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Evaluation of Three Natural Coagulant from *Moringa Oleifera* Seed for the Treatment of Synthetic Greywater

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Abstract

The scarcity of water has become a growing problem worldwide. The search for new sources has therefore intensified, and one of these sources is greywater. The objective of this article is to evaluate the effectiveness of three different coagulants obtained from *Moringa oleifera* seed (seed husk, ground seed, and degreased) in synthetic greywater. The methodology is planned in stages; in the first stage, these seeds were selected, unsheathed, and dried in the sun for 24 hours, and the coagulant was synthesized by a 1 M NaCl solution. In the second stage, the synthetic greywater was prepared in a laboratory and included personal cleaning products and additional chemical components. Finally, a statistical test was employed to evaluate the removal of turbidity and the incidence and behavior of the turbidity, pH, alkalinity, and dissolved oxygen over seven periods and the type of coagulant. It was found that the coagulant degreased obtained the highest percentage of removal (85%) and the coagulant from seed husk had the lowest efficiency with 75%. On the other hand, it was found that parameters such as pH and dissolved oxygen depend on the type of coagulant, while conductivity and alkalinity do not depend on time or the type of coagulant.

Keywords: Water Treatment; Moringa Oleifera; Coagulation; Dissolved Oxygen; Greywater.

1. Introduction

Water is one of the most important substances for life and for daily activities, and humans have therefore been forced to settle in areas where water is available [1]. However, in many parts of the world, many people do not have access to sufficient amounts or quality of water, and this has led to more than a third of the population suffering from water stress [2]. It has been estimated that this water-stressed population will increase from one billion in 1990 to four billion in 2050 [3]. Hence, the search for new, alternative sources of water has intensified worldwide. One of these new alternatives is greywater, which comes from sources such as showers, sinks, dishwashers, and washing machines [4]. The use of this type of water has been successful in homes, gardens, and agriculture [5–8], as it presents high volumes due to its daily generation and the ease of treatment as it is not in contact with wastewater [9]. However, in order for its use to be effective, it is necessary to carry out a pre-treatment that allows the relevant physico-chemical conditions for the specific purpose to be met. Previous studies have investigated the application of physical, chemical, and biological treatments for greywater [10, 11], and one of the most commonly used of these is coagulation [12].

The use of coagulants of natural origin has been widely studied and applied to drinking water [13–17] and wastewater [18–22], and this approach has shown high levels of effectiveness in turbidity removal, profitability, low variation in pH, and biodegradability [23]. There are reports of the use of natural coagulants derived from the seeds of *nirmali*, *mesquite*, *beans*, *Cactus latifaria*, *Cassia angustifolia*, and various species of legumes. However, the material that has

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recently received the most attention is *Moringa oleifera* (MO) seed [20]. This is a plant native to India that contains cationic proteins with a molecular weight ranging from 6.5-30 kDa and with isoelectric point above pI 10 [24, 25]. It has been found that MO is mainly composed of globulin and albumin type proteins, which represent 53% and 44% of total proteins, respectively. This active component of the seed that causes the coagulation of cloudy, turbid water is a soluble protein that acts as a natural cationic polyelectrolyte [26].

Its effectiveness has been demonstrated in removing a wide variety of contaminants, such as turbidity, heavy metals, emerging contaminants, *Escherichia coli*, and organic matter, among others [18, 19, 21, 22, 27–33]. Additionally, the sludge produced by this seed during coagulation is not only harmless but also has a volume four to five times lower than that produced by coagulation with alum [34]. In economic terms, the use of a coagulant produced from this seed can mean a reduction in overall construction costs, operation, and the time required for the treatment of water [35].

Several authors have proposed the use of MO as a natural coagulant in greywater; for example, Chitra & Muruganandam [36] used dehydrated seeds and evaluated the removal of turbidity in synthetic (85.6 NTU) and real (92.7 NTU) greywater, where they found efficiencies of 85.3% in synthetic and 83.7% in real greywater and higher efficiency in pH between 6 and 7. On the other hand, Rodriguez et al. [37] used dehydrated seeds to evaluate the removal of turbidity in greywater (73.2–99.1 NTU) from washing machines, from the experiments performed, they found removals of 96.22% with optimum pH between 6.8 and 7.2. Kwabena Ntibrey et al. [38] in their experiment with greywater from schools were able to determine turbidity (227.8 - 287.4 NTU) removals of 97.4%. Thanki & Singh [39] used two types of coagulants from Moringa oleifera, one filtered and the other unfiltered. From this study, they determined that the filtered coagulant showed better removals of 98.8%, while unfiltered values were close to 90% (>200 NTU). Alfa et al, [40] evaluated its effectiveness in the removal of coliforms from graywater and found turbidity (134 NTU) performances ranging from 96% to 97.1%. On the other hand, Igboro et al. [41] evaluated the coagulant efficiency in graywater of Moringa oleifeira, Parkia biglobosa, and Agave sisalama, finding that for OM an efficiency of 76.3% was achieved for a turbidity of 76 NTU. Finally, Al-Gheethi et al. [42], when treating greywater (58-68 NTU) from a university laundry, determined efficiencies of 83.63%. This study presents the evaluation of the efficiency and behavior of three types of coagulants from Moringa oleifera seed (complete seed, seed without shell, and degreased seed), since the literature mainly shows experiments with defatted seed.

2. Material and Methods

The proposed methodology was developed through different stages, which are shown in Figure 1 and described below.

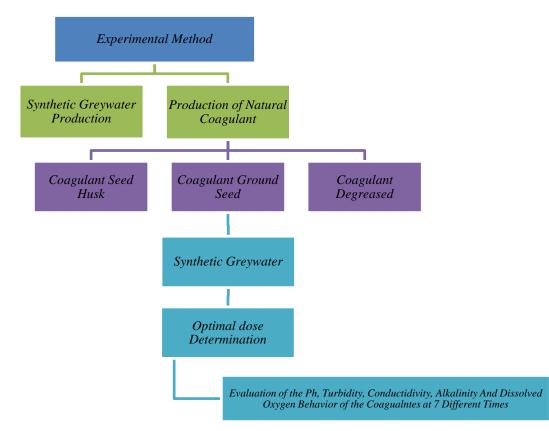


Figure 1. Flow chart for experimental method process

2.1. Stage 1

For use with synthetic greywater, the methodology proposed by Diaper [43] was followed, and the components of the greywater are shown in Table 1.

Ingredient	Quantity in 100 L (g)
Sunscreen	1.5
Facial cream	1
Toothpaste	3.25
Deodorant	1
Na_2SO_4	3.5
NaHCO ₃	2.5
Na_2PO_4	3.9
Vegetable oil	5
Shampoo	0.7
Hand soap	72
Laundry soap	15
Boric acid	0.14
Lactic acid	2.8

Table 1. Components and quantities of synthetic greywater

Seeds of the MO species were used to create the coagulant. These seeds were selected, unsheathed and dried in the sun for a period of 24 hours. Once the seeds were dry, we used the methodology proposed by Okuda et al. [44] in which a coagulant was synthesized by means of a 1M NaCl solution. Table 2 summarizes the three types of coagulants made from different compounds of these seeds (seed husk (CS), ground seed (WS) and degreased (D)) and the procedures carried out, and the final coagulant. The coagulants see in Figure 1.

Table 2. Coagulants	generated	from Moringa	oleifera seed

Coagulant	Process
Seed husk	$25g$ of husks of MO was prepared, macerated in a mortar, and then diluted with saline under constant agitation at 200 rpm for a period of 30 minutes. The solution was passed through a filter with a pore diameter of $45 \mu m$ in a vacuum, giving a milky-coloured solution.
Ground seed	$25g$ of MO seed powder was processed in a hand mill, and then diluted with saline under constant agitation at 200 rpm for a period of 30 minutes. The solution was passed through a filter with pores of 45 μ m diameter in a vacuum, giving a milky-coloured solution.
Degreased seed	25g of MO seed powder was extracted and passed through a Soxhlet extractor for eight hours, using n-hexane as solvent. The resulting frame was dried in an oven at a temperature of 105°C for 24 hours and stored in a desiccator over the test period. Subsequently it was diluted with saline under constant agitation at 200 rpm for a period of 30 minutes. The solution was passed through a filter with a pore diameter of 45 μ m in a vacuum, giving a translucent yellow solution.



Seeds of Moringa Oleifera

Seed husk (SH)

Ground seed (GS)

Degreased seed (DS)

Figure 2 . Coagulants from different process from Moringa oleifera

2.2. Stage 2

To determine the optimal dose of the coagulant, 12 different concentrations were used (2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5 and 30 mg/L), which were evaluated using a brand jar test. The samples were subjected to rapid mixing at 200 rpm for one minute, followed by slow mixing at 40 rpm for 30 minutes. Each of the samples used for the different tests was subjected to turbidity tests with a previously calibrated Hanna HI93703 turbid meter.

To evaluate the efficiency of the use of the coagulant generated in the different MO processes, a parallel test was carried out with aluminum sulfate using 6 different concentrations (0.75 mg/L; 1.5 mg/L; 3 mg/L; 6 mg/L; 12 mg/L; 24 mg/L). The samples were subjected to rapid mixing at 300 rpm for 1 minute, followed by slow mixing at 45 rpm for 5 minutes. The behaviour of the coagulants at optimal doses was evaluated over various time periods; turbidity removal calculations, pH and conductivity were made at seven different time intervals (0, 2, 4, 6, 96, 192 and 240 hours), with zero being the time closest to the preparation of the coagulants. This was in order to evaluate the possible effects on the removals associated with the state of the coagulant after its elaboration.

Finally, physical-chemical tests were carried out (pH, conductivity, turbidity, alkalinity and dissolved oxygen) in order to determine the characteristics of the water samples with respect to the coagulant.

3. Results and Discussion

Synthetic water was obtained at a pH of 6–6.4, a conductivity of $504-564 \mu$ s/cm, a turbidity of 58-62 NTU, SST of 55-68 mg/L, alkalinity of 20-23 mg/L and a DO of 6.5-7 mg/L. Regarding the optimal dose, it was found that for the three types of coagulant, the volume of 17.5 ml showed the greatest removal of turbidity. Removal rates were 75.3% for the coagulant with seed husk, 79.13% for the coagulant without the husk and 81.6% for the degreased coagulant (see Figure 2). These removals are mainly because the water-soluble cationic proteins in the MO help the coagulation and flocculation process [45].

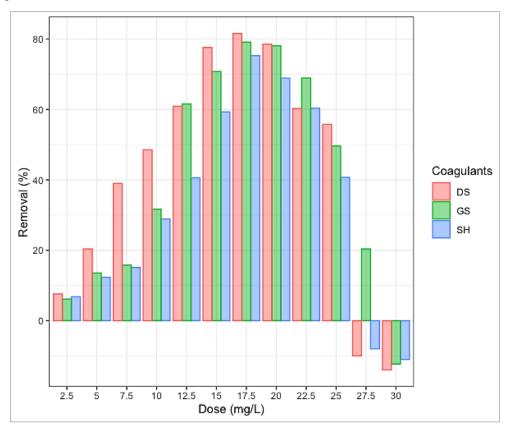


Figure 3. Determination of optimum dose of Moringa oleifera coagulant

These values agree with those presented by Chitra & Muruganandam [36] and Al-Gheethi et al. [42] with a range removal from 83.3–85.7%. Water-soluble cationic proteins from MO seed extracts that enhance coagulation and flocculation. It is important to mention that this article, as well as the references mentioned above, present values lower than 100 NTU of turbidity, and that is why the removals found are between 80 and 85%, values that can be considered high, since it has been determined that the removal of this coagulant is highly altered with low turbidity values, decreasing its efficiency [44, 46, 47].

Similarly, the results show that the coagulant with the highest efficiency is the coagulant with defatted seed, since of the 12 concentrations used, 8 showed the best percentage of removal, and in second place is the coagulant GS with 3 of the best efficiencies and one negative of the three coagulants. On the other hand, when evaluating the pH behavior of the synthetic gray water samples, Figure 4 shows a neutral behavior of the coagulant seed husk and degreased seed. The values for the coagulant seed husk remained between 7.24 and 7.31 units; for the coagulant degreased seed, values were found between 6.91 and 7.2 units. These values are related to the electrical piezo point of the MO, which is located between 10 and 11 [48, 49], which indicates that this protein is cationic; additionally, the active component with a negative charge of the coagulant can attract the bivalent cations, forming a network in which the solids in suspension are trapped.

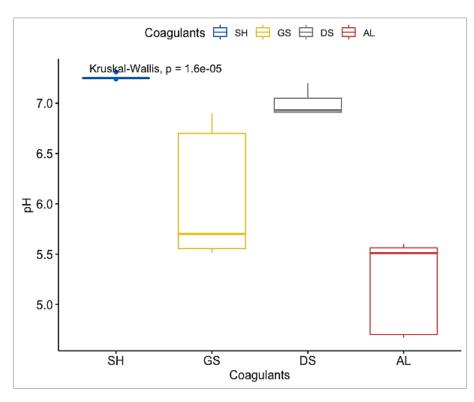


Figure 4. Kruskal-Wallis analyses and significance by coagulants

These values are similar to those presented by Kwabena Ntibrey et al. [38] who determined an average pH of 7.53; on the other hand, Thanki & Singh [39] found that the resulting pH for the Moringa coagulant with filtration was 7.8 and without filtration was 7.6 units. When comparing the resulting pH values with aluminum sulfate, a very noticeable difference was found, since values between 4.67 and 5.7 were found, which is a clear advantage of natural coagulants, since the costs of adjusting the pH in a treatment system would be lower. A Kruskal-Wallis comparison of means between coagulants showed a significant difference between them (p-value<0.05). Similarly, to evaluate the independence between the type of coagulant and pH, a Chi-square analysis was performed (p-value<0.05) and it was found that pH is dependent on the type of coagulant.

In the evaluation over time of the coagulant made from the husks of the MO seed, average removal values of 75.3% were recorded in the first six hours of coagulant preparation; however, after 96 hours, the percentage of removal increased by a further 10%, registering values of 15 NTU. This indicates a removal rate of around 82.363%, and the most efficient removal of the coagulant took place after 10 days. For the uncoated coagulant, higher values were recorded for the removal of turbidity than for the previous coagulant, and a 5% higher removal rate was observed in the first six hours of application (79.2%). After 96 hours, the removal capacity increased by 6%, registering values of 12.9 NTU. This indicates a removal rate of around 84.82%, with a final removal rate that was the same as for the previous coagulant. Finally, the results for the degreased seed showed the highest values for the removal of turbidity compared to the other two coagulants, reaching a removal rate of between 5% and 10% higher in the first six hours of application (maximum value 81.562%). After 96 hours, the removal capacity had increased by a further 10%, achieving values of 11 NTU or a removal rate of around 87.05%. In order to achieve the most efficient removal, the coagulant should be used for 10 days. These results are shown in Figure 5.

When evaluating the pH behavior during the different times, we found that the pH values for the seed husk and degreased coagulants show a constant trend, and no effect is observed for the time of operation, while the ground seed coagulant shows different behavior, with a slight increase from time 0 to time 4 hours (6. 6 - 6.9) and after this time it decreases 1 pH unit (5.7 - 5.5), as shown in Figure 6. As mentioned above, pH is dependent on the type of coagulant; however, when evaluating pH with the time interval, it was found that time does not show a significant dependence on pH (p-value > 0.05). To evaluate the mean of each parameter measured by coagulant at different times versus the initial value of the synthetic gray water, a one-sample t-test analysis was performed, with values of less than 0.05 as significance. For pH, it was found that there are significant differences in the coagulant SH and DS, since there is an increase of at least one unit concerning the initial value, and there is no significance with the coagulant GS, since it increases the first three times and decreases the other 4 final times. Dalvand et al. [50] and Rai et al. [51] found that the final pH was very close to the initial value (7) and that the coagulant did not drastically affect the pH values, these results are similar to those presented in this article where the mean values per coagulant do not differ by more than one unit, mainly in the SH and DS coagulants. This analysis is shown in Table 3.

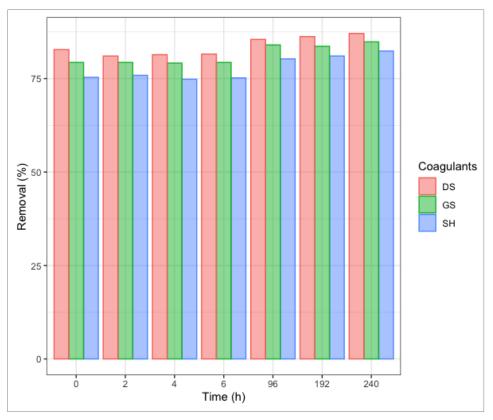


Figure 5. Comparison of removal rates of coagulants over time in hours

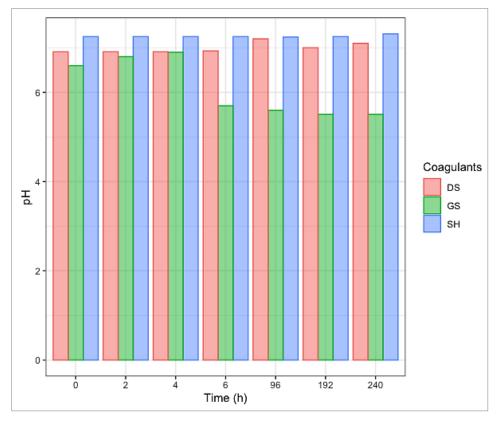


Figure 6. Comparison of the pH behavior of coagulants over time in hours

Table 3. One sample t-test for pH and type of coagulant

Coagulant	Mean (pH unity)	Confidence interval (95%)		P-Value
SH	7.25	7.23	7.27	< 0.05
GS	6.08	5.49	6.68	> 0.05
DS	6.99	6.88	7.10	< 0.05

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As for the conductivity found after coagulation, it rises significantly since the initial value is between the range of $504-564 \mu$ s/cm and after the three types of coagulants it doubles, this is reaffirmed with the one t-test analysis, since it was found that the three coagulants present a significant difference with the initial state (See Table 4). This growth can be attributed to two fundamental aspects, firstly to the addition of NaCl which is used to synthesize the coagulant, and secondly, the MO seeds contain many minerals and inorganic compounds, which dissolve in the water [52]. Similarly, these values are consistent with those found by Kwabena Ntibrey et al. [38] where growth in conductivity magnitudes is observed. Regarding the behavior with the different time intervals, two trends were found, in the coagulant GS it increased directly with time. These behaviors can be seen in Figure 7. When evaluating the dependence of conductivity with time and type of coagulant, it was found that neither the type of coagulant nor the interval is significant with this parameter (p-value > 0.05).

	Coaguiant	Mean (µs/cm)	Confidence in	(10) (10)	1 - value	
	SH	1249.68	1247.23	1252.142	< 0.05	
	GS	1403.14	1392.14	1414.14	< 0.05	
	DS	1228.92	1199.13	1258.721	< 0.05	
Conductivity (Jus/cm)						Coagulants DS GS SH
ġ) 2	4	6 96	192	240	
		Tir	ne (h)			

Table 4. One sample t-test for conductivity and type of coagulant

Coagulant

Mean (µs/cm)

Confidence interval (95%)

P-Value

Figure 7. Comparison of the conductivity behavior of coagulants over time in hours

For the alkalinity parameter, there was a little increase in the treated water at the different times established since the initial values were between 20 to 23 mg CaCO₃/L. The coagulant SH presented the largest values, with ranges between 30 to 34 mg CaCO₃/L and a tendency to increase in the first two times to decrease and increase its magnitude again, this behavior is similar to that presented by Kwabena Ntibrey et al. [38]. Both pH and alkalinity do not vary sharply (although it is not significant) due to the relationship between these two parameters [17, 53–55]. As for the coagulant GS, it grows linearly with time from 26 to 32 mg CaCO₃/L, the coagulant DS at times 0, 2, and 4 remains constant at 26 mg CaCO₃/L and grows with times 6 and 96, and then stabilizes at 30 mg CaCO₃/L until time 240. These behaviors can be seen in Figure 8. Finally, the dependence of alkalinity on the type of coagulant and time was verified by Chi-square analysis, and it was determined that neither of these two variables significantly affects alkalinity (p-value > 0.05). When evaluating the mean of each coagulant at different times with the alkalinity values, a significant difference was found between each coagulant and the basal state of water alkalinity, as shown in Table 5.

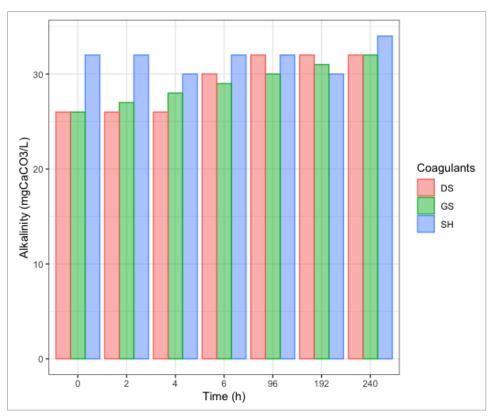


Figure 8. Comparison of the alkalinity behavior of coagulants over time in hours

Coagulant	Mean (mg CaCO ₃ /L)	lean (mg CaCO ₃ /L) Confidence interval (95%)		
SH	31.71	30.43	33.99	< 0.05
GS	29	27	30.99	< 0.05
DS	29.14	26.34	21.93	< 0.05

Table 5. One sample t-test for alkalinity and type of coagulant

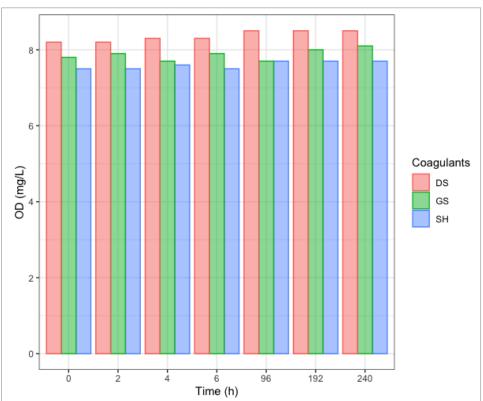


Figure 9. Comparison of the dissolved oxygen behavior of coagulants over time in hours

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Finally, dissolved oxygen was evaluated according to the different time intervals. In all three coagulants, there was an increase in oxygen, mainly in the coagulant DS, where the range of values was from 8.2 to 8.5 mg/L; in second place, the coagulant GS with values between 7.7 to 8.1 mg/L and finally, the coagulant SH with values between 7.5 to 7.7 mg/L, as seen in Figure 9. Adeniran et al. [56] also identified this interval oxygen growth behavior and concluded that the maximum growth time is 2 weeks (approximately 240 hours). Shan et al. [22] also found an improvement in dissolved oxygen, but the reason for the increase in dissolved oxygen concentration is still unknown. As for the Chi-square analysis, it was found the existence a dependence of dissolved oxygen with the type of coagulant with a p-value of 0.01, but on the other hand, there is no dependence on time (p-value > 0.05). When determining the one sample t-test analysis, it was determined that there are significant differences between each coagulant and the basal state of dissolved oxygen, as shown in Table 6.

Coagulant	Mean (mg/L)	Confidence interval (95%)		P-Value
SH	7.6	7.5	7.69	< 0.05
GS	7.87	7.73	8.0	< 0.05
DS	8.35	8.22	8.48	< 0.05

Table 6. One sample t-test for dissolved oxygen and type of coagulant

4. Conclusions

In terms of the development and evaluation of natural coagulants, it can be concluded that Moringa oleifera seed has strong coagulant characteristics, with high percentages of removal and maximum values in the range of 75% to 82%. It is important to note that these removals are within the range reviewed in the literature and that these values below 90% are mainly due to the low turbidity found in synthetic water, so it can be concluded that there is no standard value or range of removals since in greywater turbidity can be found between 16 to 500 NTU.

The data obtained for the efficiency of turbidity removal of coagulants made from *Moringa oleifera* seed show that the coagulant with the highest percentage of removal was the one made from the degreased seed. However, the difference in the removal rates for these three coagulants was less than 10% at optimum doses, indicating the potential use of any of these three types of coagulant. Regarding the time taken to achieve turbidity removal, the performance of these coagulants was very high, but their effectiveness was increased after 96 hours of application, showing differences of almost.

Regarding the analyses by parameter, it was found that the pH of the treated greywater is influenced by the type of coagulant, mainly seed husk and degreased seed, since in these two coagulants there is an increase in pH. However, it is important to mention that this increase does not require a post-treatment of pH since the values are still of neutral pH. As for conductivity, this parameter increased twice as much with the three coagulants at all the times evaluated; additionally, conductivity showed no association with either the time interval or the type of coagulant. Alkalinity showed growth in all the coagulants; neither the time nor the type of coagulant showed any incidence on the values after treatment; on the other hand, for the ground seed coagulant, the growth of alkalinity showed a linear trend, very similar to that shown by the degreased seed coagulant; as for the seed husk coagulant, it showed a behavior of decreases and increases, which does not show a clear trend with time. Finally, dissolved oxygen shows an improvement in the three coagulants, mainly degreased seed, in agreement with other authors where it was identified that there is a growth in oxygen during the first two weeks, as in our case, and after this time there is a decrease in oxygen.

5. Declarations

5.1. Author Contributions

Conceptualization, P.G.C. and O.G.B.E.; methodology, P.G.C. and O.G.B.E.; formal analysis, O.G.B.E.; writing—original draft preparation, P.G.C. and O.G.B.E.; writing—review and editing, P.G.C. and O.G.B.E. All authors have read and agreed to the published version of the manuscript.

5.2. Data Availability Statement

The data presented in this study are available in the article.

5.3. Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

5.4. Conflicts of Interest

The authors declare no conflict of interest.

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