# Potential of *Citrullus lanatus* seeds as a natural coagulant in drinking water treatment

## Potencial como coagulante natural de las semillas de *Citrullus lanatus* en el tratamiento de agua potable

## Potencial como coagulante natural de sementes de *Citrullus lanatus* no tratamento de água potável

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Rev. Fac. Agron. (LUZ). 2025, 42(2): e254224
ISSN 2477-9407
DOI: https://doi.org/10.47280/RevFacAgron(LUZ).v42.n2.VIII

**Environment**

Associate editor: Professor Beltrán Briceño
University of Zulia, Faculty of Agronomy
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Received: 19-02-2024
Accepted: 30-04-2025
Published: 20-05-2025

**Keywords:**
Coagulation
Natural coagulants
Rapid mixing
Watermelon seeds
Turbidity

## Abstract

Natural coagulants have received significant attention due to their biodegradable nature, cost-effectiveness, and abundance of sources. The objective of the research was to analyze the efficiency of *Citrullus lanatus* seeds treating synthetic waters with three levels of initial turbidity (13, 75, and 200 NTU), applying four rapid mixing times (1, 2, 4, and 5 min) in the purification process. The coagulant was prepared with 5 g of the seed previously ground, sieved, defatted, and diluted in a volume of 1 L of distilled water. Jar tests were performed using doses of 50, 70, 90, 100, 150, 200, 250, 300, 400, and 500 mg.L$^{-1}$ using true color and turbidity as control parameters. The coagulation was performed at a speed of 100 rpm, while flocculation lasted 20 minutes at 30 rpm, and the resting phase was 30 minutes. It was obtained that the optimal doses of *C. lanatus* in the treatment process were 50, 150, and 300 mg. L$^{-1}$ for waters with 13, 75 and 200 NTU, respectively, obtaining the highest percentages of turbidity removal (96.6 %) and true color (94.4 %) in water with 75 NTU. Furthermore, it was concluded that increasing the time in the rapid mixing phase caused an increase in the levels of turbidity and true color; being the time of 2 min the one that generated water that meet the values desired by the current Venezuelan drinking water regulations, which contemplates 1 NTU for turbidity and 5 UC Pt-Co for true color.

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## Resumen

Los coagulantes naturales han recibido una atención significativa debido a su naturaleza biodegradable, rentabilidad y abundancia de fuentes. El objetivo de la investigación fue analizar la eficiencia de las semillas *Citrullus lanatus* tratando aguas sintéticas con tres niveles de turbidez inicial (13, 75 y 200 UNT) aplicando cuatro tiempos en la fase de mezcla rápida (1, 2, 4 y 5 min) en el proceso de clarificación. El coagulante se preparó con 5 g de la semilla previamente molida, tamizada, desengrasada, y diluida en un volumen de 1 L de agua destilada. Se realizaron pruebas de Jarra utilizando dosis de 50, 70, 90, 100, 150, 200, 250, 300, 400 y 500 mg.L$^{-1}$, utilizando el color verdadero y la turbidez como parámetros de control. La coagulación se realizó a una velocidad de 100 rpm, mientras que, la floculación duró 20 minutos a 30 rpm y la fase de reposo fue de 30 minutos. Se obtuvo que las dosis óptimas de *C. lanatus* en el proceso de tratamiento fueron 50, 150 y 300 mg.L$^{-1}$ para aguas con 13, 75 y 200 UNT, respectivamente, obteniendo los mayores porcentajes de remoción de turbidez (96,6 %) y color verdadero (94,4 %) en el agua con 75 UNT. Además, se concluyó que el aumento del tiempo en la fase de mezcla rápida ocasionó un incremento en los niveles de turbidez y color verdadero; siendo el tiempo de 2 min, el que generó aguas que cumplen con los valores deseados por la normativa venezolana vigente de agua potable que contempla 1 UNT para la turbidez y 5 UC Pt-Co para el color verdadero.


**Palabras clave**: coagulación, coagulantes naturales, mezcla rápida, semillas de sandía, turbidez.


## Resumo

Os coagulantes naturais têm recebido atenção significativa devido à sua natureza biodegradável, custo-benefício e abundância de fontes. O objetivo da pesquisa foi analisar a eficiência de sementes de *Citrullus lanatus* tratando águas sintéticas com três níveis de turbidez inicial (13, 75 e 200 NTU) aplicando quatro tempos rápidos de mistura (1, 2, 4 e 5 min) no processo de purificação. O coagulante foi preparado com 5 g da semente previamente moída, peneirada, desengordurada e diluída em volume de 1 L de água destilada. Os testes de frascos foram realizados nas doses de 50, 70, 90, 100, 150, 200, 250, 300, 400 e 500 mg.L$^{-1}$, utilizando cor verdadeira e turbidez como parâmetros de controle. A coagulação foi realizada a uma velocidade de 100 rpm, enquanto a floculação durou 20 minutos a 30 rpm e a fase de repouso foi de 30 minutos. Obteve-se que as doses ótimas de C. lanatus no processo de tratamento foram 50, 150 e 300 mg.L$^{-1}$ para águas com 13, 75 e 200 NTU, respectivamente, obtendo-se os maiores percentuais de remoção de turbidez (96,6 %) e cor verdadeira (94,4 %) em água com 75 NTU. Além disso, concluiu-se que o aumento do tempo na fase de mistura rápida provocou aumento nos níveis de turbidez e cor verdadeira; O tempo foi de 2 min, o que gerou águas que atendem aos valores desejados pela atual regulamentação venezuelana que contempla 1 UNT para turbidez e 5 UC Pt-Co para cor.


**Palavras-chave**: coagulação, coagulantes naturais, mistura rápida, sementes de melancia, turbidez.

## Introduction

The demand for drinking water is increasing globally along with the increase in population (Dahasahastra *et al.,* 2022). Water pollution is becoming more frequent, which has implications for the development of communities, both economically and socially (United Nations Children's Fund [UNICEF], 2014). The lack of safe water for human use, sanitation and cleanliness represents a major and urgent health problem; therefore, improving water resources management could prevent approximately 10 % of all diseases that occur (World Health Organization - United Nations Children's Fund [WHO-UNICEF], 2015), especially in poor and vulnerable groups, particularly children under five years old (Roy, 2023).

Currently, there is a lack of access to safe water in rural and semi-urban regions, where a significant part of the population uses turbid raw water. In addition, it is estimated that climate change will affect the quality of drinking water, impacting public health (Mishra, 2023). That is why the elimination of colloidal and suspended particles from water is advantageous since it mitigates the inconveniences caused by turbidity. In this regard, the Official Gazette of the Republic of Venezuela (GORBV) No. 36,395 dated February 13, 1998, establishes 1 NTU (nephelometric turbidity units) as the desired value and 5 NTU as the maximum permissible limit in waters intended for human consumption.

There are several conventional treatment methods for turbidity removal, including coagulation-flocculation, chemical adsorption, and filtration. These methods effectively remove colloidal and suspended particles larger than 5 μm (Salinas *et al.,* 2023). The coagulation that involves the use of specific chemicals, such as alum, has been employed for the removal of colloids. However, research has highlighted the potential risks associated with residual aluminum intake, including neurodegenerative disorders and Alzheimer's disease. Therefore, it is of great importance to find an option to replace chemical coagulants. Natural substances represent an option for use in water clarification due to their abundant availability, low toxicity, high biodegradability, and low rate of sewage sludge production (Balbinoti *et al*. 2024).

Natural coagulants can be produced using as raw material the seeds of plants with high protein content that contain carboxyl, hydroxyl, and amino groups with a significant affinity towards various contaminants. Organic coagulants have been made from various parts or seeds of plants such as *Moringa oleifera* (Junho *et al.*, 2021), *Cassia fistula* (Arias *et al*., 2020), *Tamarindus indica* (Carrasquero *et al.,* 2019), *Cucumis melo* (Abuda *et al.*, 2021), and *C. lanatus* (Sathish *et al.*, 2018), which are known for their natural coagulant properties that remove turbidity, BOD, TSS, and COD, and also possess antimicrobial power (Ugwu *et al*., 2017).

The objective of the research was to analyze the efficiency of *C. lanatus* seeds in treating synthetic waters with three initial turbidity levels (13, 75, and 200 NTU), applying four rapid mixing times (1, 2, 4, and 5 min) in the clarification process.

## Materials and methods

### Preparation of synthetic turbid water (STW)

To prepare the STW, the procedure recommended by Okuda *et al.* (2001) was used. Five grams of kaolinite clay were weighed and added to 1 L of distilled water. Subsequently, the different turbidity values tested (13, 75, and 200 NTU) were obtained through successive dilutions, with the following true color levels of 9, 19, and 32 UC Pt-Co, respectively.

**Coagulant solution**

*C. lanatus* seeds were collected from residues from producing fruit juices and salads in Maracaibo, Zulia state, Venezuela. It should be noted that the endosperm was used; so, the seeds were washed, then manually peeled and dried in an oven (Hamilton Beach, Mod. 31105, USA) at a temperature of 60 ± 5 °C for 2 h, then processed in a manual mill (Corona Nacional, Venezuela) until obtain a flour, which was sieved through a 60 sieve (0.250 mm pore diameter) and stored in amber jars for subsequent characterization, defatting and use.

The flour obtained was defatted using the Soxhlet method. Twenty-five grams (25 g) were weighed and placed in an extraction cartridge, and 150 mL of hexane (Merck, Purity 98.5 %) was added to the flask; the extraction was performed for 8 h at a temperature of 45 °C. Once the seeds were defatted, they were dried in an oven (Hamilton Beach, Mod. 31105, USA) for 4 h at 50 °C (Association of Official Analytical Chemists [AOAC], 2005). Then, they were characterized by the following parameters: Humidity (Venezuelan Commission of Industrial Standards [COVENIN], 1980), ash content (COVENIN, 1981a), and extractable oils and fats (COVENIN, 1981b).

The coagulant was prepared with 5 g of the seed previously ground, sieved, and defatted, in a volume of 1 L of distilled water. Subsequently, the evaluated doses were obtained by dilution: 50, 70, 90, 100, 150, 200, 250, 300, 400, and 500 $mg.L^{-1}$ as recommended by Carrasquero *et al.* (2019). Coagulation-flocculation tests were performed using the Jar test in variable agitation equipment (Phipps and Brid Inc., Mod. 300, USA), treating synthetic waters of low, medium, and high turbidity, i.e., 13, 75, and 200 NTU, simulating drought and rainfall conditions that can cause turbidity variations in natural water bodies (Bina *et al.,* 2009).

**Efficiency of the natural coagulant in the clarification process**

Five hundred (500 mL) of STW were poured into the beakers, then the different quantities of coagulant were dosed, and rapid mixing was initiated, which corresponded to coagulation, at 100 rpm for 2 min, followed by flocculation with a slow mixture at 30 rpm for 20 min, and, finally, sedimentation was carried out for 30 min. At the end of this time, a sample of treated water was extracted, and filtered in a vacuum pump using Whatman paper with a pore size of 25 µm, for the determination of the physicochemical parameters: true color (platinum-cobalt method), turbidity (nephelometric method), total alkalinity (volumetric method), pH (standard potentiometric method), total dissolved solids (gravimetric method), and total solids (gravimetric method), following the *Standard Methods for the Examination of Water and Wastewater* (Lipps *et al*., 2022).

To determine the effect of coagulation time in the rapid mixing phase, Jar tests were performed by applying the doses that generated the lowest values of turbidity and true color obtained in the previous section under different times (1, 2, 4, and 5 min).

**Experimental design**

The results of the physicochemical parameters measured in seed characterization were expressed using descriptive statistics, indicating the values of central tendency (mean) and dispersion (standard deviation). The results of the removal of physicochemical parameters were studied using an analysis of variance and comparison of means through Tukey's test, using the statistical program IBM SPSS Statistics. Before performing the ANOVAs, both the homogeneity of the variances (Bartlett's test) and the normal distribution of the residuals (Kolmogorov-Smirnov Test) were checked, complying with these precepts without requiring mathematical transformation.

In the first stage, the effectiveness of the coagulant solution in removing turbidity and true color for different initial turbidity levels was evaluated, using a completely randomized design with a total of 9 assays (3 turbidity levels × 3 repetitions). For this statistical analysis, a one-way ANOVA was applied, complemented with Tukey's mean separation test. In the second stage, the effect of mixing time on coagulant effectiveness was studied, applying a 3×4 factorial design (three turbidity levels by four stirring times), with a total of 12 treatments. In this case, a two-way ANOVA was used, followed by Tukey's test for the comparison of means.

## Results and discussion

**Physicochemical characteristics of *C. lanatus* seed**

The preliminary characterization of the seed was carried out by determining moisture, oils and fats, and ashes, as physicochemical parameters (table 2).

**Table 2. Physicochemical characteristics of *C. lanatus* seed.**

| Parameter ( % ) | Arithmetic mean value ± standard deviation |
|---|---|
| Removable oils and fats | 25.40 ± 1.76 |
| Moisture | 5.43 ± 0.42 |
| Ash content | 4.07 ± 0.15 |

n:3. N: Number of repetitions.

The average percentage of oils and fats was 25.40 %, a value higher than that reported for other seeds used in the clarification process, such as *Mangifera indica* L. (15.00 %), and *Tamarindus indica* (7.63 %) (Carrasquero *et al*., 2019), which confirms the need to defat the seed before performing clarification tests because the natural coagulant could incorporate dissolved particles, fats and oils from the seed (Carrasquero *et al*., 2015). Regarding the percentage of moisture, an average value of 5.43 % was found, which is positive, because a higher water content would accelerate the appearance of decomposition reactions, which would cause the deterioration of the seed.

**Effectiveness of *C. lanatus* seeds by applying various doses in the treatment of synthetic waters with three levels of initial turbidity**

At the end of the treatments, a decrease in turbidity was obtained compared to the initial values. However, the increase in coagulant dose was associated with slight increases in measured residual turbidity, although they always remained below the original levels, evidencing the effectiveness of the seed in turbidity removal (figure 1).

The results obtained showed a turbidity elimination range of 46.6 to 96.8 % during the analysis of all synthetic turbid waters. For an initial turbidity of 13 NTU, values were obtained after the coagulation-flocculation process, ranging from 0.82 to 7.43 NTU, applying doses between 50 and 500 $mg.L^{-1}$. The highest percentages of turbidity reduction, 91.8 and 92.5 %, were obtained using doses of 50 and 70 $mg.L^{-1}$, respectively, presenting statistical differences ($p \leq 0.05$) with the rest of the doses applied.

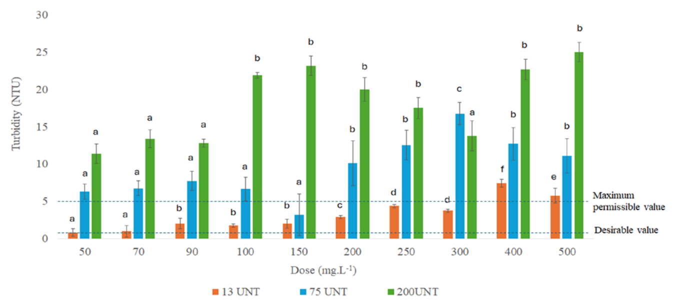


**Figure 1. Variation in the turbidity of the treated water as a function of the dose of *C. lanatus* seeds.** Vertical bars indicate the arithmetic mean ± standard deviation for n:3. Mean followed by different letters in each bar of the same color indicates significant differences according to Tukey's test (p≤0.05)

According to the statistical analysis carried out, in waters of 13 NTU, it is possible to identify five groups of doses that present residual levels of turbidity with significant differences: 50-70, 90 -150, 200, 250-300, 400, and 500 mg.L$^{-1}$. It is inferred that low doses are effective in significantly reducing the initial low turbidity. For all the doses evaluated in this type of water, removal percentages greater than 90 % were obtained.

From a dose of 90 mg.L$^{-1}$, a partial saturation effect could be observed, where the removal efficiency begins to decrease. Partial saturation is a phenomenon that occurs in coagulation processes when an excessive amount of coagulant is added (such as *C. lanatus* seeds in this case), exceeding the point at which the removal of suspended particles is maximized, without yet reaching complete supersaturation (Arciniega *et al*., 2024).

For waters with a turbidity of 75 NTU, the maximum removal of 95.7 % was achieved with a dose of 150 mg.L$^{-1}$, obtaining a residual value of 3.2 NTU. Concerning the waters of 200 NTU, even though turbidity removals greater than 86.5 % were obtained, the final values of this parameter did not comply with the maximum value allowed in the Venezuelan drinking water regulations (GORBV No.36.395, 1998).

The oscillating trend of the removal percentages obtained for the natural coagulant and the increase in turbidity produced with doses greater than 300 mg.L$^{-1}$ confirms that seed removal occurs because the seed provides high molecular weight polymers (cationic proteins) that can chemically adsorb the colloidal particle at fixed adsorption sites. As this phenomenon occurs between different colloids, the particles agglomerate in groups. According to Mandizvo *et al.* (2022), *C. lanatus* seeds are a rich source of protein, with protein fractions including globulins (63.7 %), albumins (18.6 %), and glutelins (14.0 %).

A true color reduction was obtained for waters of 75 NTU, ranging from 92.7 to 97.3 % with doses between 50 and 150 mg.L$^{-1}$, obtaining residual values between 2.0 and 5.5 UC, presenting significant differences (p≤0.05) with the rest of the doses applied (figure 2). For waters with 200 NTU, the dose range with the lowest residual values was obtained when 50 and 200 mg.L$^{-1}$ was used with residual color values ranging from 10 to 15 UC.

The increase in color may be because the seeds of *C. lanatus* caused the dispersal of colloids that generated color in the water; therefore, the seeds were ineffective when doses greater than 300 mg.L$^{-1}$ were used, achieving the highest residual value (6.7 UC Pt-Co) and the lowest percentage of color removal (33.3 %) for a dose of 500 mg.L$^{-1}$ in waters of 13 NTU.

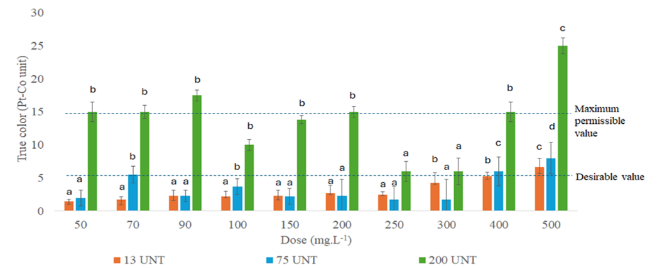


**Figure 2. Variation of true color in treated water as a function of *C. lanatus* seed dose**. Vertical bars indicate the arithmetic mean ± standard deviation for n:3. Mean followed by different letters in each bar of the same color indicates significant differences according to Tukey's test (p≤0.05).

**Comparison of the effectiveness of *C. lanatus* coagulant for different turbidity levels using optimal doses**

Table 3 shows the selected optimal doses with the residual values of pH, total solids, total dissolved solids, and total alkalinity. For waters with a turbidity of 13 NTU, the dose of 50 mg.L$^{-1}$ was selected, which allowed obtaining a final true color value of 1.5 UC Pt-Co and a turbidity of 0.82 NTU, while for waters with 75 NTU and 200 NTU, the doses of 150 and 300 mg.L$^{-1,}$ were chosen, which generated residual values after treatment of 2.60 NTU and 2.2 UC Pt-Co, and 13.80 NTU and 6 UC Pt-Co, respectively.

It was shown that the natural coagulant did not produce significant changes in the residual pH of the treated water, maintaining this parameter between 6.76 and 6.93 units. Awad *et al*. (2013) also found that natural coagulants did not affect the pH of the water after treatment.

Regarding alkalinity, values between 70 and 143 mg $CaCO_3$.L$^{-1}$ were obtained, being in the typical range for drinking water, which ranges between 50 and 200 mg.L$^{-1}$ $CaCO_3$ (Pérez, 2016). When waters have lower alkalinities, they are prone to contamination, because they do not have the capacity to resist modifications that generate pH decreases.

**Effect of rapid mixing time variation on the effectiveness of *C. lanatus* seeds**

Increasing the rapid mixing time from 1 to 5 minutes raised the residual turbidity levels for the three types of water analyzed, when comparing the results obtained for each time of the coagulation phase (figure 3). This may be because the precipitated particles that formed the floc have been resuspended (Adugna and Gebresilasie, 2018). For an initial turbidity of 13 NTU, the lowest turbidity values after treatment (0.84 and 0.92 NTU) were achieved with rapid mixing times of 1 and 2 min, with no significant differences (p>0.05) between these times.

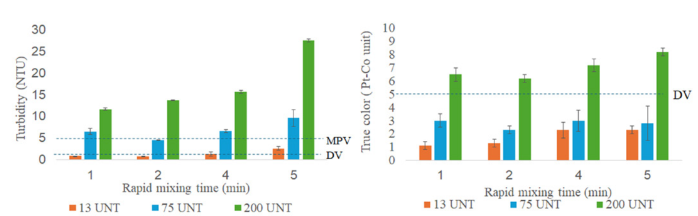


**Figure 3. Variation of residual turbidity and true color as a function of rapid mixing time and dose of the solution of watermelon seed *(C. lanatus)* for waters of different turbidity levels.** Vertical bars indicate the arithmetic mean ± standard deviation for n:3. MPV: Maximum permissible value. DV: Desirable value.

**Table 3. Comparison of watermelon seed coagulant (*C. lanatus*) for different turbidity levels using optimal doses**

| IT (NTU) | Dose (mg.L$^{-1}$) | % Removal of turbidity | % Removal of true color | pH | Total solids (mg. L$^{-1}$) | Total Dissolved Solids (mg.L$^{-1}$) | Total Alkalinity (mg $CaCO_3$.L$^{-1}$) |
|---|---|---|---|---|---|---|---|
| 13 | 50 | 93.7 ± 1.7$^b$ | 86.6 ± 2.5$^b$ | 6.76$^a$ ± 0.15 | 200 ± 17 | 103 ± 5 | 70 ± 17 |
| 75 | 150 | 96.5 ± 1.1$^a$ | 88.4 ± 2.1$^a$ | 6.93$^a$ ± 0.61 | 240 ± 35 | 95 ± 4 | 140 ± 36 |
| 200 | 300 | 93.2 ± 1.8$^b$ | 81.3 ± 5.0$^b$ | 6.91$^a$ ± 0.33 | 402 ± 67 | 99 ± 6 | 143 ± 12 |

IT: Initial turbidity. N:3. Mean followed by different letters in each row indicates significant differences according to Tukey's test (p≤0.05)

Regarding true color values, a behavior similar to that reported for turbidity was obtained; that is, an increase in the residual color values was observed. The lowest residual color values, 1.1 and 1.3 UC Pt-Co, were obtained with mixing times of 1 and 2 min in low turbidity waters (13 NTU). These values are lower than the maximum limit established by Venezuelan regulations (15 UC Pt-Co).

The highest percentages of turbidity (91.6 – 93.9 %) and true color (80.7 – 88.2 %) reduction were recorded when the times in the 1- and 2-min rapid mixing phase were used (figure 4).

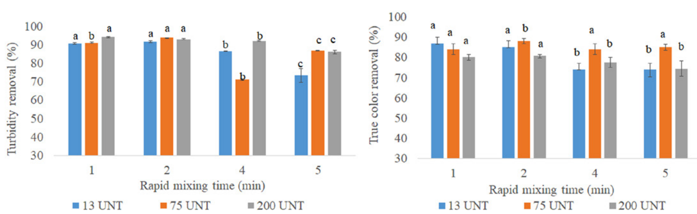


**Figure 4. Variation in the percentage of turbidity and true color removal as a function of the rapid mixing phase in the treatment process with *C. lanatus* for waters with different levels of turbidity.** Vertical bars indicate the arithmetic mean ± standard deviation for n=3. Mean followed by different letters in each bar of the same color indicates significant differences according to Tukey's test (p≤0.05).

## Conclusions

The characterization of the watermelon seeds showed low moisture content (5.43 %), ash content of 4.07 %, and a concentration of oils and fats of 25.40 %, which indicates that the seed, once the lipids have been extracted, can be used as a coagulant in the treatment of drinking water.

The optimal doses of *C. lanatus* seed for the removal of turbidity and true color in the coagulation-flocculation process were 50, 150, and 300 mg.L$^{-1}$ for waters of 13, 75, and 200 NTU. The highest percentages of turbidity (96.6 %) and color (94.4 %) removal for water with 75 NTU for a dose of 150 mg.L$^{-1}$.

When analyzing the quality of the water treated with *C. lanatus* seeds as a coagulant, it was possible to reduce turbidity values to levels lower than the maximum limits established by the sanitary standards of drinking water quality of 13 and 75 NTU. While for waters of 200 NTU, none of the doses used managed to obtain residual values below this limit.

Increasing the rapid mixing time caused a rise in the residual values of turbidity and color during the treatment of the waters of 13, 75 and 200 NTU; being the times of 1 and 2 minutes, which presented significant differences compared to the others tested and resulted in treated waters that meet the maximum values established in the Venezuelan regulations for drinking water.

## Acknowledgment

The authors express their gratitude for the support provided by the Water Resources Sanitation Network using Innovative and Sustainable Technologies (RED-AMARU) of the Ibero-American Program of Science and Technology for Development (CYTED).

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