POTENTIAL APPLICATION AS A BIO-COAGULANT FOR *Moringa oleifera* SEEDS

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Abstract

This study focused on producing a natural bio-coagulant from moringa seeds. Moringa seed extract is a natural coagulant for the treatment of water containing suspended solids and colloids and is obtained through grinding, oil extraction, protein extraction from the solids using 2 M NaCl solution, protein separation by centrifugation followed by filtration. This study investigated the production and use of bio-coagulants. Parameters investigated when producing the bio-coagulant include the effects of temperature on the removal of moisture content in the moringa seeds, the type of solvent on the oil yield, and varying the volume of n-hexane for oil extraction. Parameters investigated when using the bio-coagulant include the effect of dosage of bio-coagulant on pH and turbidity removal efficiency, and the performance of the bio-coagulant at low, medium, and high turbidity levels. The optimum temperature and time obtained by drying the moringa seeds was at 100 ^{o}C for 30 min and the highest oil yield obtained was 31% when using *n*-hexane as the solvent. Turbidity removal efficiency went up to 96.4% when using 50 mg/L of the bio-coagulant at a pH of 6.1. High turbidity removal was achieved at low bio-coagulant dosages which was regarded as a breakthrough finding for this research.

Keywords: *Moringa oleifera* seeds, *n*-hexane, bio-coagulant, water treatment.

Introduction

Background information

Water availability for all is the ultimate realization of Sustainable Development Goal Number 6, which thrusts upon member states the obligation to ensure availability and sustainable management of water by at least decreasing the number of people lacking access to safe potable water by half [1]. Before water is distributed to consumers, conventional methods, such as coagulation, flocculation, adsorption, and activated sludge are applied for water and wastewater treatment [2,3]. In addition, there is a high demand for using efficient and inexpensive methods from biomaterials, like biosorption and adsorption, and this is giving rise to the use of inexpensive biosorbents to reduce costs [1,4,5]. Despite the remarkable role of chemical and synthetic coagulants in water purification, they are associated with carcinogenic and neurotoxic effects due to their residues in treated water, such as aluminum [1,3,6]. The development of sustainable and eco-friendly coagulants as an alternative to chemical coagulants has been reported [7]. The presence of numerous bioactive compounds has associated the Moringa plant with multiple nutritional and healthy benefits [1,2,8]. Unwanted particles from wastewater can be separated by the seed extract [6]. Over 90% of bacteria can sediment from raw water by using the unprocessed seed powder, thus they can be potentially used as an anti-microbial treatment. The seed extract acts as a coagulant and flocculent to clarify turbid water. Moringa seed extract destabilizes colloidal particles and thus has a good potential to lower the *E. coli* count [8] and *Moringa oleifera* has been used to treat wastewater from paper mills [6]. Therefore, this project focuses on the coagulation potential of *Moringa oleifera* seed extract in the treatment of water.

Coagulants used in water and wastewater treatment

Coagulants are either natural or inorganic based. Natural coagulants are economical since a variety of users can use them at lower dosages without losing their efficacy and they require far less sludge to be disposed of, which further lowers expenses [4,8]. Low-turbidity raw water can be effectively treated with inorganic coagulants that are primarily based on aluminum or iron. Examples include polyaluminum chloride (PAC), sodium aluminate, aluminum sulfate (Alum), ferric sulfate, and ferric chloride [2,3].

Aluminum-based coagulants are primarily used in water and wastewater treatment to: (i) remove heavy metals like cadmium (Cd), lead (Pb), arsenic (As), and iron (Fe), (ii) remove phosphorus (chemical precipitation), (iii) remove chemical and biological oxygen demands (COD) and BOD, (iv) remove color pigments, and (v) for sludge conditioning and dewatering [9]. When using inorganic coagulants, a larger dosage is needed to cause coagulation, and a lot of sludge containing dangerous chemicals is produced as a result [2].

Moringa oleifera

A perennial woody plant of the *Moringa* family, *Moringa oleifera* is indigenous to tropical and southern subtropical areas that are semiarid or arid [10]. The robust and swiftly expanding *Moringa oleifera* Lamarck (family *Moringaceae*) tree is extensively grown because of its great degree of environmental adaptability [11]. It is regarded as one of the world's most useful plants because practically every part of it can be used for industrial, traditional medicinal, and food purposes [12] and is known to be drought-resistant [13]. Many countries that are tropical or subtropical grow a lot of *Moringa oleifera* because practically every part of the plant can be used for food or other purposes as it is highly valued [14].

Moringa plant is classified as an organic coagulant (bio-coagulant) and it has a variety of bioactive chemicals, from tree seeds, leaves, roots, and flowers [15], which have been linked to several health and nutritional advantages [1,8,9]. The polyphenol content of *Moringa oleifera* from different regions indicates high genetic diversity, likely due to differences in cultivation conditions, climate, or soil environment, resulting in the accumulation of various polyphenols and enhanced drought resistance [16]. Moreover, *Moringa oleifera* seeds have drawn interest due to their high turbidity removal efficiency, affordability, and low toxicity, as well as the possibility of on-site preparation [17]. Studies on seeds are much accentuated on the purification of water and oil extraction [18]. Research has demonstrated that powdered *Moringa oleifera* seeds are a cost-effective, low-impact, and sustainable substitute for costly conventional chemicals [19] economical, producing less pH and biodegradability [20], low toxicity, and high cost-efficiency [9] and eliminate dangerous bacteria from water bodies [21]. In Zimbabwe, the *Moringa oleifera* tree grows quickly and is commonly grown, especially in tropical areas like the Binga district. In addition to producing proteins that function as efficient coagulants in the treatment of water and wastewater, Moringa seeds have high-quality edible oil content—up to 40% by weight [1]. The seed extract can separate unwanted particles from wastewater sedimenting impurities [3,9]. The raw seed powder can filter out over 90% of microorganisms from raw water, making it a viable anti-microbial treatment [8]. To clear turbid water, the seed extract works as a flocculent and coagulant [3,4,9].

Ajav and Fakayode [22] aimed to ascertain the physical characteristics of Moringa seeds with respect to an oil expeller's design. Oloyede et al. [23] identified the engineering characteristics of moringa seeds and pods that are important for designing a moringa sheller. Arreola et al. [24] aimed to identify the characteristics of the coagulating proteins. Barakat and Ghazal [11] identified the physicochemical characteristics of *Moringa oleifera* seeds grown in various Egyptian regions. Nwaiwu and Bello [19] determined the optimum combination for *Moringa oleifera* and alum, XX [21] examined a few of the *Moringa oleifera* seed's physical characteristics at different moisture contents. Du et al. [25] investigated the effects of different NaCl concentrations and Madrona et al. [26] used electrophoresis to determine the protein's molecular weight in the *Moringa oleifera* seed. This study aimed to evaluate the efficiency of *Moringa oleifera* seed extract for water treatment. This investigation aimed to separate the proteins from moringa seeds into a biocoagulant and ascertain the ideal timing and temperature for drying the moringa seeds, conduct seed oil extraction, and determine the efficiency of turbidity removal in conditions of low turbidity, medium turbidity, and high turbidity using canteen and laundry wastewater.

Materials and methods

Materials

The wastewater samples used for testing the bio-coagulant were collected from Harare Institute of Technology's municipality water, laundry, and canteen water and were characterized according to the Environment Management Act (Effluents and Solid Waste Disposal) S1 6 of 2007. Moringa seeds (shelled) used were sourced locally in Zimbabwe and it was utilized as the primary ingredient in the study (Figure 1).

Experimental method

The study was carried out at the Harare Institute of Technology, Zimbabwe. The raw material used was Moringa seeds and the bio-coagulant extract can be obtained through processes of washing, drying, grinding and drying, oil extraction, protein extraction, centrifugation, and filtration. The methodology (Figure 2) is similar to the one adapted from [2,8,9].

Drying and grinding

The moringa seeds were dried at 100 °C for 20 min and then ground using a mortar and pestle until a fine powder was obtained. Afterwards, the moisture content can be determined as:

$$Moisture\ content = \frac{\text{difference in weight of seeds}}{\text{weight of seeds before drying}} \times 100\% \quad (1)$$

Oil extraction

The solvent used for oil extraction was *n*-hexane with a ratio of moringa solids to hexane of 1:5 w/v for 24 h. The oil was separated by precipitation and then filtration.

Protein extraction and centrifugation

The protein in moringa seeds was extracted by 2 M NaCl solvent. The solid residue was removed by filtration and the remaining supernatant was centrifuged at 4000 rpm for 30 min to remove the sediment and remain with the Moringa liquid extract. NaCl present in the *Moringa oleifera* solution improves coagulation efficiency because it helps to release the coagulant protein fraction present in the solution. Moreover, the coagulation process enhances this extraction by about 7 times higher than that extracted by distilled water [6].

Coagulation tests

To determine optimum turbidity removal percentages and dosage, standard sedimentation jar test equipment was used in this study which comprised six 1000 mL jars that can simultaneously test each sample [5]. The procedure involved using a 1000 mL graduate and pouring the necessary amount of well-mixed raw water into one of the transparent jars. Afterwards, identical paddles were placed in each beaker followed by setting the filled jars on the gang stirrer. For 30 seconds, beakers were agitated at 40 to 50 rpm until all dosages of the bio-coagulant (mg/L) had been added. For 30 seconds, the mixing was accelerated to a quick mix of 100–125 rpm, and the slow mix time was increased to twice as long as the rapid mix and then lowered the mixing speed to 40 rpm. The mixer was turned off for the mixture to settle. After 15 minutes of settling (sample point height of 23 cm), the relative speeds of settling and the appearance of the supernatant were noted whereby the water's initial turbidity sample determined the settling rate with *Moringa oleifera* seed. Afterward, turbidity and/or absorption measurement of each sample was performed in triplicate, and turbidity was measured using a hand-held turbidimeter with an accuracy of ± 2%.

Coagulation efficiency

The efficiency of using MO as a bio-coagulant was determined using turbidity and it was calculated using the following expression:

$$Turbidity\ removal = \frac{Turbidity_{before/raw} - Turbidity_{after/treated}}{Turbidity_{before/raw}} = 100\% \qquad (2)$$

Results and discussion

Wastewater characterization

Table 1 shows the characterization results of a sample of wastewater for turbidity, oxygen absorbed, total dissolved solids, dissolved oxygen, and total suspended solids as 80.20 NTU, 90 mg/L, 1722 mg/L, 36.8 saturation% and 114 mg/L, respectively. Higher collision frequencies, greater van der Waals attractions, and quicker aggregation make coagulation frequently more effective in high-turbidity water than in low-turbidity water. Total suspended solids can include harmful substances and cause turbidity, which reduces the amount of sunlight that reaches the water.

Production of bio-coagulant

Drying of Moringa seeds for moisture removal

The mass change was monitored as the temperature was changed and the results are shown in Figure 3. The mass of Moringa seeds decreased rapidly with an increase in temperature until there was a slight decrease from 100 °C to 120 °C hence this means that the moisture content in the seeds was removed and the optimum temperature was 100 °C. The Moringa seeds' moisture content was determined as 6.8%. 7.31% moisture content on a wet basis [22], 6.3% moisture content on a dry basis [23], and 4.74% moisture content was found in the literature [27]. Moisture content has a huge effect on the storage life of the seeds as the physical characteristics of the seed, such as its width, length, geometric mean diameter, thickness, surface area, sphericity, angle of repose, true density, and coefficient of friction, are influenced by its moisture content. These properties are crucial information for designing effective processes and equipment [21] as well as forecasting the stability and safety of seeds in terms of microbial growth based on moisture content. Therefore, it is important to note the moisture content in order to predict a reasonable possible life storage of each accession.

Effect of the type of solvent on oil extraction from Moringa seeds

The oil in moringa seeds is highly soluble in n-hexane compared to other solvents such as ethanol. From the results, the oil yield obtained from hexane is 31 ± 0.06% slightly higher than that of ethanol 28.1%. In a study, petroleum ether was determined as the best solvent (33.9%) followed by n-hexane at 27.1% [2]. Methanol was also tested and it was at 26.3% extraction.

Effects of varying volumes of n-hexane on oil extraction

n-Hexane was used to extract the oil and the effect of changing its volume was investigated at constant Moringa seed powder mass to determine the ratio of seed solid to *n*-hexane to be used. The results for the change of *n*-hexane against the oil extracted are presented in Figure 4. As the volume of *n*-hexane increased, the amount of oil extracted also increased as seen by the mass of oil extracted by *n*-hexane. Change in mass of oil extracted increased slowly after 450 mL giving a ratio of seed solid to hexane of 1:5 w/v.

Validation of bio-coagulant

Effect of bio-coagulant dosage on turbidity reduction efficiency

The effects of bio-coagulant dosage on turbidity reduction efficiency can be seen in Table 2 and Figure 5a. From Figure 5a, the turbidity removal percentage increased from a dosage of 20 mg/L to 50 mg/L and then decreased slightly from 60 mg/L and 80 mg/L where the graph plateaus. The optimum dosage is determined as 50 mg/L (turbidity removal percentage 96.4%). When the *Moringa oleifera* dosage goes above the optimum of 50 mg/L, the turbidity removal efficiency decreases. This is attributed to the increased turbidity because of the neutralization and precipitation of all colloids with an optimum dosage; therefore, water turbidity is brought on by the excess coagulants as they did not interact with colloidal particles which are oppositely charged [6]. *Moringa oleifera* was used to treat paper mill effluent at different coagulant dosage rates (50–300 mg/L) and the optimum dosage rate of 150 mg/L was determined when it had a turbidity removal efficiency of 96% [6]. Moringa oleifera was also used to treat synthetic water and high residual nephelometric turbidity units (NTU) were determined at lower dosages (0–10 ml/L) of *Moringa oleifera* [2]. This was in contrast to the results of the present study.

Moringa oleifera and alum were used to treat two different natural waters (Santa Helena brook and Picacha brook) at different concentrations (mg/L) [5]. The performance for the Santa Helena brook natural water was: highest turbidity removal of 90% at three different concentrations of 63, 94, and 125 mg/L for *Moringa oleifera* and the lowest dosage rate of 63 mg/L was taken as the optimum in this instance, and this was slightly higher than what was determined in this study. Of note, is that *Moringa oleifera* performed better than alum in this study as the highest turbidity removal for alum was 80% at an 18.8 mg/L dosage rate. The performance for the Picacha brook natural water was: highest turbidity removal of 100% at five different concentrations of 250, 281, 312, 344, and 375 mg/L for *Moringa oleifera* where the lowest dosage rate of 250 mg/L was taken as the optimum in this instance. This was higher than what was determined in this study. Of note, is that *Moringa oleifera* performed similarly to alum in this study as the highest turbidity removal for alum was also 100% at 219, 250, and 281 mg/L dosage rates. *Moringa oleifera* was investigated as a bio-coagulant on synthetic water at different dosages from 0 to 200 mg/L[10]. They determined that the highest turbidity removal of ~79% was reached at 50 mg/L just as was observed in the present study.

The native protein responsible for coagulation is a 13 kDa dimeric with roughly 6.5 kDa subunits [2]. According to Arreola et al. [24], proteins with molecular weights ranging from 6 to 5 kDa form the active agents of *M. oleifera* salt solutions, which combine to form a heterodimer with a molecular weight of 11–12 kDa. The presence of polar amino acids with both positive and negative charges in large quantities in moringa seed extracts causes the rupture brought on by the effect of salt, which permits a greater number of radical groups to exist in the product. As a result, brine solutions become more efficient in the coagulation process by encouraging the creation of chemical bridges and adsorption between the coagulant and colloidal materials.

According to the literature, protein extractability increased significantly starting at 0.25 M of salt concentration; after that, there was no discernible change in extractability. After the salt concentration reached 2.0 M, extractability rose. Additional salt concentration (>2.0 M) had a negative impact on protein extraction. Significant changes were observed in the protein extractability upon additional elevation of the salt concentration [28].

A greater concentration of NaCl during extraction was beneficial for low molecular weight *Moringa oleifera* salt-soluble protein [25]. Small proteins have a coagulating mechanism whereby their positive charge binds to the surface of negatively charged particles. This causes particles with distinct charge zones to form, and as a result of their collisions, the formation of follicles is encouraged [24]. Adsorption and charge neutralization are the main mechanisms of the coagulation with moringa. Studies show the protein content of *Moringa oleifera* to be 26.7% [29], 28% [30], 37.2% [31], 33.53% [12], and 40.34% [10]. Proteins combine with other substances, thus forming sediments. As the dosage of bio coagulant is increased, the settling velocity will also increase for the reason that number of polypeptides that can bind the floc will increase with an increase in dosage, which will speed up the settling process [32].

Effect of bio-coagulant dosage on pH

The effects of bio-coagulant dosage on pH can be seen in Figure 5b. From Figure 5b, the highest turbidity removal of 96% was at pH 4.5 while the least turbidity removal of 75% was at pH 6.2. Based on the experiments, the optimum pH obtained using moringa bio-coagulant is 6.1 with 89% turbidity removal efficiency. It lies within the WHO (2006) recommended pH range for water which is between 6.0 and 8.0. However, a pH of 4.4 can be used to remove the turbidity and adjust the pH afterward. This will help to remove additional turbidity up to 96%. *Moringa oleifera* was used to treat paper mill effluent at different pH values (4–9) and the optimum pH range was determined to be 6–8 [6]. The results from the present study fell within the same range. The turbidity removal drastically dropped from a pH higher than 6.0 due to alkaline conditions causing a decrease in protein stability [33].

Effect of different turbidity levels

The bio-coagulant was tested in water at different turbidity levels: low (5 NTU), medium (15 NTU), and high (40 NTU). The results are summarised in Figure 5c. *Moringa oleifera* bio-coagulant was found to be more effective in water of high turbidity. This is due to more frequent contact of the ions in the bio-coagulant which form heavy flocs hence providing high efficiency. The results can be seen in Figure 5c. *Moringa oleifera* was investigated as a bio-coagulant on synthetic water at different dosages from 0 to 200 mg/L from initial turbidity of 25 NTU and the highest turbidity removal of ~79% was reached at 50 mg/L [10]. The synthetic waters used in the study had higher turbidity showing similar results as in our study. The *Moringa oleifera* dose can handle higher turbidity as is in this study and still achieves good removal efficiencies.

Conclusion

The protein in moringa seeds can potentially be used as a bio-coagulant. The optimum temperature and time for drying the moringa seeds was 100 ℃ for 30 min. The results showed that the highest oil yield of 31% was obtained from n-hexane since it is a more non-polar solvent hence highly recommended for seed oil extraction. The highest turbidity removal efficiency of 96.4% was at low dosages of 50 mg/L where all the colloids were neutralized and precipitated. A high turbidity removal of 88.6% was also obtained at an optimum pH of 6.1. *Moringa oleifera* bio-coagulant was found to be more effective in water of high turbidity due to more frequent contact of the ions in the bio-coagulant and forming heavy flocs, hence providing high efficiency.

Further studies on the performance of the bio-coagulant in powder form for turbidity reduction efficiency must be carried out. Vacuum drying method or freeze-drying method can be used.

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Table captions

Table 1. Wastewater characterization

Table 2. Effect of bio-coagulant dosage on turbidity reduction efficiency.

Figure captions

Figure 1. (a) Moringa shells, (b) Moringa seeds, and (c) ground Moringa seeds.
Figure 2. Graphical methodology for the process.
Figure 3. Determination of moisture content in moringa seed.
Figure 4. Effects of varying moles of *n*-hexane for oil extraction.
Figure 5. (a) Effects of bio-coagulant dosage on turbidity removal efficiency, (b) effect of bio-coagulant dosage on pH, and (c) performance of bio-coagulant dosage on different turbidity.

Table 1:

Parameter	Method	Quantity determined
Total dissolved solids	Gravimetric SOP/CM11	1722mg/l
Total suspended solids	Gravimetric SOP/CM35	114mg/l
Dissolved oxygen	Electrode SOP/CM51	36.8 saturation%
Oxygen absorbed (PV)	Titrimetric SOP/CM25	90mg/l
Chloride	Titrimetric SOP/CM07	14mg/l Cl
Copper	AAS Flame SOP/CM22	<0.01mg/l Cu
Chromium	Spectrophotometric SOP/CM08	<0.01 mg/l Cr^{6+}
E. Conductivity	Electrode SOP/CM12	18.55 uS/cm
Iron	AAS Flame SOP/CM22	7.74 mg/l Fe
Lead	AAS Flame SOP/CM22	0.31 mg/l Pb
Nitrates	Spectrophotometric SOP/CM23	18.68mg/l N
Phosphates	Spectrophotometric SOP/CM28	2.8 mg/l P
Potassium	Flame photometric SOP/CM31	10.9 mg/l K
Sodium	Flame photometric SOP/CM31	575.70 mg/l Na
Sulphate	Turbidimetric SOP/CM33	120 mg/l SO_4
Zinc	AAS Flame SOP/CM22	0.23 mg/l Zn

Table 2 :

Biocoagulant dosage (mg/L)	Initial Turbidity (NTU)	Final Turbidity (NTU)	Turbidity Removal (%)
20	38.6	4.41 ± 0.02	88.6
30	38.6	2.53 ± 0.05	93.4
40	38.6	1.42 ± 0.03	96.3
50	38.6	1.39 ± 0.04	96.4
60	38.6	1.47 ± 0.05	96.2
70	38.6	1.62 ± 0.05	95.8
80	38.6	1.75 ± 0.03	95.5

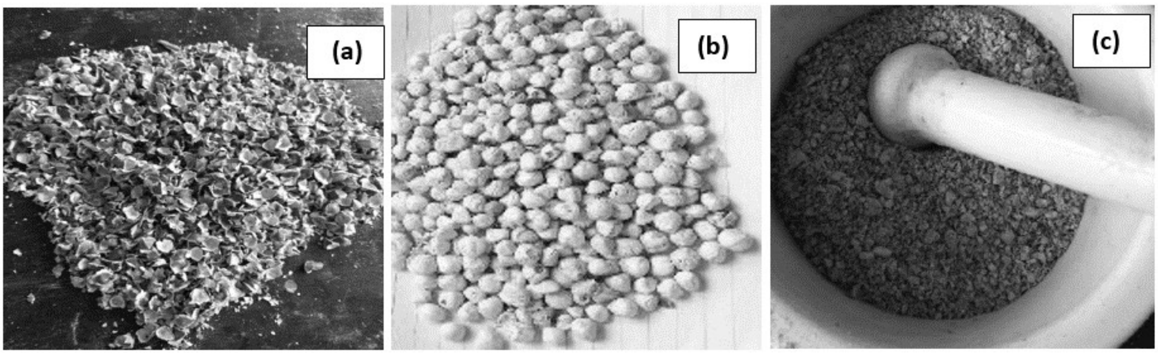


Figure 1

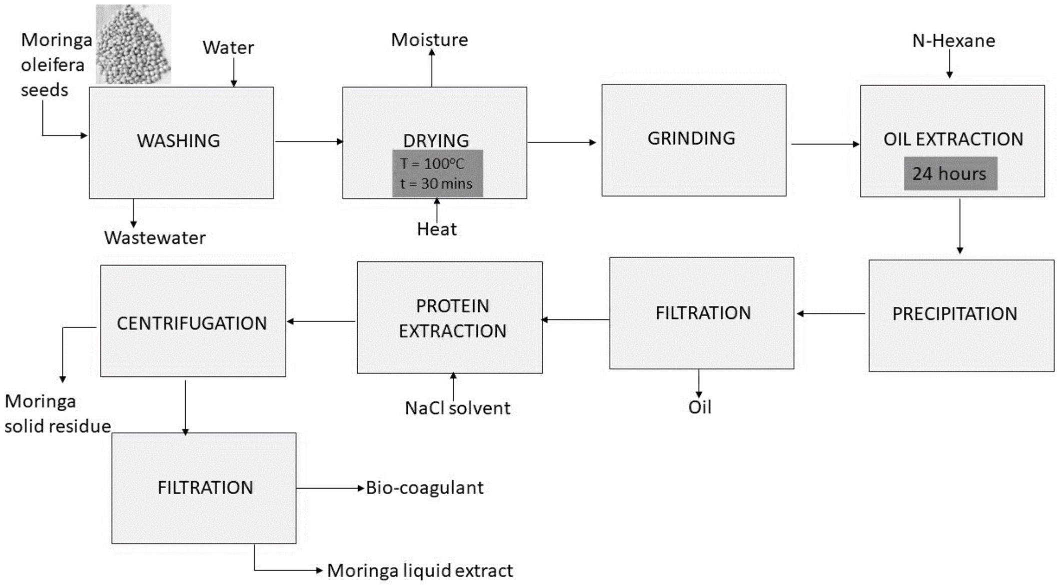
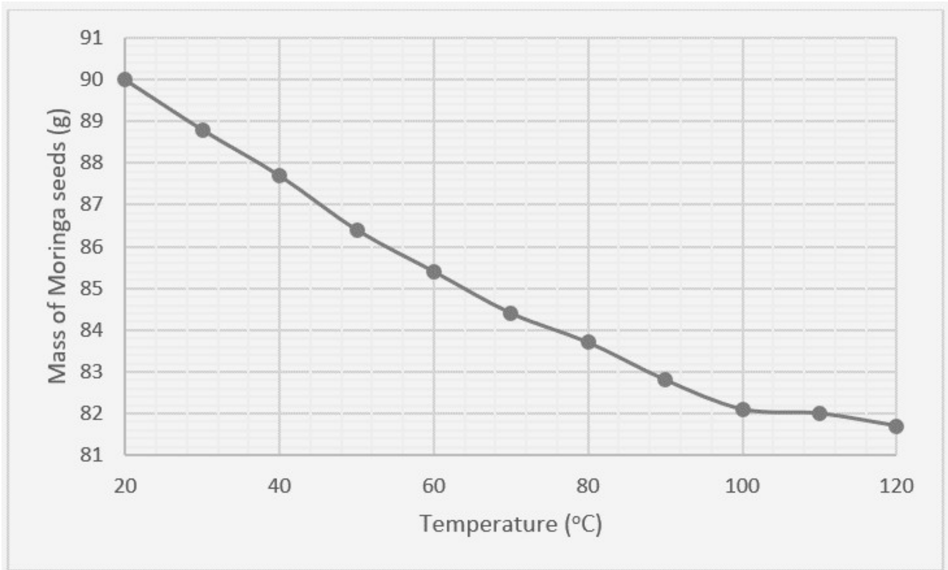

Moringa
oleifera
seeds
Water
WASHING
Wastewater
Moisture
DRYING
T = 100°C
t = 30 mins
Heat
GRINDING
N-Hexane
OIL EXTRACTION
24 hours
PRECIPITATION
FILTRATION
Oil
PROTEIN
EXTRACTION
NaCl solvent
CENTRIFUGATION
Moringa
solid residue
FILTRATION
Bio-coagulant
Moringa liquid extract

Figure 2



91
90
89
88
87
86
85
84
83
82
81
Mass of Moringa seeds (g)
20
40
60
80
100
120
Temperature (°C)

Figure 3

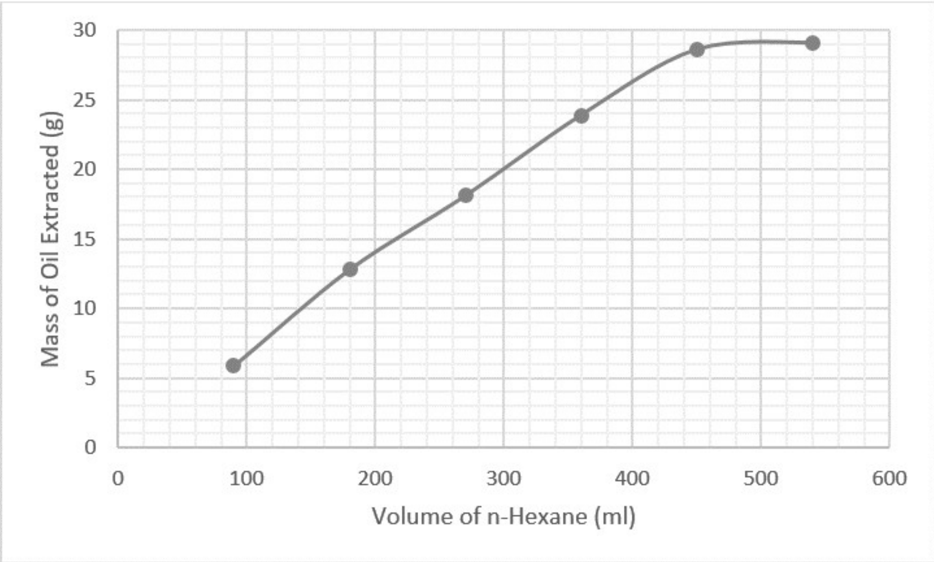
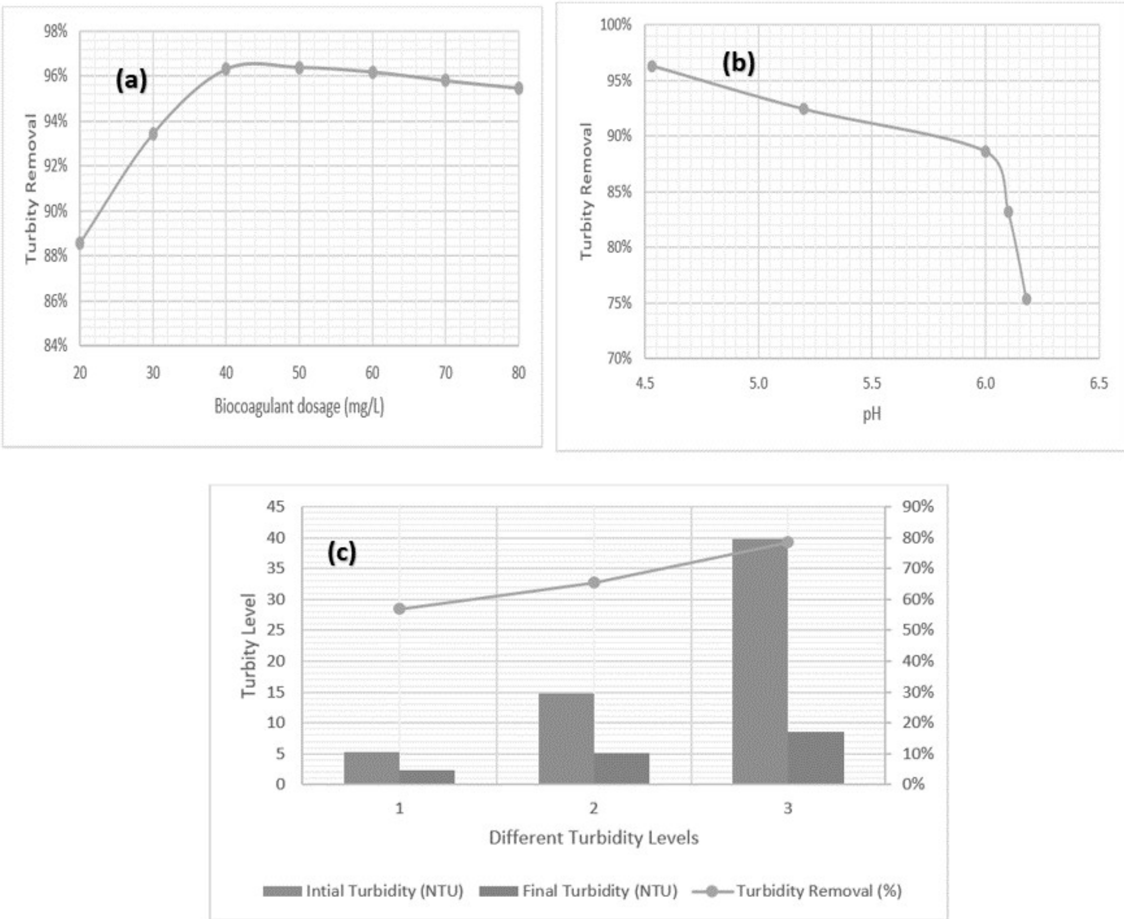

Mass of Oil Extracted (g)
30
25
20
15
10
5
0
0
100
200
300
400
500
600
Volume of n-Hexane (ml)

Figure 4



(a)
98%
96%
94%
92%
90%
88%
86%
84%
Turbity Removal
20
30
40
50
60
70
80
Biocoagulant dosage (mg/L)
(b)
100%
95%
90%
85%
80%
75%
70%
Turbity Removal
4.5
5.0
5.5
6.0
6.5
pH
(c)
45
40
35
30
25
20
15
10
5
0
Turbity Level
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
2
3
Different Turbidity Levels
Intial Turbidity (NTU)
Final Turbidity (NTU)
Turbidity Removal (%)

Figure 5

POTENCIJALNA PRIMENA SEMENA *Moringa oleifera* KAO BIO-KOAGULANTA

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Ova istraživanja su fokusirana na dobijane prirodnog bio-koagulanta iz semena moringe. Ekstrakt semena moringe je prirodni koagulant za tretman vode koja sadrži suspendovane čvrste materije i koloide i dobija se mlevenjem, ekstrakcijom ulja, ekstrakcijom proteina iz čvrstih materija pomoću 2 M rastvora NaCl i odvajanjem proteina centrifugiranjem i filtracijom. Istraživani parametri dobijanja bio-koagulanta uključuju uticaj temperature na uklanjanje sadržaja vlage u semenu moringe, vrstu rastvarača na prinos ulja i variranje zapremine *n*-heksana za ekstrakciju ulja. Parametri vezani za korišćenja bio-koagulanta uključuju uticaj doze bio-koagulanta na pH i efikasnost uklanjanja zamućenosti, kao i performanse bio-koagulanta na niskim, srednjim i visokim nivoima zamućenosti. Optimalna temperatura i vreme sušenja semena moringe bili su 100 °C tokom 30 minuta, a najveći prinos ulja bio je 31% kada se koristi *n*-heksan kao rastvarač. Efikasnost uklanjanja zamućenosti porasla je do 96,4% kada se koristi 50 mg/L bio-koagulanta pri pH vrednosti 6,1. Visok stepen uklanjanja zamućenosti postignut je pri niskim dozama biokoagulanta, što se smatra revolucionarnim otkrićem ovog istraživanja.

Ključne reči: Seme *Moringa oleifera* , *n*-heksan, bio-coagulant, treatman vode.