

Article

Chitosan Production from Fish Scales and Its Application as a Natural Coagulant for Surface Water Treatment: Experimental and Statistical Evaluation

José Lugo-Arias ^{1,*} , Javier Carpintero ¹, Salvador Villamizar ², Jorge Luis Pacheco Yepes ^{3,*}, Ruben Cantero-Rodelo ¹, Leandro Gómez-Plata ⁴ and Keila Isabel Cruz ¹

¹ Department of Civil and Environmental, Universidad de la Costa, Barranquilla 080002, Colombia; jcarpint3@cuc.edu.co (J.C.); rcantero@cuc.edu.co (R.C.-R.); keilacruzj@gmail.com (K.I.C.)

² Grupo de Investigación en Procesos Ambientales (GIPROAM), Department of Environmental Sciences, Universidad Francisco de Paula Santander, Cúcuta 540002, Colombia; salvadorenriquevm@ufps.edu.co

³ Mechanical Engineering Program, Faculty of Engineering, Universidad Simón Bolívar, Barranquilla 080002, Colombia

⁴ Programa de Química, Facultad de Ciencias Básicas, Universidad del Atlántico, Barranquilla 080001, Colombia; lgomezplata@mail.uniatlantico.edu.co

* Correspondence: jlugo@cuc.edu.co or joselugoarias@gmail.com (J.L.-A.); jorge.pachecoy@unisimon.edu.co (J.L.P.Y.)

Abstract

The progressive deterioration of surface water quality due to natural and anthropogenic factors, together with the limitations associated with conventional chemical coagulants, has driven the development of natural coagulants as sustainable alternatives for water treatment. In this context, the present study analyzed the production and application of a chitosan-based natural coagulant obtained from *Oreochromis niloticus* fish scales through a chemical method. The first phase involved biopolymer extraction through depigmentation, deproteinization, demineralization, and deacetylation; the second phase evaluated its performance as a coagulant using jar tests with water from the Magdalena River; and the third phase consisted of statistical analysis of the results using ANOVA. Yields of 78%, 78.20%, 88.52%, and 30% were obtained for each processing stage, and the chitosan achieved a degree of deacetylation of 76.87%, confirming its potential for water treatment applications. Optimal conditions were determined as a coagulant dosage of 300 mg/L and a flocculation time of 30 min, while ANOVA results indicated that both variables significantly influenced turbidity removal ($p < 0.05$). Under these conditions, a turbidity reduction of 76.30% was achieved. However, the final turbidity and color values did not meet Colombian regulatory standards, which was attributed to the presence of residual minerals and a moderate degree of deacetylation. Overall, the results demonstrate that chitosan derived from fish scales represents a sustainable alternative to chemical coagulants; however, process optimization and complementary treatment stages are required to meet drinking water standards.

Keywords: coagulation; chitosan; natural coagulant; fish scales; surface water treatment



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1. Introduction

Water is an essential resource for the development and sustainability of life on Earth. Its availability has played a fundamental role in the evolution of human societies, supporting the establishment and growth of settlements [1]. However, at present, the quality of

this resource has progressively deteriorated due to increasing water pollution, primarily associated with industrial development and rapid population growth. These activities generate large volumes of contaminants that exceed the natural self-purification capacity of water bodies, thereby reducing the availability of water suitable for human consumption [2].

Surface water sources, such as rivers and reservoirs, typically contain suspended solids, colloidal particles, natural organic matter, and various dissolved compounds, primarily of inorganic origin, at variable concentrations that hinder compliance with drinking water quality standards. For this reason, water treatment requires the implementation of physical and chemical processes to ensure safe sanitary conditions, as well as acceptable organoleptic characteristics [3].

Among the most relevant parameters in water quality assessment is turbidity, which is caused by the presence of suspended and colloidal particles. To control this parameter, coagulation and flocculation processes are widely employed in the water treatment industry for solid removal, clarification, and the elimination of compounds such as phosphates, oils, color, and odor, among others [4].

Coagulation aims to destabilize suspended particles through charge neutralization, facilitating their subsequent aggregation. In turn, flocculation promotes contact among these destabilized particles through slow mixing, enhancing the formation of larger flocs that can be easily removed by sedimentation and filtration processes. Together, these processes constitute one of the most critical stages in conventional drinking water treatment [5].

Traditionally, coagulation is carried out by the addition of chemical coagulants, such as metal salts and polyelectrolytes. However, their use presents significant drawbacks, including high operational costs, the generation of large volumes of sludge, and alterations in parameters such as the pH of the treated water [6]. Additionally, some studies have suggested a potential relationship between the use of aluminum-based coagulants and health risks due to the presence of residual aluminum in treated water [7].

In this context, there has been growing interest in the development and application of natural coagulants as sustainable alternatives. Biopolymers of organic origin, characterized by their high molecular weight and polymeric structure, represent an eco-friendly and efficient option for water treatment [8]. Among them, chitosan has been widely studied due to its cationic nature, its ability to interact with negatively charged particles, and its abundance in natural sources [9].

Chitosan is primarily obtained from chitin present in residues from the fishing industry, such as shells, heads, and crustacean exoskeletons, which account for approximately 75–85% of the total weight of these organisms and constitute a significant environmental concern, as this residual biomass is generally discarded without adequate treatment [10]. The improper disposal of these organic residues can generate negative environmental impacts, such as odor emissions, the proliferation of pathogenic microorganisms, and the contamination of surface water resources [11,12]. However, these residues contain between 14% and 35% chitin, considered the second most abundant natural biopolymer after cellulose, which provides a valuable opportunity for their recovery and valorization. Consequently, their utilization not only mitigates environmental impacts but also promotes the development of sustainable solutions for water treatment [10].

Chitosan has been widely investigated in previous studies, achieving removal efficiencies exceeding 90%, often outperforming conventional metal salts and other natural coagulants [13–16]. Previous studies [17–21] have evaluated coagulant dosages ranging from 0.5 to 30 mg/L for treating waters with turbidity levels between 5 and 200 NTU, reporting removal efficiencies between 25% and 98%. These studies considered key operational variables, including pH (5–10), rapid mixing time (1–3 min), slow mixing time (15–30 min),

rapid mixing speed (100–180 rpm), slow mixing speed (25–50 rpm), and sedimentation time (5–30 min).

However, the combined effects of these variables, along with temperature and influent characteristics [13,22], remain insufficiently explored through systematic experimental design approaches. Studies employing experimental design approaches that simultaneously evaluate process variables—such as the slow mixing gradient—remain limited, particularly for high-turbidity waters under tropical conditions, such as those found in the lower Magdalena River basin (Colombia), representing a significant knowledge gap.

In particular, limited studies have explored the use of fish scales as a raw material and the simultaneous optimization of key operational variables through experimental design approaches, especially the effect of flocculation time and its interaction with coagulant dosage under high-turbidity conditions. These limitations highlight the need for further research to optimize the performance of this type of coagulant under real wastewater conditions.

Therefore, this study differs from existing research by integrating the use of fish scale-derived chitosan with a response surface methodology to optimize key operational variables under real high-turbidity conditions.

Therefore, the objective of this study was to produce chitosan from fish scales as a natural coagulant and alternative for high-turbidity water treatment, and to optimize key process variables—namely coagulant dosage and flocculation time—using a response surface methodology supported by analysis of variance (ANOVA). Moreover, this study provides experimental evidence on the performance of chitosan under real conditions of the lower Magdalena River basin, contributing to a better understanding of its applicability in industrial scenarios.

In this context, the present study evaluated the use of fish scales as a raw material for the production of a natural coagulant with potential application in drinking water treatment as an alternative to conventional chemical coagulants. Available evidence indicates that natural coagulants represent an efficient, safe, and environmentally sustainable option, as they exhibit high turbidity removal capacity, lower sludge generation, and reduced impact on physicochemical parameters such as pH, conductivity, and dissolved oxygen. This behavior is mainly associated with adsorption and charge neutralization mechanisms, which promote the destabilization and removal of suspended and colloidal particles.

2. Materials and Methods

2.1. Chitosan Extraction and Preparation

Fish scales were obtained from local fish markets located in the downtown area of Barranquilla, Colombia. Extraneous biological material (viscera) was removed prior to processing. The scales were then washed with abundant tap water heated to 80 °C and sun-dried for approximately 3 h, followed by oven (Memmert GmbH + Co. KG, Schwabach, Germany) drying at 90 °C for 5 h. The dried scales were ground using a laboratory mortar and sieved through a 0.43 mm mesh. The resulting powder was subsequently subjected to the following processes based on [23–25]:

Chemical depigmentation: The ground fish scales were treated with a 4.5% (*w/v*) NaClO solution (Sigma-Aldrich, St. Louis, MO, USA) under magnetic stirring (Heidolph Instruments, Schwabach, Germany) at 300 rpm for 1 h at room temperature (22 °C). The resulting mixture was then filtered under vacuum (Welch, Niles, IL, USA) and washed with distilled water until a near-neutral pH was achieved. Subsequently, the washed biomass was dried in an oven at 60 °C for 4 h.

Deproteinization: A 10% (*w/v*) NaOH solution (Sigma-Aldrich, St. Louis, MO, USA) was prepared and applied at a solid-to-liquid ratio of 1:10 (g of solid per 10 mL of solution), with stirring at 300 rpm for 3 h at room temperature, in order to remove the proteins

present. The deproteinized material was then filtered under vacuum and washed with distilled water until a neutral pH was reached, removing any residual base from the process. Subsequently, the material was dried in an oven at 60 °C for 4 h to eliminate moisture from the deproteinized biomass.

Demineralization: The deproteinized material was treated with 0.5 N hydrochloric acid (HCl) (Sigma-Aldrich, St. Louis, MO, USA) at a solid-to-liquid ratio of 1:10 (*w/v*), under stirring at 300 rpm for 3 h at room temperature. After the reaction, the sample was washed with distilled water until a neutral pH was reached, then filtered under vacuum and dried in an oven at 60 °C for 4 h. The resulting solid (chitin) was weighed using an analytical balance (Mettler Toledo, Columbus, OH, USA).

Deacetylation: This process was carried out using a homogeneous chemical method. The obtained chitin was deacetylated by treatment with a 40% (*w/v*) NaOH solution at a solid-to-liquid ratio of 1:10, followed by incubation in an ice bath at 5 °C for 20 h. Subsequently, the mixture was stirred at 300 rpm for 4 h at 80 °C. The alkaline filtrate was discarded, and the resulting product was washed with distilled water until a neutral pH was achieved, removing any residual base from the process.

Subsequently, the deacetylated product was dried in an oven at 60 °C for 4 h to remove moisture from the material, yielding chitosan.

2.2. Preparation of Chitosan Solution as a Coagulant

A total of 1 g of chitosan was added to 100 mL of 0.1 M acetic acid (Merck KGaA, Darmstadt, Germany) and stirred at 100 rpm for 1 h. The resulting solution was then diluted to 1 L with distilled water to obtain a 0.1% (*w/v*) chitosan stock solution.

The degree of N-deacetylation was determined by potentiometric titration using a pH meter (HI 98107, HANNA Instruments, Woonsocket, RI, USA). The procedure consisted of dissolving chitosan in a known excess of HCl, followed by titration with sodium hydroxide (NaOH). A pH versus NaOH volume curve was obtained, showing two inflection points identified from the first derivative (dpH/dV). The volume difference between these points corresponds to the acid consumed for the protonation of amino groups and allows the determination of the degree of deacetylation (%NH₂), which was calculated using Equation (1).

$$\% NH_2 = \frac{16.1 (Y - X)}{W} * F \quad (1)$$

where Y represents the volume at the higher inflection point and X represents the volume at the lower inflection point; [F] is the molarity of the NaOH solution; [W] is the mass of the sample (g); and 16.1 is a constant factor associated with the type of protein considered.

2.3. Jar Test

- Water sample collection

Raw water samples were collected from the Magdalena River in Barranquilla, Colombia, at a sampling point located at coordinates 10°56'46.95" N and 74°45'30.98" W. Sampling was conducted during a single campaign on 28 May 2025. A total volume of 50 L of raw water was collected.

The samples were stored in clean polyethylene containers, immediately refrigerated (approximately 4–5 °C), and transported to the Water Treatment Laboratory of Universidad de la Costa. All analyses and experiments were conducted on the same day to minimize physicochemical changes in the water prior to treatment.

- Water quality characterization

The physicochemical parameters of the water were characterized in accordance with the Standard Methods for the Examination of Water and Wastewater established by the

American Public Health Association (APHA), the American Water Works Association (AWWA), and the Water Environment Federation (WEF) [26]. The evaluated parameters included turbidity, color, pH, hardness, alkalinity, and temperature, in order to establish the initial conditions of the water to be treated.

- Coagulation test

The coagulation process was evaluated using jar tests to determine the optimal coagulant dosage, employing 1 L water samples in beakers using a jar test apparatus (Flocculator SW6, Stuart Equipment, Stone, UK). The tests were conducted using raw water collected from the Magdalena River (Barranquilla, Colombia).

Different coagulant dosages ranging from 100 to 450 mg/L were applied. A rapid mixing stage was then performed at 140 rpm for 3 min to ensure homogeneous dispersion of the coagulant. Subsequently, slow mixing was carried out at 45 rpm for 30 min to promote floc formation and growth.

After the mixing stages, the samples were allowed to settle for 20 min. Aliquots were then collected from each beaker to determine turbidity, pH, and color. The optimal coagulant dosage was established based on the lowest residual turbidity, corresponding to the highest turbidity removal efficiency.

The removal efficiency of turbidity and color was calculated using Equation (2):

$$\text{Coagulation efficiency (\%)} = \frac{C_0 - C_t}{C_0} * 100 \quad (2)$$

where $[C_0]$: is the initial concentration of turbidity or color and $[C_t]$: is the residual concentration after a given contact time.

- Effect of slow mixing time on coagulation efficiency

The slow mixing time was varied to determine its optimal value in the coagulation process. Initially, the water sample was characterized, and the previously determined optimal coagulant dosage was applied.

In this test, the stirring speed was fixed at 45 rpm, and different slow mixing times (25, 30, and 35 min) were evaluated. After this stage, the samples were allowed to settle for 20 min to enable the sedimentation of the formed flocs.

At the end of the process, samples were collected to determine water quality parameters (turbidity, pH, and color), in order to compare the results with the initial conditions. The optimal flocculation time was defined as the time that produced the lowest residual turbidity.

2.4. Statistical Analysis of the Data

To evaluate the effect of operational variables on turbidity removal, a full factorial experimental design (3^2) was implemented. The independent variables were flocculation time (A: 25, 30, and 35 min) and coagulant dosage (B: 250, 300, and 350 mg/L). Each experimental condition was conducted in duplicate, resulting in a total of 18 experimental runs.

The selection of the ranges for the two operational variables (coagulant dosage and flocculation time) was based on preliminary jar test experiments (Section 2.3), in which the highest turbidity removal efficiencies were observed. A broader coagulant dosage range of 100–450 mg/L was initially evaluated, with the best performance (region of interest) identified within the selected range used for optimization, corresponding to flocculation times between 25 and 35 min. Additionally, this flocculation time range was selected in accordance with the design criteria for flocculators established by Colombian Resolution 0330 of 2017 for drinking water and basic sanitation systems.

The response variable was turbidity removal efficiency (%). Mean values of the replicates were used for the statistical analysis, and the experimental error was estimated from the variability between replicates. The process variables kept constant throughout the experiments were a rapid mixing time of 3 min, an initial water turbidity of 335 NTU, and stirring speeds of 140 rpm and 45 rpm for rapid mixing (coagulation) and slow mixing (flocculation), respectively.

A second-order polynomial model was used to describe the relationship between the independent variables and the response, including linear, quadratic, and interaction effects (Equation (3)):

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 + \varepsilon \quad (3)$$

where Y is the predicted turbidity removal (%), β terms are regression coefficients, x_1 represents flocculation time, x_2 denotes coagulant dosage, and ε is the random error. Equation (3) was fitted using Statgraphics Centurion version 19. Analysis of variance (ANOVA) was performed at a 95% confidence level to evaluate the statistical significance of the model and its terms. Prior to ANOVA, the assumptions of normality, independence, and homoscedasticity of residuals were verified to ensure the validity of the statistical analysis. All statistical analyses were carried out using the same software.

3. Results and Discussion

3.1. Chitosan Production and Characterization

- Depigmentation process:

Table 1 presents the results of the depigmentation process, in which a yellow, sand-like powder was obtained. A noticeable color change from light gray to a yellowish tone was observed due to contact with sodium hypochlorite, resulting in an overall yield of 78%. According to [27], the depigmentation process occurs exothermically due to gas release.

Table 1. Depigmentation process results.

Initial Weight (g)	NaClO Solution (%)	Chitin/NaClO Ratio (w/v)	Temperature (°C)	Agitation Time (h)	Final Weight (g)	Yield (%)
100	4.5	1:10	25	1	78	78

Upon comparison of the yield obtained in this study with that reported by [28] (27%) using a similar procedure, it can be inferred that the type of fish scales used in this study does not readily degrade upon exposure to sodium hypochlorite, leading to a significantly higher removal efficiency than that reported by [28].

Since the main objective of this process is to remove pigments from the sample, ref. [28] reported that particle size plays a significant role in depigmentation efficiency. When smaller particles are used, as in this study (40 mesh, 0.43 mm), 4–5 washing cycles are typically required, with an average duration of 15 min per wash. In contrast, when larger particles are used, 6–8 washing cycles are needed. This is attributed to the action of sodium hypochlorite, which progressively breaks down the raw material, ultimately yielding a fine, whitish-yellow powder. However, caution must be exercised when using this reagent, as it has been reported to induce morphological modifications in chitin [29].

- Deproteinization process:

Table 2 presents the results obtained from the second stage of the methodology, corresponding to deproteinization. The mass yield of this stage was 78.2%. During the process, foam formation and a characteristic cooked fish odor were observed. After the washing

steps required to achieve neutral pH, a noticeable change in texture was identified, shifting from a fine to a more granular structure. Compared to the previous stage (depigmentation), where the material exhibited a very fine texture, the sample in this stage appeared more compact.

Table 2. Results of the deproteinization process.

Initial Weight (g)	Solution	Chitin/NaOH Ratio (w/v)	Temperature (°C)	Agitation Time (h)	Final Weight (g)	Yield (%)
78	NaOH 10%	1:10	25	4	61	78.20

A process yield of 78% was obtained in this stage, indicating that the hydrogen bonds linking protein molecules were effectively disrupted, allowing their solubilization without significant disintegration of the raw material. In contrast, ref. [28] reported a yield of 92% under similar operating conditions. This difference may be attributed to variations in the composition of the fish scales used, as that study employed rainbow trout scales, whereas the present work used Mojarra lora scales.

To more accurately assess the efficiency of the process, protein content was determined using the Kjeldahl method, based on total nitrogen quantification and its conversion into protein using an appropriate conversion factor. The analysis included acid digestion, followed by distillation and titration to determine the nitrogen content [30,31]. The results are presented in Table 3. Comparison with other raw materials indicates that the operating conditions used in this study were appropriate, as the low final protein content confirms the effectiveness of the deproteinization process, with most of the protein fraction successfully removed from the scales. Although rainbow trout scales showed a higher yield (92%) than that obtained in this study (78%), this may be due to incomplete deproteinization, resulting in less structural degradation of the raw material.

Table 3. Comparison of protein content (%) from different chitosan sources.

Source	Initial Protein (%)	Initial Protein (%)	Initial Protein (%)
Mojarra lora scales	49.12%	<2.45%	This study
Rainbow trout scales	62.89%	6.94%	[28]
Commercial chitosan (Acros)	-	39.91%	[32]

Furthermore, when compared with commercial chitosan (ACROS brand), which exhibited a higher protein content (39.91%) than that obtained from Mojarra lora scales (<2.45%), the results suggest that the operating conditions applied in this study were more effective in removing protein residues.

- Demineralization process:

Following the demineralization stage, a fine white powder was obtained. This material exhibits morphological characteristics comparable to the chitin reported by [28] from rainbow trout scales and by [27] from shrimp shells, indicating the effectiveness of the mineral removal process. The quantitative results of this stage are summarized in Table 4. The demineralization yield reached 88.52%, reflecting a high process efficiency. This value is notably higher than the 77% yield reported by [28] under similar operating conditions, suggesting a more effective removal of inorganic components in the present study.

Table 4. Demineralization test results.

Initial Weight (g)	Solution	Ratio (w/v)	Temperature (°C)	Agitation Time (h)	Final Weight (g)	Yield (%)
61	HCl 0.5N	1:10	25	3	54	88.52

Chitin is a biopolymer that can undergo denaturation when exposed to elevated temperatures. Therefore, it is essential to avoid high-temperature treatments, as they may lead to polymer degradation [33]. For this reason, this stage was conducted at room temperature, consistent with the previous processing steps. According to [28], the use of a complexing agent such as EDTA can help prevent degradation and further improve the efficiency of this stage.

Another critical step is the washing process. If residual acid remains trapped within the solid structure, it may cause cleavage of the chitin polymer chains [27]. To prevent this, small amounts of an alkaline solution should be added until the supernatant reaches a neutral pH, particularly if the deacetylation step to obtain chitosan is not performed immediately [28].

Table 5 presents the results of ash content determination, which was used to evaluate the efficiency of the demineralization process. The observed results can be attributed to the relatively high ash content, likely due to the significant mineral composition of the mojarra lora scales. This elevated mineral content reduces the overall efficiency of the demineralization stage.

Table 5. Comparison of ash content (%) from different chitosan.

Source	Initial Ash (%)	Final Ash (%)	Reference
Mojarra lora scales	75.70	40.52	This study
Rainbow trout scales	26.71	75.15	[28]

The high ash content may also be associated with the limited penetration of hydrochloric acid into the raw material. Therefore, future studies could explore the use of alternative acids to assess their effect on ash removal efficiency and overall process yield.

- Deacetylation process:

This stage constituted the final step in the production of chitosan. The chitin initially exhibited a white, granular structure; however, after the process, a very fine white powder was obtained, corresponding to chitosan. During this stage, the derivatization of chitin takes place, with the main reaction being the hydrolysis of acetamide functional groups, leading to the formation of the deacetylated polymer, chitosan, in which acetyl groups are replaced by amino groups [27].

The results of this procedure are presented in Table 6. A yield of 30% was obtained, which is lower than the values reported by [28] (38.81%) and [27] (41.14%). This reduced yield may be attributed to insufficient reaction time, as longer durations are often required to obtain chitosan that is soluble in acidic media. Furthermore, it is important to note that the yield is influenced by the increase in reaction temperature, which enhances the degree of deacetylation. However, higher temperatures also promote degradation reactions in polysaccharides, resulting in a reduction in polymer molecular weight.

Table 6. Deacetylation test results.

Initial Weight (g)	NaOH Solution (%)	Chitin/NaOH Ratio (w/v)	Temperature (°C)	Agitation Time (h)	Final Weight (g)	Yield (%)
30	40	1:10	80	4	9	30

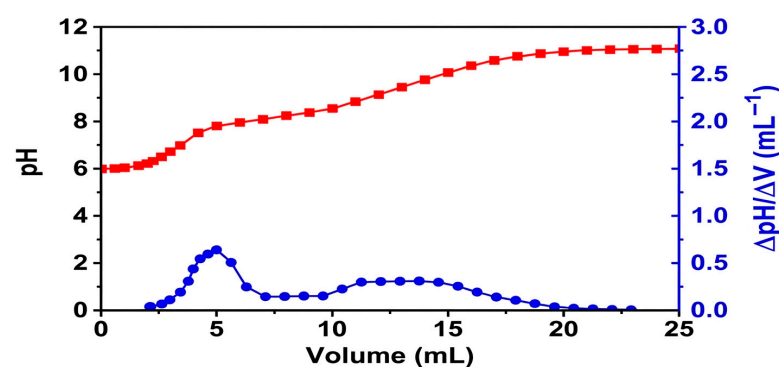
This decrease in molecular weight can be mitigated by conducting the reaction in the absence of oxygen or by incorporating antioxidants, thereby minimizing depolymerization processes [34].

- Potentiometric titration:

The degree of deacetylation (DD) is directly related to the amount of free amino groups and represents a key parameter in chitosan production, as it strongly influences its physico-chemical properties, reactivity, and biodegradability. Table 7 presents a comparison of DD values reported for chitosan obtained from different sources, including two commercial-grade samples. The potentiometric titration results obtained in this study are shown in Figure 1.

Table 7. Comparison of degree of deacetylation (%) from different chitosan sources.

Source	DD (%)	Reference/Brand
Mojarra lora scales	76.87	This study
Rainbow trout scales	87.01	[28]
Shrimp shells	87.92	[27]
Commercial chitosan (Sigma-Aldrich)	<75.0	Sigma-Aldrich
Commercial chitosan (Acros)	88.61	[28]

**Figure 1.** Potentiometric titration curve ($\Delta\text{pH}/\Delta V$).

The chitosan produced from mojarra scales in this study exhibited a lower degree of deacetylation compared to commercial chitosan (88%) and to chitosan derived from shrimp shells and rainbow trout scales. Nevertheless, the obtained material meets the minimum requirement of a DD higher than 75% to be classified as chitosan. Therefore, it remains suitable for a wide range of applications, particularly in industrial processes and water treatment.

3.2. Jar Test

3.2.1. Raw Water Characterization

Table 8 presents the initial characterization of the water sample used to evaluate the effectiveness of the obtained chitosan as a coagulant. The analyzed parameters included turbidity, pH, alkalinity, color, and temperature.

Table 8. Characterization of water sample from the Magdalena River.

Parameter	Unit	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Turbidity	NTU	335	333	328	332	2.94
Color	PCU	470	500	500	490	17.32
pH	pH units	8.45	8.23	8.06	8.24	0.16
Alkalinity	mg/CaCO ₃	80	80	80	80	0.00
Temperature	°C	24.8	24.5	24.8	26.03	0.68

3.2.2. Determination of the Optimal Coagulant Dose

Figure 2 illustrates the variation in turbidity and removal efficiency as a function of the applied chitosan dose. As observed, increasing the coagulant dosage leads to higher turbidity removal, reflected in a decrease in residual turbidity. When the dosage increased from 100 to 450 mg/L, the average turbidity decreased from approximately 335 to 113 NTU, corresponding to removal efficiencies ranging from 30% to 72%.

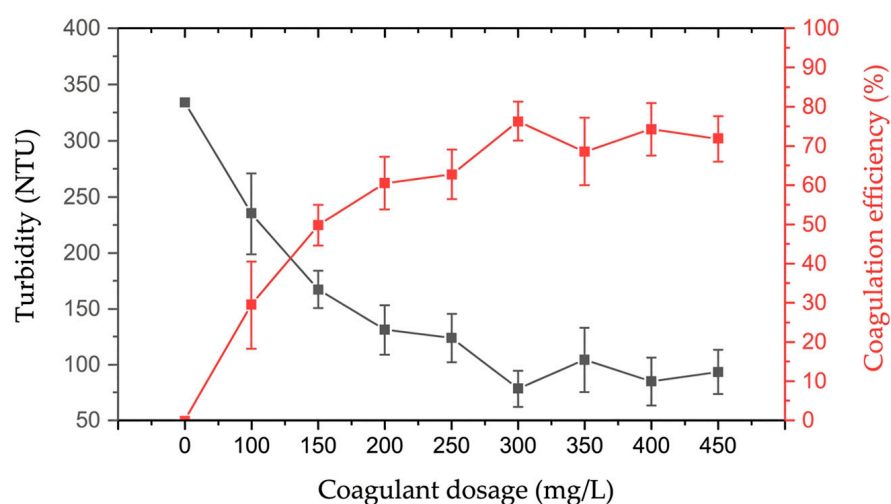


Figure 2. Effect of coagulant dosage (100–450 mg/L) on residual turbidity (NTU) and coagulation efficiency (%), at natural pH (8.4), and initial turbidity of 335 NTU.

However, no significant improvements in turbidity removal were observed beyond a dosage of 300 mg/L. At higher doses (350–450 mg/L), turbidity increased slightly. This behavior may be attributed to an overdosing effect, whereby excess coagulant induces [17,18,35]. As a result, previously destabilized particles may re-disperse, leading to the formation of a large number of very small microflocs with low settling velocities. Consequently, the residual turbidity remains relatively high. Based on these results, a dosage of 300 mg/L was identified as the optimal coagulant dose for the coagulation process.

The identified optimal dosage confirms the aforementioned behavior, indicating that higher dosages may reduce turbidity removal efficiency, as previously reported in the literature. However, those studies were conducted using water matrices with different characteristics. For instance, the coagulant dosages evaluated ranged between 0.5 mg/L and 30 mg/L for treating turbidity levels between 5 NTU and 200 NTU, under different operational conditions [17–21]. These differences may explain the variations in removal efficiencies reported in those studies compared to the present results, as such factors significantly influence the coagulation process.

Therefore, the coagulant dosage range selected in this study (250–350 mg/L) for the response surface experimental design differs from those reported in previous studies and

was defined to expand the region in which the coagulant exhibited the highest efficiency (see Figure 2). This range was strategically chosen to enable a detailed evaluation of the effect of dosage on turbidity removal efficiency. Lower dosages were not considered, as preliminary tests indicated that they were not effective for the water matrix studied and were therefore excluded from further analysis.

Although no physicochemical characterization of the coagulant or the generated sludge was performed in this study, the turbidity removal mechanisms of chitosan can be inferred based on previous literature. The removal process is mainly attributed to charge neutralization and adsorption–bridging mechanisms, in which positively charged amino groups interact with negatively charged colloidal particles, promoting their destabilization and aggregation into larger flocs. These mechanisms are consistent with the turbidity removal efficiencies observed in this study [36–38]. However, further research involving detailed physicochemical characterization is required to confirm these mechanisms under the specific operating conditions evaluated.

Although relatively high coagulant doses were required to achieve high turbidity removal efficiency, the economic viability of chitosan as an alternative coagulant should be carefully evaluated. Despite the preparation of chitosan as a coagulant involving multiple processing steps, as previously described, these can potentially be optimized or integrated at an industrial scale [19,39]. Considering the abundant availability of fishery waste as a low-cost raw material, production costs can be reduced while simultaneously adding value through sustainable practices that mitigate the costs associated with waste management. Under these conditions, chitosan-based coagulants may become competitive with conventional chemical coagulants, which are often considered relatively expensive, such as aluminum sulfate. Therefore, chitosan represents a sustainable alternative for water treatment applications [17,20,40,41].

3.2.3. Effect of Slow Mixing Time on Process Efficiency

After establishing the optimal coagulant dose, the flocculation time was evaluated. Figure 3 presents the turbidity (NTU) and turbidity removal efficiency obtained for flocculation times of 25, 30, and 35 min.

Based on the results, the optimal flocculation time was determined to be 30 min, as this condition achieved a turbidity reduction of approximately 45%. Beyond this time, flocs may begin to break apart, which explains the increase in turbidity observed at 35 min. In contrast, at 25 min, the flocculation time was insufficient, preventing complete floc formation and resulting in lower removal efficiency.

These findings indicate that 35 min of flocculation is excessive for effective floc formation. This behavior can be attributed to the fact that slow mixing intensity and flocculation time are critical factors influencing the coagulation process when natural coagulants such as chitosan are used. Under non-optimal conditions, these factors may negatively affect floc formation and stability, thereby reducing turbidity removal efficiency [17,20,21]. Therefore, the flocculation time range evaluated in this study can be considered relatively narrow and consistent with values previously reported in the literature [20].

Table 9 summarizes the flocculation times reported for commercial chitosan, chitosan obtained from different sources, and related compounds. For most chitosan types, optimal flocculation times range between 20 and 30 min; since beyond this interval, flocs tend to break apart and particle re-aggregation becomes difficult.

It is important to highlight that not only the optimal coagulant dose but also the flocculation time is influenced by the degree of deacetylation (DD). For instance, ref. [42] reported high removal efficiencies using similar flocculation times; however, their chitosan exhibited a higher DD (85%) than that obtained in this study. Similarly, refs. [27,43] used

shrimp shell-derived chitosan, indicating that the origin of the raw material also plays a significant role in turbidity removal efficiency and other performance parameters.

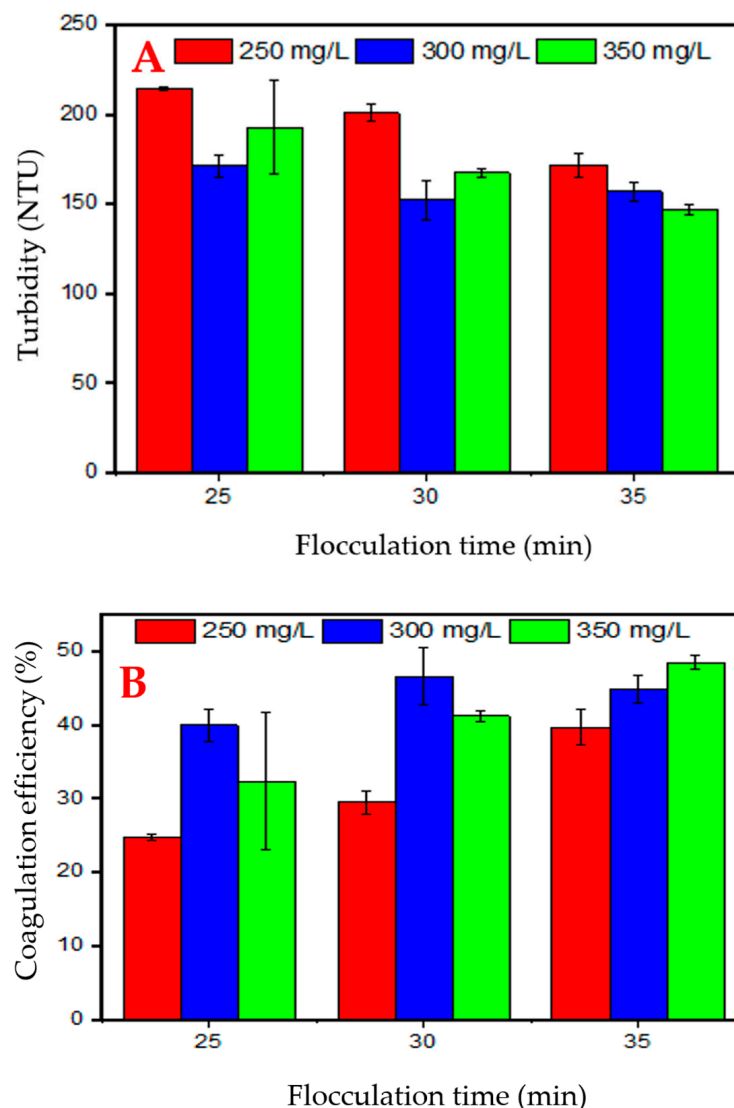


Figure 3. Effect of flocculation time (25–35 min) on (A) residual turbidity (NTU) and (B) coagulation efficiency (%), at chitosan doses of 250, 300, and 350 mg/L, natural pH (8.4), and an initial turbidity of 335 NTU.

Currently, chitosan derived from shrimp exoskeletons is widely used in various industrial applications due to its typically high degree of deacetylation. However, in the present study, chitosan obtained from mojarra scales showed promising results, including a relatively high degree of deacetylation and favorable performance. Moreover, when compared across different stages of the production process with commercial chitosan and shrimp shell-derived chitosan, it demonstrated improved protein removal and overall process efficiency. Additionally, it achieved a significant reduction in turbidity in river water samples treated for potable use.

From an environmental perspective, the implications of sludge generation should also be considered. Sludge generation and its management were not characterized in this study. However, previous studies have shown that natural coagulants, such as chitosan, tend to generate biodegradable sludge with a lower environmental impact compared to conventional chemical coagulants, enhancing its potential for reuse within a circular economy framework [44]. Nevertheless, further research is needed to characterize sludge production

and composition, as well as to assess appropriate management strategies, including reuse or disposal. For instance, the resulting sludge could be valorized as a precursor for biochar production for soil amendment or for biomass-based energy generation through processes such as incineration [45–47].

Table 9. Comparison of flocculation times for chitosan from different sources.

Chitosan Source	Flocculation Time (min)	Reference
Mojarra lora scales	30	This study
Chitosan sulfate	30	[48]
Shrimp shells	5	[48]
Commercial chitosan (Sigma-Aldrich)	3	[49]
Commercial chitosan and shrimp shells	20	[43]
Shrimp shells	20	[27]

3.3. Statistical Analysis

To evaluate the effect of coagulant dose and flocculation time on water turbidity, an analysis of variance (ANOVA) was performed. Table 10 presents the estimated effects and their corresponding interactions.

Table 10. Analysis of variance for turbidity (ANOVA).

Source	Sum of Squares	df	Mean Square	F-Ratio	p-Value
A: Flocculation time (min)	10,472.1	1	10,472.1	58.72	0.0000
B: Coagulant dosage (mg/L)	6426.69	1	6426.69	36.04	0.0000
AA	53.4815	1	53.4815	0.30	0.5865
AB	16.6667	1	16.6667	0.09	0.7611
BB	5911.12	1	5911.12	33.15	0.0000
Total error	8559.85	48	178.33		
Total (corrected)	31,439.9	53			

The ANOVA table partitions the variability in turbidity into contributions associated with each factor and their interactions. The statistical significance of each effect is then assessed by comparing its mean square with an estimate of the experimental error. In this case, three effects showed *p*-values lower than 0.05, indicating that they are statistically significant at a 95% confidence level. These results suggest that both coagulant dose and flocculation time have a significant effect (*p*-value < 0.05) on turbidity removal efficiency.

Furthermore, the ANOVA results indicate that the observed variations in performance are primarily attributed to the studied process variables rather than external factors.

These findings are further supported by the Pareto chart (Figure 4), which ranks the input factors and their interactions according to their relative importance. The flocculation time was identified as the most influential factor affecting turbidity removal, followed by the optimal coagulant dose, and finally their interaction. Effects exceeding the standardized reference line in the Pareto chart are considered statistically significant in relation to the observed turbidity response.

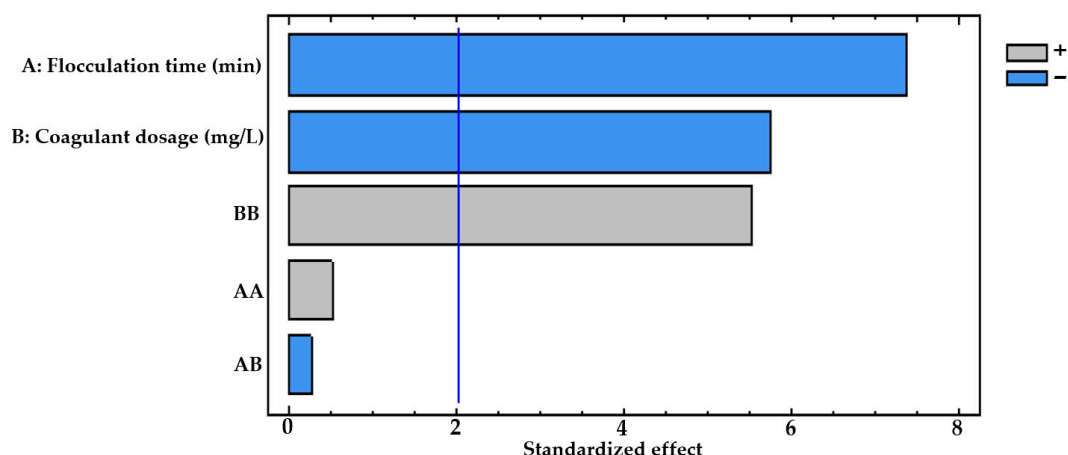


Figure 4. Standardized Pareto chart for turbidity.

Figure 5 illustrates the relationship between the evaluated factors. It was observed that increasing the flocculation time led to a decrease in turbidity, indicating an inverse relationship between these variables. In contrast, the coagulant dose exhibits a second-order (quadratic) relationship with turbidity. Specifically, as the coagulant dose increases, turbidity initially decreases due to enhanced particle destabilization and aggregation. However, beyond the optimal dose of 300 mg/L, no further improvement is observed. At this point, the coagulant becomes ineffective, as there are no remaining particles available for interaction, and overdosing effects may occur.

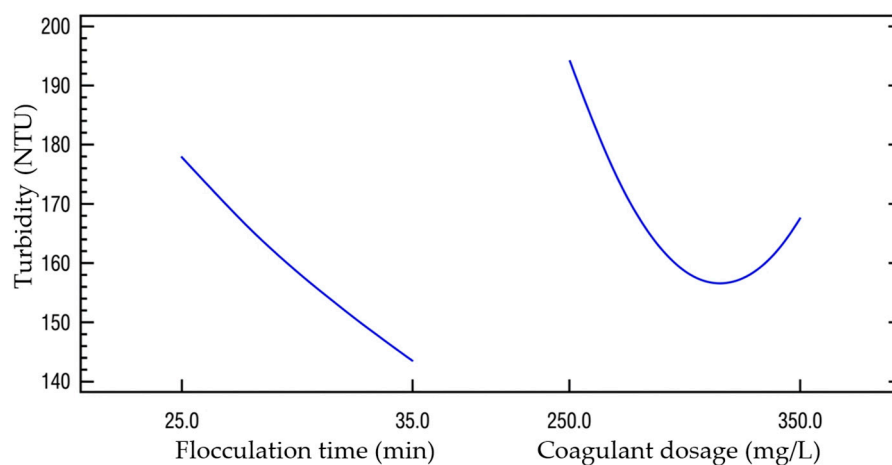


Figure 5. Main effects for turbidity: (a) flocculation time (min) and (b) coagulant dose (mg/L).

3.4. Regulatory Comparison

A comparison with the Colombian drinking water quality standard (Resolution 2115 of 2007) (Table 11) indicated that turbidity and color did not meet the maximum permissible limits. The non-compliance of these parameters may be associated with factors such as the degree of deacetylation (DD) of the chitosan. Achieving a higher DD (>76%) could enhance turbidity removal, potentially allowing compliance with the regulatory standards.

Additionally, the mineral composition of fish scales should be considered, as it can influence impurity retention and overall treatment performance. A high mineral content may reduce the efficiency of the demineralization process; therefore, the use of alternative solvents, beyond hydrochloric acid (HCl), should be explored to improve mineral removal. Despite not meeting the limits established by Resolution 2115, a turbidity removal efficiency of 76.30% was achieved. Furthermore, previous studies on different types of fish scales

have reported the presence of heavy metals such as Cd, Cr, Cu, Fe, Hg, and Pb, which contribute to higher ash content [50,51].

Table 11. Comparison with Resolution 2115 of 2007.

Parameter	Unit	Final Sample	Standard (Resolution 2115)	Compliance
Turbidity	NTU	76.30	2	Non-compliant
Color	PCU	370	15	Non-compliant
pH	pH units	7.13	6.5–9.0	Compliant
Alkalinity	mg/CaCO ₃	80	200	Compliant

It is highly probable that these metals are also present in the fish scales used in this study. Therefore, a proximate and compositional analysis is recommended to identify the types and concentrations of metals present in the raw material. Subsequently, a post-treatment analysis of the water after the coagulation–flocculation process should be conducted in order to assess and minimize the presence of these metals. This approach would contribute to improving turbidity and color removal efficiencies.

Furthermore, it is important to note that the treatment process evaluated in this study is not complete, as it only includes the coagulation–flocculation and sedimentation stages, while excluding the filtration step. Filtration plays a critical role in removing remaining impurities, thereby facilitating a further reduction in color and turbidity levels [52].

4. Conclusions

Chitosan was successfully obtained from *Oreochromis niloticus* scales through a chemical process, achieving satisfactory yields at each stage, particularly during demineralization (88.52%). The resulting material exhibited a degree of deacetylation of 76.87%, which is suitable for its application as a coagulant in water treatment. The application of chitosan in coagulation–flocculation processes enabled turbidity removal efficiencies of up to 76.30% under optimal operating conditions (300 mg/L and 30 min of slow mixing). Statistical analysis confirmed that coagulant dose and flocculation time are significant variables affecting process efficiency. However, the treated water did not meet the regulatory limits for turbidity and color established by the Colombian standard (Resolution 2115 of 2007), indicating that the coagulation–flocculation process alone is insufficient to achieve potable water quality. The presence of residual minerals and the degree of deacetylation of chitosan were identified as key factors influencing its performance. For future studies, it is recommended to optimize the deacetylation process to increase the degree of deacetylation, implement purification steps to remove residual metals, and complement the treatment with additional processes such as filtration. These improvements would enhance the efficiency of chitosan and support its potential as a sustainable alternative in water treatment applications.

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