necessary to also adjust the parameters of the coagulation process, such as blending rates and the period of placement in blending and separation basins.

An interesting criterion for discussions of possible replacements of conventional coagulants for natural polymers may also be their cost. The prices of all three coagulants are subject to fluctuations depending on the location of use. Our research did not establish the price of the optimum dosage, as the natural polymers used for the purpose of our study were obtained under price-inefficient conditions at low quantities.

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