International
Journal
of Advanced Research in Engineering& Management (IJAREM)
ISSN: 2456-2033 || PP. 19-25

Impact of Heat-treatment and Storage Duration on Kenaf Seed Extracts in Water Treatment

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Abstract: Access to clean drinking water is a fundamental human right. Yet, improved drinking water is still a major challenge in many developing countries today, retarding progress and human development. This study investigated the coagulation performance of denatured kenaf crude extract (KCE) in water treatment using a jar tester. To assess its coagulation performance, KCE suspension samples were heated at 60, 97 and 140 °C for 2, 4 and 6 h while the remaining samples were stored for 3, 5, 7, 10 and 14 days respectively prior to the test. The results show that maximum turbidity removal of approximately 98, 97 and 99% was achieved with sample heated to 60, 97 and 140 °C from 86% recorded with the unheated sample. Interestingly, KCE which had been stored for a 3-day duration achieved maximum turbidity removal of 84% compared with the 88% recorded with the fresh sample, although at a dosage, far lower (40 mg/l) than that of the fresh sample (80 mg/l). Therefore, the use of denatured KCE by heating is advantageous for people in developing countries which resulted in improved turbidity removal performance. Conversely, storing the crude sample did not improve the efficiency but its performance was remarkable at lower dosage.
Keywords: Extract, kenaf seed, water treatment, developing countries, turbidity

1.0 Introduction

Many developing countries are faced with drinking waterchallenges because of cost issues related to potable water production. Water treatment is capital intensive and the quality of river water is varied due to suspended and colloidalparticles load resulting from human activities and high storm and runoff events (Ali et al., 2010). In many parts of the world, highly turbid river water is used for drinking purpose thereby posing some serious health challengeto the population, especially those living in rural areas who are the most vulnerable.

To address this challenge, the united Nation came up with 17 goals, tagged Sustainable Development Goals (SDG) in order to improve upon the achievement of themillennium development goals (MDGs) by the year 2030. One of the key aspects of the SDG is the implementation of the Integrated Water Resource Management (IWRM) following the MDG goal 6 to half the population without clean drinking water by 2015. The 2030 SDG agenda (for goal 6) recognizes the importance of clean drinking water in bringing development in other areas such as health, education and poverty reduction (UN, 2016). Additionally, the SDG is to address holistically the water management cycle with more countries facing the challenge of being below the 25% threshold, which is the first stage of water stress (UN, 2016).

In view of the above, there are ongoing studies to improve water supply in developing countries using non-conventional materials. In this regards, severalnaturally-occurring macromolecules,especially proteins, polysaccharides and mucilage have received considerable interest as potential water and wastewater treatment materials(Agarwal et al., 2003, Anastasakis et al., 2009). Study has shown that natural extracts are biodegradable and cost-effective and are readily available(Agarwal et al., 2003). Among the natural extracts, *moringa oleifera* (MO) is the most studied to date(Jahn Samia, 1998, Ghebremichael et al., 2005, Ingale and Gandhi, 2016).Furthermore, several animal products havebeen widely used in water purification. Kawamura (1991)investigatedthe application of chitosan, a cationic compound, and sodium alginate, a natural polysaccharide(anionic compound) of animal origin, as coagulants in water treatment which resulted in improved water qualitycomparable to that of alum at a similar dose. Rural people have used animal-based products such asisinglass and chitosan obtained from shredded sturgeon fish and crustaceans respectively to treat contaminated water (Bratby, 2006, Choy et al., 2015).Hu et al. (2013)also used low doses of chitosan in

combination with AS to produce successful turbidity removal with low sludge volume compared to sludge generated by alumas a primary coagulant.

The work reported here aimed to investigate the application of kenaf seed extract as alternative coagulant to the traditional chemicals in water treatment. Additionally, its potential in terms of turbidity removal in water is of interest.

Kenaf *(Hibiscus Cannabinus* Linn) is a member of the *Malvaceae*family plants. It is an annual herbaceous plant that grows under a range of weather and climatic conditions (Meints and Smith, 2003). It is widespread in most tropics and is found in Ethiopia, Zimbabwe, Mozambique and Uganda (Katende et al., 1999). It is also widely distributed in Cameroon (Agbor et al., 2005) and in northern Nigeria where both the leaves and the seed are consumed as a vegetable soup (Meera and Agamuthu, 2012).

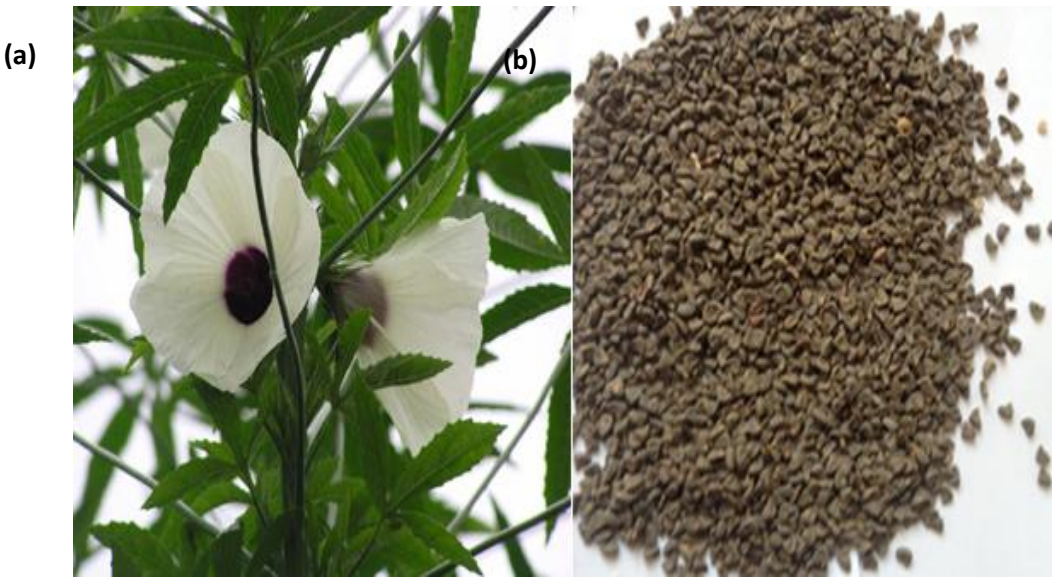

Figure 1 Kenaf flowering plant (a) with fruits and (b)dried seed kernels.

Additionally, the plant has appreciable protein contents which is processed and used by village women to improve breast milk.Mariod et al. (2010)characterized the concentration of protein in defatted Kenaf seed using petroleum ether and found the percentage of protein in the seed to be 13.04%. The protein istraditionally used in the preparation of coffee and other local food (Falasca et al., 2014).Agbor et al. (2004) reported the evidence of phenol, tannin, saponin, alkaloids and steroids in kenaf seed. Recently, similar observation was also reported byCheng et al. (2016) but included essential oil and fatty acids. The presence of protein and other compounds in Kenaf seed makes it imperative to be evaluated in water treatment as coagulant. Some properties such as saponin and RIP can inactivate microbes as they foam when in contactwith water (Shaheed et al., 2009).

2.0 Materials and methods

2.1 Collection of Kenaf seeds

Kenafseeds were obtained in Hawul local government area of Borno State-Nigeria. Fresh dried seeds were sorted, packaged and labelled appropriately for ease of identification and transported to the Civil Engineering laboratory at the University of Birmingham, UK for processing, preparation and analysis. The seed was cleaned by washing with tap water and damaged seeds removed. The cleansed seeds were then dried in an oven at 60ºC for six hours before grinding.

2.2 Extraction of the active coagulant compound

Kenaf seeds were ground to fine powder using a laboratory disc miller (Tema mill, Germany) for two minutes to obtain the desired powder. The seed powders obtained were sieved through a set of stainless steel sieves (600 to 212µm) according to (Jones and Bridgeman, 2016). The powders retained on the 212 and 300µm were combined and used in the study.

One mole sodium chloride (1.0 M NaCl) solution was used in this experiment to extract the coagulating compounds in the seed. KCE was prepared from the ground seed powders according to Jones and Bridgeman (2016) where 1.0 M NaCl solution was added to the seed powder to make 2% (w/v) suspension, i.e. 2g of the seed powder in 100 ml NaCl. The suspension was vigorously stirred using a magnetic stirrer for 15 min at room temperature (19±2ºC). The suspension was then centrifuged at 4000 rpm for 10 min using a Heraeus Megafuge16 (Thermo Scientific, Germany). The suspension was decanted, and the residual solids were dried in an oven at 50ºC overnight. The weight of the dried solid material was measured to ascertain the amount of seed powder used in making the suspension. The decanted suspension was then filtered through a Whatman No. 42 filter paper and the filtrate used as coagulants.

2.3 Denaturation process adopted

All processes were adopted from Jones and Bridgeman (2016) where Kenaf salt extracts were heated at temperatures of 60, 97 and 140^{o}C for 6, 4 and 2 hrs respectively, using a hot plate. The heated samples were then centrifuged at 4500 rpm for 10 min and were filtered through a Whatman no. 42 filter paper. Similarly, the extract was stored for 1, 3, 7, 10 and 14 days to denature the extract.

2.4 Preparation of the synthetic turbid water

This preparation was similarly adopted from Jones and Bridgeman (2016) in which turbid water samples for the jar test experiments were prepared by adding kaolin particles into tap water. 40g of laboratory grade kaolin (Fluka and high grade, Sigma Aldrich) was added to 400ml of tap water and the suspension stirred for 30 min using a magnetic stirrer. The suspension was made up to 1L by adding 600ml of tap water and then stirred for further 30 minutes. The suspension was allowed to stand for 24hr for the kaolin to hydrate. The suspension was vigorously mixed for five minutes and the contents mixed with 30 litres of tap water and allowed to stand overnight for particle settlement. The supernatant was decanted, and its turbidity measured. Depending on the level of turbidity required, the supernatant was either diluted with tap water or concentrated with kaolin suspension.

2.5 Running the Jar test experiment

Jar tests were conducted using a standard apparatus comprising 6 number of 1 litre beakers (Phipps and Bird, 7790-900B USA) to evaluate the optimum coagulant dose for the coagulation tests according to (Jones and Bridgeman, 2016). For effective dispersion of the coagulant the water was rapidly mixed at 200rpm for 1 minute during which various doses of the coagulant were added to the beakers. The mixing speed was then reduced to 30rpm for another 30 minutes to simulate the flocculation stage. The suspension was then allowed to stand undisturbed to facilitate settlement for 1hour. A final treated water sample (10 ml) was drawn 2cm from the top surface of the water in the beakers using a syringe. The turbidity of the water was then measured using a turbidity meter (HI 93703, Hanna) and the water pH was measured with a pH meter (Mettler Toledo SevenGO, Switzerland). All experiments were conducted at room temperature (19±2ºC) in triplicates.

3.0 Results

Laboratory measurements were conducted on the coagulation performance of KCE in a water sample with a turbidity of 200 NTU. Table 1 presents the effects of temperature on a stock solution of KCE which was heated to 60, 97 and 140 °C for 6, 4 and 2 hours respectively. Different doses (10 – 100 mg/l) of the extract were applied in the coagulation test. The results revealed that, water sample which had been treated with the heated KCE at 140ºC for 2 hours achieved the highest turbidity reduction of approximately 99% with 60 mg/l dose while the 60 and 97°C treated extracts achieved 98 and 97% performance with a reduced dose of 40 mg/l respectively. At a higher coagulant dose of 80 mg/l, the native sample achieved only 91% performance. The performance of KCE after heat treatment was significantly higher because, at 10 mg/l dose, all the samples recorded significant efficiencies of > 91% while the 60 °C treated sample achieved > 95% performance. An increase in dose resulted in increased performance throughout the coagulation process, although, there was reduced performance beyond 60 mg/l across all the heated extracts. However, the overall coagulation performance of the sample was impressive even at starting dose of 10 mg/l.

One notable feature of KCE was that heating to 60 °C improved its performance across all doses. Similarly, KCE heated to 140 °C showed the same trend but its performance deteriorated slightly at 100 mg/l

dose, from 98.4 to 98.5%. However, the general performance of denatured KCE deteriorated after the optimum dose of 80 mg/l except for the native sample which only deteriorated at 100 mg/l to achieve 85% efficiency.

Table 1 Effect of heat treatment on the performance of KCE on 200 NTU turbidity removals.

Coagulant Dose (mg/l)	ΔT=0°C %Reduction	ΔT=60°C %Reduction	ΔT=97°C %Reduction	ΔT=140°C %Reduction
10	77.7	95.1	91.1	93.5
20	80.0	97.3	95.0	96.2
40	84.8	98.2	96.5	98.1
60	85.9	97.5	95.7	98.7
80	85.5	97.3	95.0	98.4
100	85.1	95.6 93.898.5		

A further investigation was conducted on the performance of KCE in order to identify the most appropriate time for stock solution before any deterioration in quality as a coagulant. Figure 2 shows the efficiency of KCE as a coagulant after storing the stock solution for 1, 3, 7, 10 and 14 days. The results revealed that by storing KCE samples for these days did not yield any significant performance of extracts.

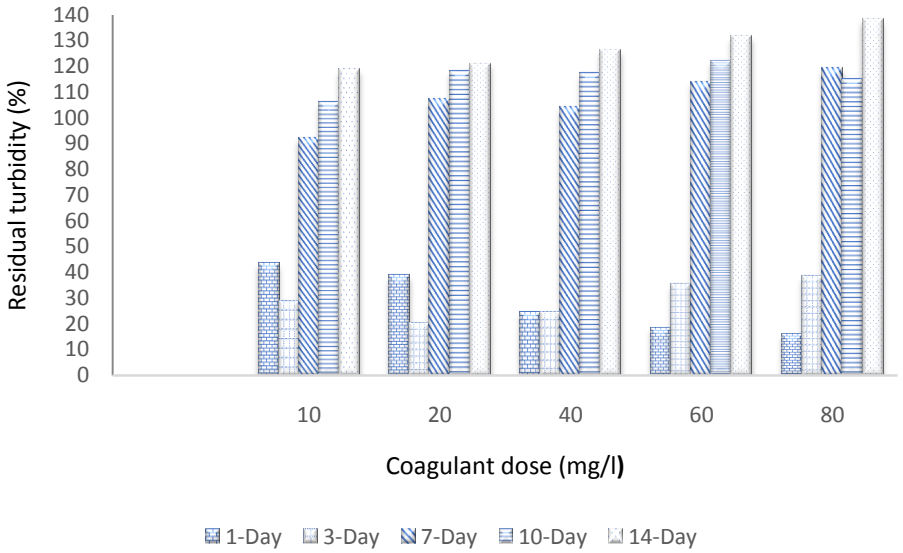

Figure 2 Effect of storage duration on the performance of KCE in 130 NTU turbidity removal.

The performance of fresh KCE at 80 mg/l was approximately 88% turbidity removal while KCE, which was stored for 3days, yielded turbidity reduction of 84.4% with 20 mg/l dose. Additionally, KCE which had been stored for 7, 10 and 14 days yielded no meaningful coagulation performance. With higher doses, residual turbidity in treated water was found to be more than the initial turbidity, especially with the 14 days stored extract.

However, at 40 mg/l dose, fresh and the 3-day stored extract achieved 81% turbidity removal performance, residual turbidity was less than 25 NTU, from 130 NTU in the water. Similarly, maximum turbidity reduction was observed with a dose of 80 mg/l in water treated with the 10-day than in the 7-day extract. It was clear that storing KCE for a lengthy time did not in any way improve its performance, rather its water treatment potential deteriorated. Although the efficiency of the 3-day stored sample was lower than the fresh extract, it required only half of the fresh sample dose to achieve the 84% removal efficiency. The result further shows that the 3−day sample demonstrated good performance compared with the other extracts across the doses used in the treatment process.

The effect of storage duration of raw seeds was also considered in river water with a low turbidity of 19 NTU (result not presented in Figure). The seeds were stored at room temperature of 19 ± 2 °C for 24 months. Kenaf extract obtained from raw seeds achieved up to 94% performance using a dose of 40 mg/l. The overall

performance achieved with the stored seed extracts was better than the 73% recorded with the fresh extracts after harvesting. This results, however, is at variance with that of Katayon et al. (2006) who observed a better performance with fresh MO seed. In each case, turbidity removal efficiency was significantly higher with stored samples than with the fresh seed extracts.

4.0 Discussion

Protein is said to become denatured when its folding structure is altered as a result of exposure to certain elements of physical factors (e.g. heat), causing the protein to become biologically inactive or its overall primary activity affected. Proteins can also degrade and denature upon storage, with such denaturation leading to visible aggregation and turbidity formation (Sharma and Luthra-Guptasarma, 2009). In some instances, proteins can be re-natured but in most cases the denaturation is irreversible. The work reported here evaluated the effect of denatured KCE against the native state as alternative coagulants. Their performances were assessed after heat treatment to some specified temperature ranges.

A denatured KCE sample by storage did not yield any improvement in performance due to its low protein concentration. Another possible reason for low performance in KCE is that the remaining coagulant proteins after denaturation are low compared with the particle concentration resulting in low collision efficiency. However, the results are not in agreement with the findings of Katayon et al. (2004), who noted a decrease in turbidity removal efficiency of MO extracts stored longer than a day. The performance trend in KCE is not surprising because the denaturation may have removed some proteins with limited coagulation potential in the seed, thus disrupting its random configuration, having little protein left for coagulation.

While some studies have shown that MO extract is a cationic electrolyte (Ndabigengesere et al., 1995, Kwaambwa and Maikokera, 2007), Kenaf extract were found to be anionic in this work. Certainly, proteins can be denatured within few hours of storage, and such differences in behaviour can lead to different structural conformation. A wide range of characteristics can be exhibited by denatured proteins, from reduced solubility to communal aggregation. After the tenth day, the degradation in performance was severe, extensively caused by the aggregation and precipitation. Additionally, there was another issue of physical protein agglomeration and adhesion on the container which could have added to the degraded performance after the tenth day(Jones and Bridgeman, 2016).

There are other physical factors or processes which can cause protein denaturation. Heating is one such process, and so samples were denatured at different temperatures and their coagulation performance.

A clearer understanding of the impact of protein denaturation by heating was seen in KCE. Surprisingly, after heat treatment, KCE achieved a significant improvement in coagulation efficiency. The denaturation of the proteins was seen to be more advantageous in KCE where its Non-treatedsample presented the worst performance due its low protein concentration which was further subdued by the activity of other contaminants. Furthermore, the degree of improvement was varied across the denatured samples at doses of 20-60mg/l, but improved performance was observed at higher doses across all the extracts. Thus, heating can improve the coagulation potential of kenaf. It was evident that even after the deterioration, the denatured samples still outperformed the maximum efficiency recorded by the non-treatedextract. The heat treatment yielded a considerable amount of coagulant protein occasioned by the removal of coagulant-hindering proteins. The extracts are thermo-stable after heat treatment, which is advantageous for people in rural areas where the main source of energy is firewood. Furthermore, the heating process improved the effectiveness of the filtration process. In the non-treated extracts, the lipids have the potential of clogging the filter pores.

This study shows that the denaturation of proteins that are partially sensitive to storage time and temperature in water treatment are beneficial. The processes are simple and straightforward to adopt in developing countries, requiring no technology.

The raw seeds were tested on low turbid water because natural coagulants are poor in coagulating low turbidity water (Muyibi and Okuofu, 1995).Katayon et al. (2006)investigated the effect of different storage conditions of raw MO seed and observed better performance with the fresh extract than in seed which had been stored for one month. Conversely, in this study the seed which was stored for more than 24 months was observed to outperform the fresh extracts. This result was, however, not in agreement with the previous study by Katayon et al. (2006).

5.0 Conclusion

Natural extract obtained from kenaf seeds is a potential coagulant in water treatment. Processes which could easily be adopted in rural areas such as heating and storage time improves turbidity removal efficiency of the extracts which is largely beneficial. Application of kenaf extracts in water treatment could increase access to clean drinking water in developing countries to meet the SDG target by the year 2030.

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