

## **Application of Cocoa Mucilage (*Theobroma Cacao*) In the Treatment of Wastewater from the Production of Cassava Starch (*Manihot Esculenta*)**

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### **Abstract**

The production of cassava starch is an artisan activity that is practiced in a non-technical way, in addition, the water used in the various processes is poured directly into the river. The objective of this work is to apply cocoa mucilage (*Theobroma cacao*) as a partial substitute for ferric chloride in the treatment of wastewater from the production of cassava starch (*Manihot esculenta*). After performing the jar test to determine the best cocoa mucilage concentrations, the respective substitutions were established. It was possible to analyze that the treatment with 10 mg L<sup>-1</sup> of cocoa mucilage and 30 mg L<sup>-1</sup> of FeCl<sub>3</sub> was effective in removing the pollutant load from the wastewater, so much so that in the case of TTS it was removed in a 92%, TS 83.04%, BOD<sub>5</sub> 97.21%, COD 93.17%, TKN concentration 92.39%, P 51.81% and cyanide group 90.91%. It was determined that the application of cocoa mucilage is an organic and environmentally sustainable alternative when purifying this type of wastewater.

**Keywords:** Cassava starch, Coagulant based on cocoa mucilage, Pollutant load, Removal, Wastewater.

### **Introduction**

The cultivation of cocoa stands out above all in countries with a tropical climate, which are located at 15° both north and south of the equatorial line; becoming the source of income for 40 million people settled in Africa, Asia, Central and South America (Vassallo, 2015). About Ecuador, the areas with the highest cocoa cultivation are in the coastal provinces such as Los Ríos, Guayas, Manabí, Santo Domingo de los Tsáchilas and the Amazon province of Sucumbíos; These areas have unique agroclimatic conditions that stimulate this crop, giving it sensory characteristics of aroma and flavor that make it desirable worldwide (Guerrero,

2016).

The cocoa processing activity is very broad, likewise, this activity generates a wide range of by-products that are important for some activities that today are useful to carry out certain human activities without causing environmental damage, these by-products are organic matter, antioxidants, flavonoids, sugars, antioxidants, settled and dissolved solids (Omodele *et al.*, 2020).

On the other hand, in the rural area of the parish of Canuto, specifically in the Tarugo site, one of the main activities of the population that resides in this site has as its main activity, the production of yucca starch, the same as It is carried out in an artisanal way and in a not very technical way, since the water used in the various processes is poured directly into the river, which causes contamination of this important water source as well as a severe impact on the surrounding environment (Torres *et al.*, 2010).

According to Zambrano (2016), there are currently approximately 220 artisanal producers on this site and only 2 comply with the technical processing standards, where the work is carried out by small machinery, thus achieving more than 10 quintals of production per week. In this way, the National Water Secretariat (SENAGUA), carries out constant monitoring of the water flows in various places to determine the impact on them; since this activity, discharges its liquid discharges with many proteins, which results in a heating of water bodies.

It should be noted that a large amount of wastewater is generated from the starch extraction process from cassava, especially in the washing-peeling stages where a large amount of waste such as peels is produced, which has an impact on a decrease in COD levels; while in the sedimentation stage and which is the most critical since high levels of COD and BOD<sub>5</sub> are produced that adversely affect the environmental health of the surrounding aquatic channels (Dudu *et al.*, 2019).

For this reason, García & Gutiérrez in (2016), determine that the integral management of water resources is a concern that currently advocates for all humanity, and it is in this context where the saving, reuse, and non-contamination of water, are they constitute an inherent part of all semi and industrial activity within the sustainable development of the area and an integrated management of the hydrographic basins.

The objective of this research is to apply cocoa mucilage (*Theobroma cacao*) in the treatment of wastewater from the production of cassava starch (*Manihot esculenta*), in the Tarugo area

to lower the pollutant load of the river and make this activity is sustainable in space and time.

### Materials and methods

For the present study, residual water samples were used from cassava rallanderias (starch production) extracted from the Tarugo site whose geographic coordinates are UTM: 0602594 E, 9912716 N; The same that were transferred for their previous characterization, to the FCZ-LAB physicochemical and microbiological analysis laboratory at kilometer 2 ½ via Chone-Boyacá, the day of the collection of the samples (Table 1).

**Table 1.** Analysis of the physicochemical parameters to be considered in the present study.

Parameter	Units	Methodology
pH	xxx	Potentiometry
TSS		Gravimetry
TS		Oximetry
BOD <sub>5</sub>		Digestion/spectrophotometry
COD	mg l <sup>-1</sup>	Digestion/titration
TKN		Digestion/titration
P		Digestion/titration
CN		Atomic absorption

pH: Hydrogen potential; TTS: Total soluble solids; ST: Total solids; BOD<sub>5</sub>: Biological Oxygen Demand; COD: Chemical Oxygen Demand; NKT: Nitrogen Kjeldahl Total; P: Phosphorus; CN: Cyanides

On the other hand, the cocoa mucilage was extracted by the saline method, adapted from the method proposed by Prasad, (2009), for saline extraction of coffee mucilage. Wherein, 7.31 grams of NaCl was added in 200 ml of distilled water, then this dilution was diluted in 300 ml of distilled water further thus making 500 ml. Next, about 150 grams of fresh cocoa almonds are acquired, then they are placed in a container with 500 ml of the previously prepared saline solution; then it is mixed using a stirrer for this purpose for 30 minutes. The supernatant generated by this operation was filtered using Whatmann # 5 filter paper for this purpose, the fluid received in an Erlenmeyer flask corresponds to the extracted cocoa mucilage.

Then, the cocoa mucilage concentration was determined as follows: The muffle was preheated to 110 °C, meanwhile, two porcelain crucibles were weighed, noting this value. Next, 50 ml of mucilage was added to each crucible, then both crucibles were introduced into the flask until the moisture was removed. Each of the crucibles was weighed and the difference between the final weight and the initial weight of each capsule was calculated. Finally, the two results obtained were added and the weight corresponding to the total residue

was obtained. The weight of the residues in 100 ml of cocoa mucilage was 5.9 grams (59000 mg L<sup>-1</sup>).

Once the net weight of the solids had been calculated, the volume of cocoa mucilage required to obtain the different concentrations to be tested of this bio flocculant was calculated from the optimal dose of FeCl<sub>3</sub>, commonly used in water treatment plants, with an optimal concentration of 40 mg L<sup>-1</sup>. To compare the performance of cocoa mucilage extract as a potential substitute coagulant versus FeCl<sub>3</sub>, the optimal dose was analyzed using the jar test (Phipps & Bird <sup>TM</sup>, model PB-700), mixing rapidly for 15 s at 200 rpm, at to destabilize the surface charges of the contained organic matter particles, followed by slow mixing for 25 min at 25 rpm to promote the formation of flocs and their subsequent precipitation. Once this was done, the cocoa mucilage: sulfate was mixed, as detailed in table 2.

**Table 2.** Description of the treatments to be used in the present investigation.

Treatments	Units	Cocoa mucilage	Ferric chloride
T0 (CF)		xxx	40
T1 (MC8:CF32)	mg L <sup>-1</sup>	8	32
T2 (MC10:CF30)		10	30
T3 (MC12:CF28)		12	28

For the analysis of the bio flocculant in the field 4 scale models were built with which the main variables were analyzed that allowed making subsequent adjustments to improve the design of the system and that coincides with the treatment system reported by Cuenca & Intriago (2014). For the design of the models, a single substrate was used, made up of sieved soil (5mm), in order that the substrate did not interfere with any type of organic matter that could have been present and affected the operation of the system.

The gradient of the system was constituted with a slope from 3cm at one end to 0.5cm at the other. The soil was added little by little and compacted to achieve greater impermeability and better water flow. Four units were prepared 1 control (T0) and 3 treatments (T1, T2 and T3). For the control T0, a 15 cm layer of sand from the Toachi cast was placed with a 1 cm sieve and compacted in the manner explained above for the earth. Then a layer of 15 cm of gravel (12mm in diameter) and a layer of 15 cm of shell (19 mm in diameter) was applied, it is worth mentioning that all the substrates used were previously sieved to homogenize them;

finally, 1800 mg of FeCl<sub>3</sub> was added as a chemical flocculant.

Treatment T1 had the same characteristics as that described for treatment A, apart from adding 280 mg of cocoa mucilage and 1120 mg of FeCl<sub>3</sub> for every 35 liters of residual water. Treatment T2 consisted of the same conditions as T1 except for the amount of mucilage, which in this case was 350 mg plus 1050 mg of FeCl<sub>3</sub> for every 35 liters of residual water and T3 likewise with the same conditions except for the amount of mucilage that this time was 420 mg plus 980 mg of FeCl<sub>3</sub> for every 35 liters of residual water.

It is worth mentioning that the various parameters to be considered were analyzed every 5 days until the data obtained did not vary significantly, which indicated the stabilization of the system (Raffo & Ruiz, 2014). Regarding the removal percentage of the pollutant load, Lin, Chongyu, & Wensheng, (2003), proposed the following formula:

$$\% \text{ Removal} = \frac{\text{Input conc} - \text{Output conc.}}{\text{Input conc}} \times 100 \quad (1)$$

The experimental design used for the present investigation was completely randomized (DCA), with a confidence level of 95%; the design allowed to study the effect of the concentration of cocoa mucilage used as flocculant with three repetitions.

It should be noted that the analysis of the values of the parameters characterized from the tests carried out allowed to determine if there was a statistically significant correlation effect on the response variable, which was the percentage of removal of the pollutant load, for which the software was used. statistic Statgraphics Plus 5.1; designed by the Manugistics Institute, Inc., Rockville MD, USA.

## **Results and Discussion**

### **Characterization of wastewater from the physicochemical point of view.**

Table 3 details the value of the physicochemical parameters analyzed in the residual water from the cassava starch extraction process, where a low pH value is observed, which gives this water an acidic character (Ripley, Boyle, & Converse, 1986). In this same order of things, the high number of dissolved solids makes us realize that there is a large amount of organic matter that is dissolved in the water column, so its removal is impossible only with physical treatments (Nath, Mishra, & Pande, 2020).

**Table 3.** Values of the parameters considered pollutant load in the wastewater samples from the Tarugo site.

Parameter	Unit	Mean	Standar Desviation	Variation Coefficient	min	max
pH	---	4.5	0.31	6.91	4.1	4.9
SSt		1187.5	6.63	0.56	1179	1195
St		3498.43	6.21	0.19	3239	3254
DBO <sub>5</sub>		3353.33	110.40	3.29	3248	3460
DQO	mg L <sup>-1</sup>	3489.50	10.21	0.29	3479	3508
TKN		186.98	1.07	0.57	185.9	188.9
P		20.13	0.52	2.56	19.6	20.9
CN		1.43	0.34	24.03	1.1	1.9

pH: Hydrogen potential; TTS: Total soluble solids; ST: Total solids; BOD<sub>5</sub>: Biological Oxygen Demand; COD: Chemical Oxygen Demand; TKN: Total Kjeldahl Nitrogen; P: Phosphorus; CN: Cyanides

It is also necessary to highlight that there is contamination of the water by macronutrients such as nitrogen and phosphorus, which suggests that there may be places where eutrophication processes can be carried out (Valencia, Salcedo, & Páramo, 2018).

For its part, the presence of WAD cyanides is linked to the low pH concentration of around 4.5; Therefore, in the wastewater from cassava starch plants, this compound can be found as hydrocyanic acid, cyanide ions and their complexes (Zapata, 2020).

### **Analysis of the performance of cocoa mucilage in the treatment of wastewater from cassava starch plants**

It is worth mentioning that before the analysis of the parameters considered in this study was started, the pH of the system had to be stabilized, thereby conditioning the residual water from the cassava starches.

In this way, the system began to work, after 15 days of stabilization with sodium bicarbonate (NaHCO<sub>3</sub>) in two phases, the first in a proportion of 6% (60g of NaHCO<sub>3</sub> L<sup>-1</sup>) and the second phase (500mg of NaHCO<sub>3</sub> L<sup>-1</sup>); thus, guaranteeing the buffer activity of the water, due to its acid characterization (Akhil *et al.*, 2021).

**Table 4.** Value taken by the pH after conditioning the residual water from the Tarugo site.

Parameter	Unit	Residual wáter NA	Residual wáter WA	Standard Desviation	Variation Coefficient
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pH	---	4.5	6.16	0.23	3.76
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NA: non conditioner. WA: with conditioner.

After finding the optimal stabilization conditions of the system and the optimal doses of the cocoa mucilage used as coagulating organic substance within the coagulation / flocculation process, the value of the pollutant load of TSS, TS, BOD<sub>5</sub>, COD, TKN, was determined P and CN after 20 days.

**Table 5.** Concentration of the effluent from the removal of the pollutant load of TSS and TS after having conditioned the wastewater from the Tarugo site.

Treatment	Ac*	Ec*	Permissible Limits*	ES	p-value
<b>Total Soluble Solids (TSS)</b>					
T2		95.00 <sup>a</sup>			
T3	1187.5	101.67 <sup>b</sup>	100	1.56	<0.0001
T1		116.00 <sup>b</sup>			
T0		122.00 <sup>c</sup>			
<b>Total Solids (TS)</b>					
T2		593.33 <sup>a</sup>			
T3	3498.43	605.00 <sup>b</sup>	1600	3.44	<0.0001
T1		621.00 <sup>c</sup>			
T0		634.67 <sup>d</sup>			

\*Affluent concentration mg L<sup>-1</sup>; Effluent concentration mg L<sup>-1</sup>.

As we can see for both TSS and TS, the treatment (T2) is the one with the best performance in the bioreactor during the 20 days, since when performing the ANOVA at 95% confidence level and the comparison of means by Duncan's method, we can determine that it reached the lowest values (95.00 and 593.33 mg L<sup>-1</sup> respectively).

The value for TSS is well below that reported by García in (2017) where he obtained a value of 453 mg L<sup>-1</sup>. In this vein, Tanhato, Hasan & Sebastien in (2019), reported 848.34 mg L<sup>-1</sup> for total solids. As we can see, both values are optimal to be considered as irrigation water in agriculture both for TULSMA (2015) and for the Environmental Protection Agency (EPA, 2016). The TSS and TS values reported in this research are low and therefore favor aquatic

life by promoting the photosynthetic process of phytoplankton, which are the primary producers of aquatic environments (Badawi & Zaher, 2021). Regarding BOD<sub>5</sub> and COD, we can see that there is a reduction in the concentration of the influent after 20 days of treatment, reaching the permissible limits as we can describe in table 6.

**Table 6.** Concentration of the effluent from the removal of the BOD<sub>5</sub> and COD pollutant load after having conditioned the residual water from the Tarugo site.

Treatment	Ac*	Ec*	Permissible Limits*	ES	p-value
<b>Biochemical Oxygen Demand (BOD<sub>5</sub>)</b>					
T2		93.67 <sup>a</sup>			
T3	3353.33	102.67 <sup>b</sup>	100	1.78	<0.0001
T1		109.00 <sup>c</sup>			
T0		113.33 <sup>c</sup>			
<b>Chemical Oxygen Demand (DQO)</b>					
T2		238.33 <sup>a</sup>			
T1	3489.5	257.00 <sup>b</sup>	250	2.04	<0.0001
T3		258.67 <sup>b</sup>			
T0		272.67 <sup>c</sup>			

\*Affluent concentration mg L<sup>-1</sup>; Effluent concentration mg L<sup>-1</sup>.

Regarding the results of the decrease in the concentration of the BOD<sub>5</sub> and COD load, the lowest concentrations occurred with T2 (MC10: CF30); with 93.67 and 238.33 mg L<sup>-1</sup> respectively. These results are in accordance with those determined by Chethana *et al* (2016).

Thus, we can note that the ratio of the biodegradability index (BOD<sub>5</sub> / COD) is 0.39, so the biodegradability of the effluent water is low (Saleh, Tuzen, & Sari, 2021); In this way, it can be determined that this type of water is suitable for reuse.

Likewise, in table 7, we can determine that the concentration of TKN and P had effluent values of 14.23 and 9.70 mg L<sup>-1</sup>, these values were reached with T2; reaching the values of the permissible limits for reuse in the agricultural sector (TULSMA, 2015; EPA, 2016).



**Table 7.** Concentration of the effluent from the removal of the pollutant load of TKN and P after having conditioned the residual water from the Tarugo site.

Treatment	Ac*	Ec*	Permissible Limits*	ES	p-value
<b>Total Kjeldahl Nitrogen (TKN)</b>					
T2		14.23 <sup>a</sup>			
T3	186.98	16.57 <sup>b</sup>	15	0.23	<0.0001
T1		17.40 <sup>c</sup>			
T0		19.50 <sup>d</sup>			
<b>Phosporus (P)</b>					
T2		9.70 <sup>a</sup>			
T3	20.13	12.47 <sup>b</sup>	10	0.17	<0.0001
T0		13.33 <sup>c</sup>			
T1		14.43 <sup>d</sup>			

\*Affluent concentration mg L<sup>-1</sup>; Effluent concentration mg L<sup>-1</sup>.

The values indicated here are within those determined by various studies on the removal of pollutant load from wastewater from the production of cassava starch. For example, Papong *et al.*, (2014), determined that the pollutant load produced by TKN and P after using various organic coagulants was 13.78 and 9.32 mg L<sup>-1</sup>, both for TKN and P respectively; The low concentration of these compounds in the effluent water determines low eutrophication processes due to the fact that nitrogen and phosphorous compounds are low and at the same time slow down the development of algae and higher aquatic plants, creating oxygenic conditions in the receiving water bodies (Dos Santos *et al.*, 2018).

Finally, we have cyanides, the same ones that after using T2 proved to be more effective than the other treatments, since it shows a concentration in the effluent of 0.13 mg L<sup>-1</sup>, making it a water that can be reused in agricultural activity, as classified by TULSMA, in (2015) and EPA, (2016).

**Table 8.** Concentration of the effluent from the removal of the CN pollutant load after having conditioned the residual water from the starch plants at the Tarugo site.

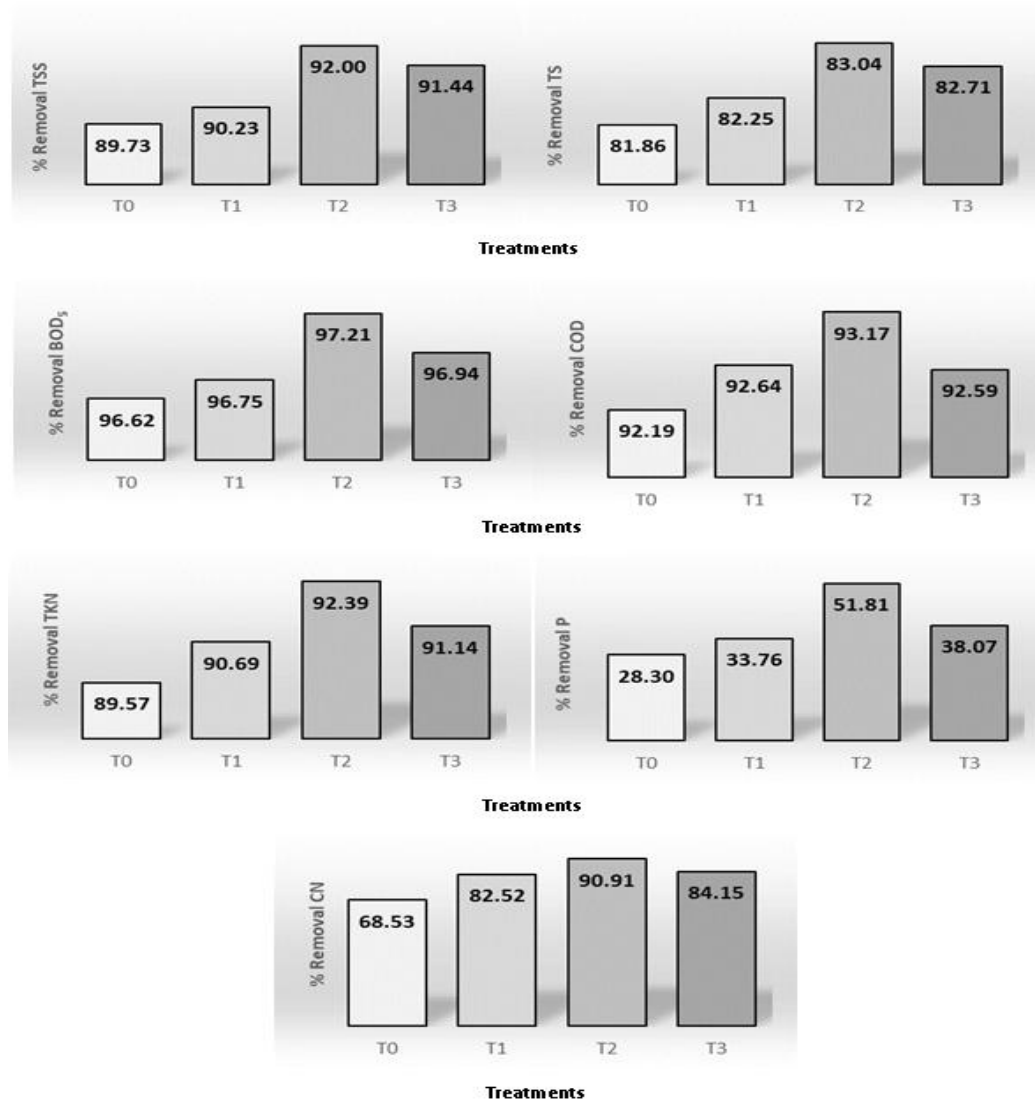
Treatment	Ac*	Ec*	Permissible Limits*	ES	p-value
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		(CN)			
T2		0.13 <sup>a</sup>			
T3		0.23 <sup>b</sup>			
T1	1.43	0.25 <sup>b</sup>	0.20	0.01	<0.0001
T0		0.45 <sup>c</sup>			

\*mg L<sup>-1</sup>

As can be seen, the value obtained from cyanides coincides with that reported by (Torres *et al.*, 2007); who determine that values in the concentration of cyanides below 0.20 mg L<sup>-1</sup> are considered inhibitory in anaerobic biological processes (Araujo *et al.*, 2020).

### Análisis del porcentaje de remoción de la carga contaminante con los diversos tratamientos.



**Figure 1.** Comparison of the various treatments and their efficiency percentage in terms of

removing the pollutant load from the residual water of the starch plants at the Tarugo site. The results of the removal of the variables studied demonstrate the behavior of each of the treatments carried out in the present study, to determine the efficacy of each of them and check which of them conforms to what is required by current regulations. (TULSMA, 2015; EPA, 2016).

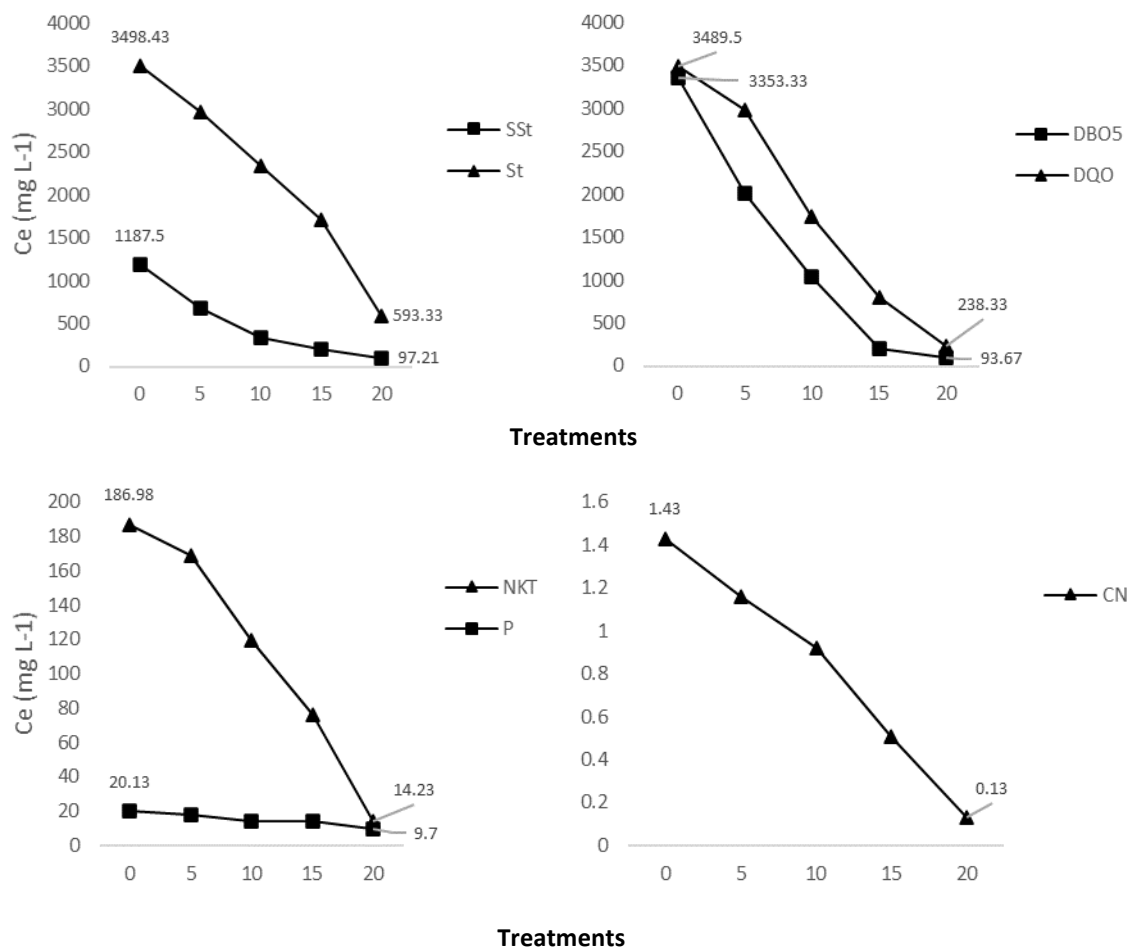
As can be analyzed, the results of the removal of the pollutant load of the variables analyzed here (TSS, TS, BOD<sub>5</sub>, COD, TKN, P and CN), are favorable and it was determined that the most effective treatment is T2, same containing 12 mg L<sup>-1</sup> of cocoa mucilage and 28 mg L<sup>-1</sup> of ferric chloride (MC12: CF28); Furthermore, this treatment complies with current national regulations for the treatment and reuse of wastewater for agricultural purposes (TULSMA, 2015; EPA, 2016).

These removal percentages are higher than those reported by Kastali *et al* (2021) in a study on wastewater treatment with natural flocculants, where they obtained removal percentages for COD of 88%, for BOD<sub>5</sub> of 43% and for TSS45%. Likewise, in a study of wastewater treatment using coagulation and flocculation techniques, Balpreet, Rajeev & Anirudh in (2021), found removal percentages of 78.1% for COD and 81% for TSS.

#### **Behavior of the wastewater treatment process from the starch plants at the Tarugo site.**

The figure 2 shows the behaviors of TSS, TS, BOD<sub>5</sub>, COD, TKN, P and CN for the control T2. This treatment shows that the proposed system works synchronously, since, according to Reed, Crites, & Middlebrooks (1995), all the parameters decrease simultaneously once the system has stabilized. In the case of this study, the occurred from day five.

It is also important to highlight that the T2 treatment demonstrated a high removal of COD, BOD<sub>5</sub>, TKN, P and CN, so its efficiency is remarkable when purifying this type of water (Vera *et al.*, 2011).



**Figure 2.** Behavior of the various treatments and their effluent concentration of wastewater from the starch plants at the Tarugo site.

## Conclusions

The treatment of wastewater from cassava starches has attempted to use a partial substitution of ferric chloride for cocoa mucilage, as a mixture between a conventional flocculant and a natural biodegradable one. The dose of ferric chloride and cocoa mucilage that had the best efficiency was 12 mg L<sup>-1</sup> of cocoa mucilage and 28 mg L<sup>-1</sup> of ferric chloride. The percentage of reduction of the parameters treated in this study with the proposed flocculant mixture could be clearly evidenced from day 5 and it remained constantly downward until day 20. It is worth mentioning that the effluent load emanating from treatment 2 met the quality criteria for wastewater treated and disposed of for use in agricultural activities in general. The treatment of this type of water with organic flocculating compounds such as cocoa mucilage is a biodegradable alternative, which consolidates the circular bioeconomy criteria.

## Conflict of Interest

No conflict of interest

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