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Removal of Chromium in Wastewater from Tanneries Applying Bioremediation with Algae, Orange Peels and Citrus Pectin

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Abstract

The dumping of tanneries is a severe pollution problem because it generated wastewater full of chemical and toxic substances which deteriorate the water sources and the health of organisms that are living there. The aim of this project was to determine the removal capacity of the total chromium (Cr) and chlorides (Cl-) present in the wastewater of the tanneries by the bioremediation with the *Chlorella sp*. and *Scenedesmus sp*. microalgaes, orange peels and citrus pectin. The methods applied were: i) the inoculation of a single or mixture microalgaes in the water of tanneries for a period of 24 or 48 hours (h), or a photoperiod 12h:12h (light:darkness); ii) orange peels with particle sizes: 0.5mm, 1.0mm and 2.0mm, were exposed to residual water during 2h, 4h or 6h with a rate of 0.5g/100mL; and iii) citrus pectin with equal parameters of those used in the orange peels. The initial concentration of residual water was Cr=352.2 mg/L and Cl- =25100 mg/L. The Scenedesmus sp. algae removed 98.63% (4.82 mg/L) of Cr and 54.18% (11500mg / L) of Cl- after 48h while the orange peel with particle size of 0.5mm and with only 2h of exposition decreased the initial concentration of Cr and Cl- in a 94% (21.1mg/L) and 59.76% (10100mg/L) respectively. Finally, the pectin at a contact time of 6h showed the highest absorption of Cr (98.23% - 6.2 mg/L). Therefore, the three different tested products presented a very high efficiency in the reduction of ion concentration of Cr and Cl- and lead new and novel alternatives for the treatment of wastewater of the tannery industries.

Keywords: bioremediation, citrus pectin, chloride, chromium, microalgae, orange peel, wastewater

1 Introduction

Tanneries use chemical substances in their processes that, when mixed with water, produce dangerous liquid waste due to the presence of chromium (Cr), which represents an environmental risk to the bodies of receiving water and damages to the health of living organisms. It is because the operations are carried out in an artisanal and illegal manner and are normally located close to the water sources to facilitate the collection and dumping [13].

The sector of tanneries in Colombia is composed mainly of microenterprises (77%) and large industry (1%), where the effluents present pH variations, presence of toxic substances and salts that significantly affect the aquatic life of the receiving water sources [16].

Cr salts include chromium alum, basic chromium sulphate and commercial chromium sulphates, which are the most used by most tanneries worldwide to obtain products with high quality and the speed of penetration of chromium in the skin [2], [9]. Even the relative low quantity required to tan a lot of skins and the low price of salt, are reasons why there is a large supply of tanning agents that makes it an attractive and viable alternative [4], [13].

The dumping of liquid waste from the activities of tanneries in water sources is carried out without any treatment, leading to the accumulation of toxic substances such as Cr that is not eliminated naturally and with a slow disintegration process. Cr tends to precipitate and mix in the sediments and mud of water bodies where it manages to interact in the trophic chain, causing chronic poisoning in organisms [9], [12].

The existing environmental problems caused by the operations of tanneries in Colombia is due to technological backwardness and poor performance of the processes. Which causes alterations in the life of aquatic ecosystems at the local and regional level, reason that supports the importance of carrying out efficient treatments for the reduction of toxic substances present in the wastewater of tanneries [23].

Due to the above, the degradation in the quality of the water by the shedding generates concern and need to look for ways of treatment of the residual waters product of the activity of the tanneries; nevertheless, this search of physical and / or chemical treatments brings disadvantages of economic type for the implementation and maintenance. Being a conditional factor and for the aforementioned operation situation, bioremediation and biomass arise as treatment technologies, more economical and environmentally beneficial as a viable alternative for environmental mitigation [6], [7].

In this sense, tanneries when they discharge to receiving streams cause changes in quality such as the excess of toxic substances, sulfides and salinity [13], [15]. The removal in the concentration of heavy metals such as Cr in these effluents has become an interest for the scientific and technological community. As for example, the orange peel when passing through a preliminary treatment or preparation becomes a potential of Cr removal due to polygalacturonic acid [1], [10].

Bioremediation is a technology that uses microorganisms, cellular components or free enzymes, in order to obtain the partial transformation of the contaminant into substances less toxic or harmless to the environment and human health [10], [20]. Biomass is another technology used to treat different pollutants, its effectiveness is influencing the removal through the ion exchange and the metal ions in a solution that stabilize the treatment present in the water [5].

Therefore, this research applied bioremediation and biomass in wastewater from the final process of the tannery, in this case, the selected microalgae are *Chlorella sp., Scenedesmus sp*. Biomass of orange peels and citrus pectin, to an adjusted method, that initially each one was worked separately and then the accomplishment of diverse combinations for the laboratory analysis to the residual water in the removal of Cr and Cl-.

The purpose of applying this technique was to evaluate the removal capacity of Cr by *Chlorella sp., Scenedesmus sp*., the biomass of orange peel and citrus pectin, as a bioremediator in a treatment for tannery wastewater, seeking to establish guidelines for its large-scale application.

2 Materials and methods

A factorial experimental design was elaborated combining random block design, giving for five assemblies and with three replicas for each of the treatments or tests used in each assembly. The analysis of the data was carried out by analysis of variance (ANOVA) and the T-Student test.

Chlorella sp. Species were used in the first assembly. and Scenedesmus sp., separately and a mixed culture of the species and inoculation times in the tannery water were 24 and 48 hours (h), constant aeration, pH 6.0 and photoperiod 12:12 Light/Darkness (L/OR).

In the second assembly, orange peels were used with particle sizes of 0.5mm, 1.0mm and 2.0mm, exposed at 2h, 4h and 6h times, with magnetic stirring of 150rpm (magnetic stirrer MSH-300 brand BOECO Germany), pH 5.0 and 0.5g of orange peel per 100 mL of residual water. The preparation of the orange peel was carried out previously, which consisted of a dehydration at 105 ° C in a drying oven (Memmert brand) for 24 hours, then the dried material was taken to grind (brand Numak model FZ-102) to obtain the required particle sizes.

In the third assembly, the citrus pectin handled was of commercial origin (Quick Reference 105 CIMPA) at a concentration of 0.5 g of pectin per 100 mL of residual water, the times and conditions given in the second assembly were taken.

In the fourth assembly, randomly it was decided to make a mixture between the mixed culture and the second assembly, exposed in times of 2h, 4h and 24h, pH 6.0 and constant aeration.
In the fifth assembly, a mixture of citrus pectin and orange peels was made, with the same conditions of the second assembly at a concentration of each component of 0.5g/100mL of residual water.

A sample was taken of the wastewater from the tanning process of a company that operates in an artisanal way in the municipality of Gigante, Huila, whose identity of the company is not required due to the confidentiality of the owner. The parameters of the quality in this type of water were determined taking into account the current regulations according to the parameters and maximum permissible limits in the punctual discharges of non-domestic wastewater - ARnD to surface water bodies of activities associated with manufacturing and manufacturing of goods. [16], the analysis were performed according to the protocol of an Accredited Laboratory.

The parameters analyzed are the total amount of dissolved solids (TSD) and the electrical conductivity (CE) in the water are closely related according to the dissolved salts. These can respond to the differences in the kinetics of removal of Cr from the tannery water with the mounts made, thus, the performance of the measurements for chlorides was added. The content of total Cr and hexavalent Cr (VI) and pH were determined to define the conditions at the time of the comparisons with the tests in water with Cr.

For the physical-chemical analysis of the assemblies, samples were taken to quantify the variables: chlorides, Cr VI, total Cr and sulfides in an accredited laboratory. On the other hand, the TSD, CE and pH variables were measured with portable multiparameter probe (reference HI 9813, Hanna Instruments brand).

2.1 Cultivation and scaling of microalgae

Original strains of Chorella sp. (CR2714-A strain Colombia) and Scenedesmus sp. (55P1589-A Colombia strain) were used. Then, the cultivation and production was carried out using the Bold Basal Medium (BBM) in the Limnology laboratory of the University Corporation of Huila, Corhuila. The mixture to prepare a liter of BBM consisted in adding 936 mL of distilled water and 64 mL of SP standard solution, the solution was made in polycarbonate containers, to avoid the adsorption of the metals to the walls of the container. The pH of the BBM medium prepared according to the above must have a conductivity of 1.4 mS/cm with adjustment of the pH to 6.6 with NaOH or HCl.

The strains were preserved in test tubes with BBM medium at a temperature of 28 ± 2 ° C, with a photoperiod of 12 hours of light and 12 hours of darkness (12:12 L / O) [14], in the same way they were cultivated with the mentioned conditions and monthly escalations were made.

The growth of the microalgae in the culture medium was at a temperature of 28 ± 2 ° C, with a photoperiod of 12:12 hours. It's constant aeration was required by an air motor with two outlets AC9902, with air flow 240L / h , connected to silicone hoses (ϕ 4,8mm) and with stone bubble diffuser terminal, to reduce size and keep the algae culture in agitation.

The growth measurement of each species was carried out daily at a specific time and in each of the prepared containers (3L volume). An Advance Optical AC85-240V 50-60 Hz microscope and a Neubauer camera were used for the counting. 0.05mL of homogeneous sample used, this was developed with the intention of comparing behavior of the microalgae when inoculated in the wastewater of tannery.

2.2 Collection and treatment of orange peels

The collection of orange peels was carried out in natural juices sale sites in the city of Neiva. 5 kg of husks were collected, which were transferred to the laboratory; there, they were washed with distilled water to remove impurities and the endocarp was extracted manually. Then the moisture content in the drying oven (Memmert brand) was reduced to 105 ° C for 24 hours. Having the material dry, it was taken to a hammer mill with screen (Numak brand model FZ102) to obtain the particle sizes required for the assemblies.

The process to determine the different sizes of particles was done by US standard ASTM E11 sieves reference 35mm, 18mm and 10mm (mesh opening: 0.5mm, 1.0mm and 2.0mm, respectively). They were stored in polyethylene covers according to the sizes obtained in order to prevent them from acquiring moisture, contagion and being in good condition until the experiments.

2.3 Obtaining citrus pectin

Commercial material (Quick reference 105 CIMPA) of an input marketer for the food industry was used. To the samples at the end of each one of the different experiments a filtration was carried out (filter paper of extra fast filtration, smooth and thick surface) to prevent the addition of material now of the collection, preservation and later analysis of the samples by the accredited Laboratory.

3 Results and Discussion

A factorial experimental design was elaborated combining random block design, giving for five assemblies and with three replicas for each of the treatments or tests used in each assembly. The analysis of the data was carried out by analysis of variance (ANOVA) and the T-Student test.

The results of the analysis indicated a total Cr concentration of 352.2mg/L and chlorides of 25100mg/L exceeding the maximum discharge limit declared in the current Colombian regulations, electrical conductivity of 10200µS/cm, TDS of 1.0 and pH of 2.1. It was necessary to use NaOH for the stabilization of the pH up to the required values; this was done with the purpose of preserving the tolerance levels of the species and the biomass to the acidity (Ardila F., 2012). In the parameters Cr VI (<0.010mg/L) and sulfides (<1.50mg/LS-2) the registered values comply with the Colombian regulations without presenting variation in concentration.

3.1 Cultivation of microalgae

The growth of each of the species at laboratory scale was observed and recorded daily for 12 days, in a volume of 2 L, which are shown in figure 1. The microalga Scenedesmus sp. presented a higher growth compared to *Chlorella sp*. for the same crop volumes, this may occur due to the difference between the initial concentrations of the microalgae.

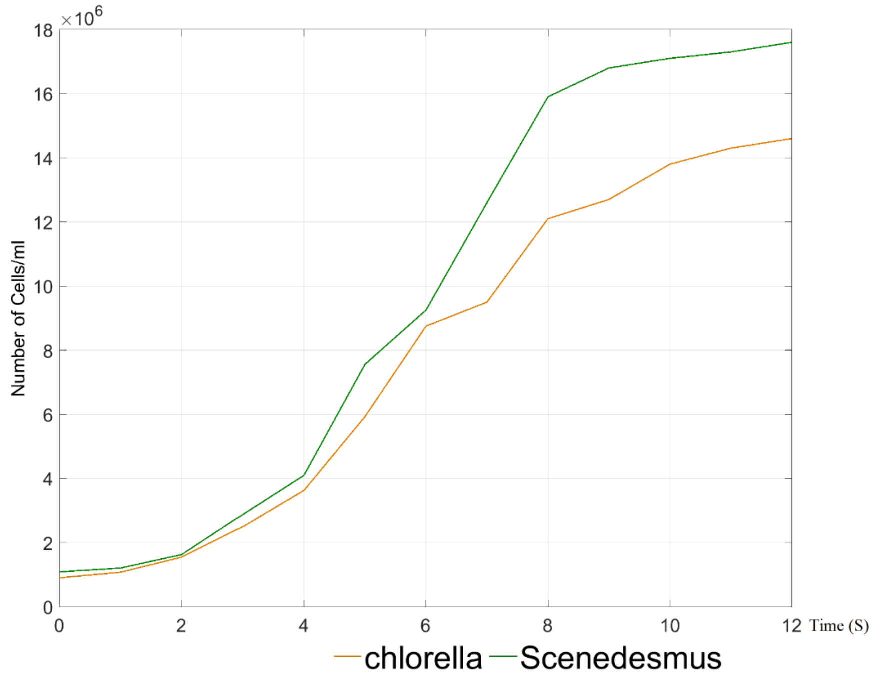

Figure 1. Growth curve of the microalgae *Chlorella sp*. and *Scenedesmus sp*. in volume of 2L

3.2 Removal of Cr and Cl- by microalgae

After having the highest record of the growth rate of microalgae were inoculated into the tannery wastewater, with the results provided by the accredited laboratory and applying the software for factor analysis and design of the 2FI model; in factor. A four levels were established (without microalgae, *Chlorella sp*., Scenedesmus sp., and mixed culture *Chlorella sp*. + *Scenedesmus sp*.) and factor B two levels (24h, 48h) with two responses (Cr, Cl-).

- Cr removal

The analysis of variance to the model applied in response to Cr with microalgae in the times used is significant ($p < 0.0004$) and indicates good accuracy and reliability of the tests performed ($CV = 0.98\%$). The analysis of Cr removal in response to the process is shown in figure 2, indicates that Scenedesmus sp. in 48h, it showed the greatest removal in 98.63% (4.82 mg/L), ratifying the findings of Marcano and collaborators (2010) of removal greater than 90% [14]. Although the concentration of *Chlorella sp*. in the removal of Cr is significant ($p < 0.0001$), it occurred only in 24h and then the removal potential is reduced [8], [10], [13].

Regarding the mixed culture, the positive removal of Cr occurred at 48 h with removal of 46.5 mg/L (86.79%), whose tests were possibly affected by cell growth, showing a decrease with respect to the species separated [9], [10] due to the presence of competing or contaminating microorganisms present in experimental crops [10], [19].

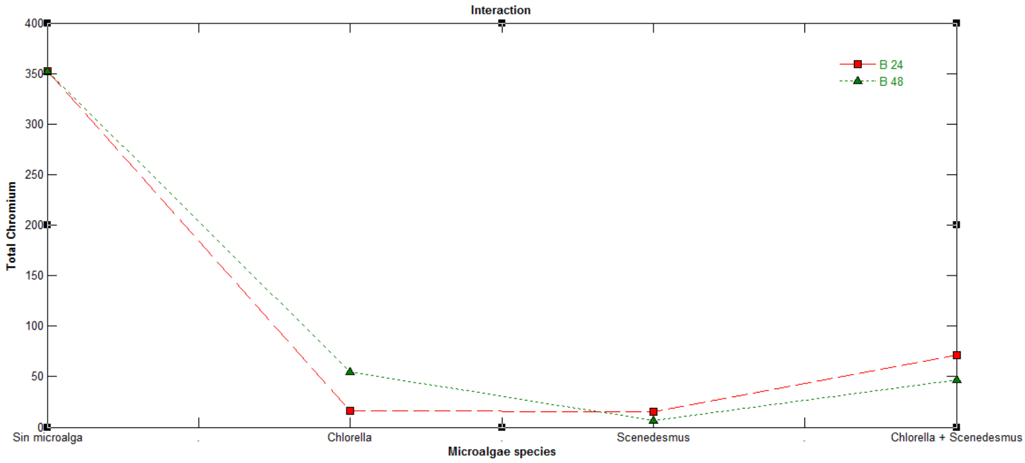

Figure 2. Response of the microalgae species to the interaction with Cr.

Removal of Cl-

The removal with the microalga Scenedesmus sp. in a time of 48h was 11500mg/L represented 54.18% (Figure 3), although the removal was considerable in any of the treatments there is no significant difference ($p = 0.2159$) in the use of the species or mixed culture for the absorption of Cl-. It is considered that the presence of salts other than Cl-, affected the removal capacity in the different treatments, so it is necessary to determine the characterization of ions of salts present in the wastewater and identify the behavior in the treatments carried out [7], [22].

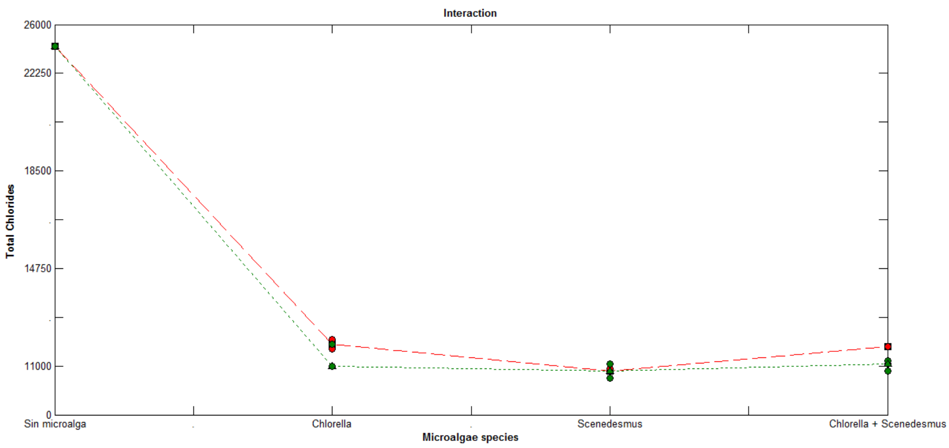

Figure 3. Response of the microalgae species to the interaction with Cl-

3.3 Adsorption of Cr and Cl- by the orange peels

In factor a four levels were established (0mm, 0.5mm, 1mm and 2 mm) and factor B three levels (2h, 4h, 6h) with responses for Cr and Cl-.

Adsorption of Cr

The analysis of variance indicated that the model applied in response to Cr with the different sizes of orange peel particles in the times used is significant ($p < 0.0001$, CV = 0.45%). The removal of Cr with the process in the different sizes of orange peel particles, shown in Figure 4, shows that the particle size 0.5mm with contact time of 2h obtained the highest Cr removal in 94% (21.1mg/L).

In spite of being removed with time, the ions on the surface of the orange peel particle saturate depending on the size [5], which is why in contact between 4h to 6h an ion release is evident of Cr reducing the removal capacity [9], [13]

So that the experiments in the conditions of removal and classification by established sizes can be used as an adsorbent material for the elimination of Cr ions and that when handling different sizes of orange peel particles in contaminated aqueous solution. Becoming an alternative for the treatment of water containing heavy metals [1], [10], [21].

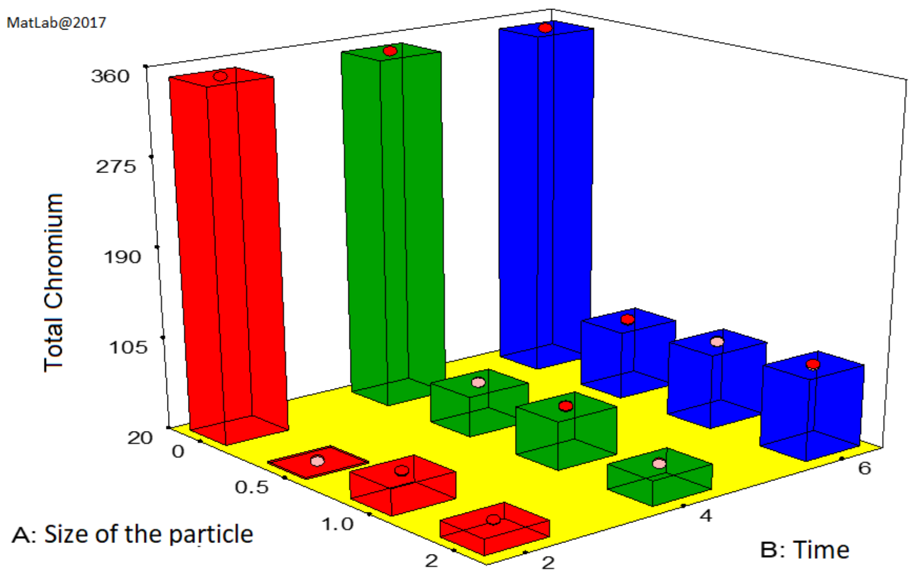

Figure 4. Interaction of the particle sizes with respect to the times for Cr

Adsorption of Cl-

Figure 5 shows that the applied interactions did not show significant differences ($p = 0.8048$, CV = 3.73%), although the greatest adsorption potential was achieved in

0.5mm and 2.0mm sizes in 2h time with a value of 10100mg/L equivalent to 59.76% removal. In the case of time 6h and size of 1.0mm the removal was 10200mg/L, the low removal potential is because the salts are residues that usually occur along with the metals, which can cause variations in the process. Because of the competition of Cl- ions and metals that interact is active spaces of the surface of the adsorbent material, the removal capacity is reduced [10].

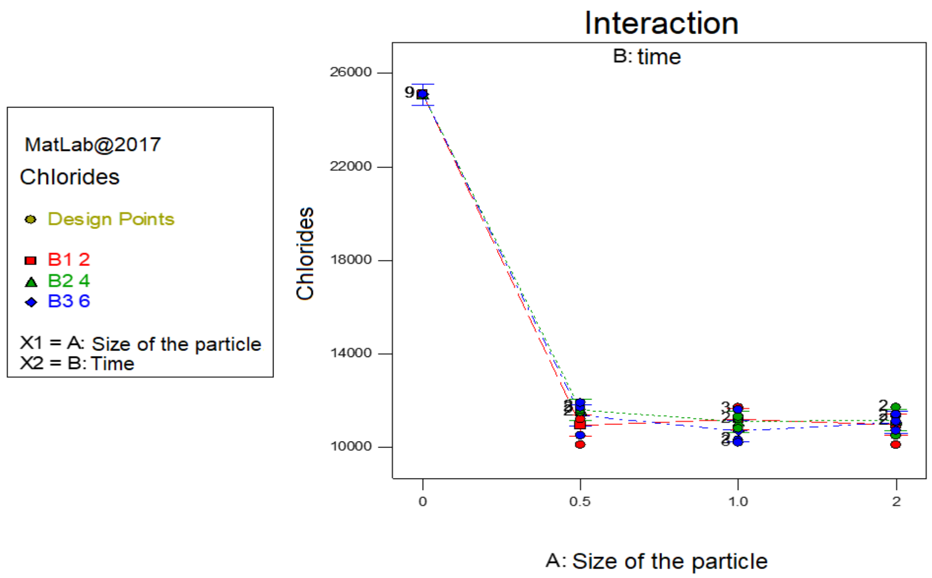

Figure 5. Interaction of particle size with respect to times for Cl-

3.4 Adsorption of Cr and Cl- by Citrus Pectin

Adsorption of Cr

The ability to remove Cr in response to the pectin process and the effects of the time it was in contact with the solution are significant (p <0.0001, CV = 0.21%), as shown in the figure 6. The pectin in a time of 2h achieved the removal of 9.21mg / L represented in a 97.38% removal and increasing the time to 6h contact was the highest absorption of 98.23% (6.2 mg/L).

In the time of 4h, no increase in removal occurred, on the contrary, the potential decreases to 95.57%, which corresponds to the fact that the fast action and constant agitation in the pectin exerts its gelling effect allowing a high removal of ions. In addition, the pectin in small-ungelded quantities is reactivated to adsorb the ions [10], [11], [13].

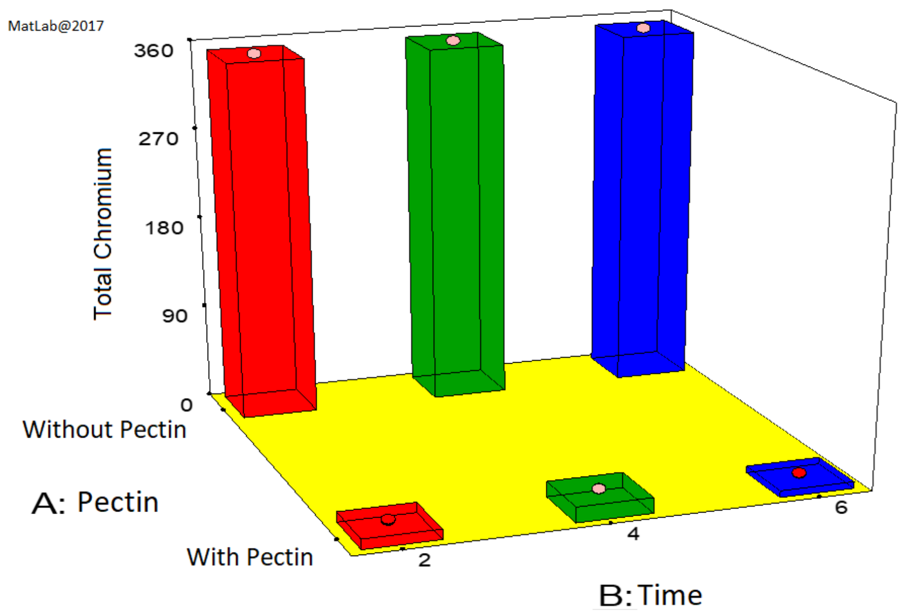

Figure 6. Interaction of citrus pectin with respect to the times for Cr

Adsorption of Cl-

The interaction of the pectin and the different times didn't present significant differences ($p = 0.0690$, CV = 1.20%), although the highest adsorption potential was achieved in the time of 2h with a value of 6200mg / L of Cl - equivalent to 75.3%, for the time of 6h the greatest removal was 6300mg / L Cl- as shown in figure 7.

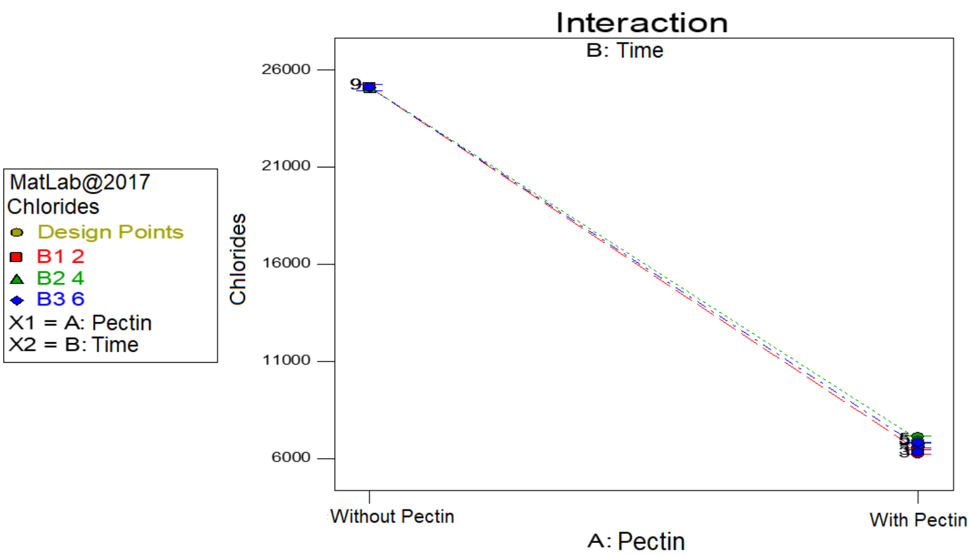

Figure 7. Interaction of citrus pectin with respect to the times for Cl-

3.5 Removal of Cr and Cl- combining mixed culture and orange peels.

Cr removal

The analysis of Cr removal in response to the process, according to the obtained model shown in Figure 8, demonstrates that the effect of the culture with the different sized shells in the removal of Cr is significant ($p < 0.0001$; CV = 0.43%). The mixed culture with orange peels of size 0.5mm the removal potential was 82.67% (59.8mg/L), compared to the size of 1.0mm the removal was of 79.86% (70.9 mg/L), although with the size of 2.0mm the adsorption was increased to 68.76mg/L. It is understood that in the mixed culture they were in the phase of adaptation to the new medium and their effect on the adsorption of Cr ions were minimal in relation to the time of 4h [9], [13].

The potential for removal of Cr ions with shells of size 2.0mm with a time of 24h achieved the highest removal within the assembly at 41.6 mg/L equivalent to 88.18%.

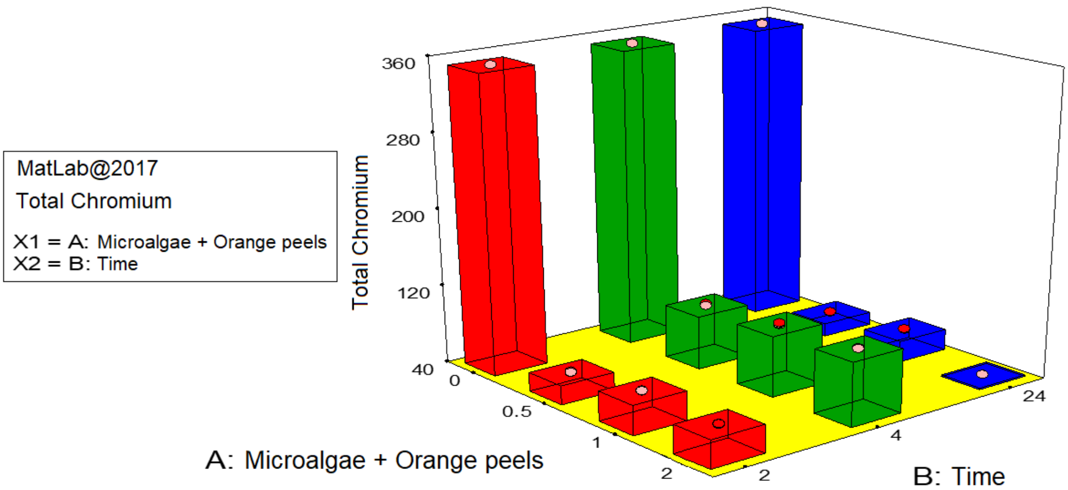

Figure 8. Interaction of mixed culture with orange peels with respect to the times for Cr

Removal of Cl-

The greatest adsorption potential was achieved in the time of 2h and size 0.5mm with value of 9480mg/L Cl- equivalent to 62.23%, and then the removal behavior decreased to 54.18% (Figure 9). The adsorption that occurred between the time of 4h and 24h and the effect with the sizes of the particles did not register values distant from each other, inferring that the reduction potential of Cl- in any of these times does not influence [13].

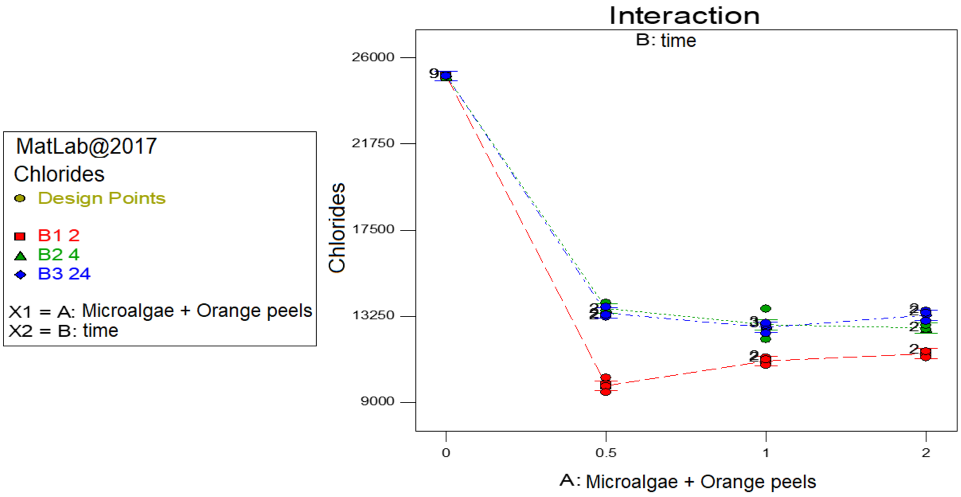

Figure 9. Interaction of mixed culture with orange peels with respect to the times for Cl-

3.6 Adsorption of Cr and Cl- combining orange peels and citrus pectin

Adsorption of Cr

The results applied in the model shown in Figure 10 show that the removal of Cr in response to the process by combining orange peels (different particle sizes) with citrus pectin is effective. Being the size of the particle 0, 5mm in a contact time of 2h its adsorption potential is 97.19% (9.87 mg/L), although the result is significant ($p < 0.0001$) the particle size 2.0mm, in the same contact time slightly increases its removal capacity by 97.68% (8.16 mg/L).

In this sense, when the time is 4h and 6h of contact with the different particle sizes, the concentration of Cr ions decreases, going from 13.9mg/L to 19.5mg/L. The behavior is confirmed by [3], in whose work they explain that having a single, long, smooth and continuous tendency, the mechanism of removal occurs by the formation of an adsorbent layer on the surface of the particle [3], [9], [10], [13].

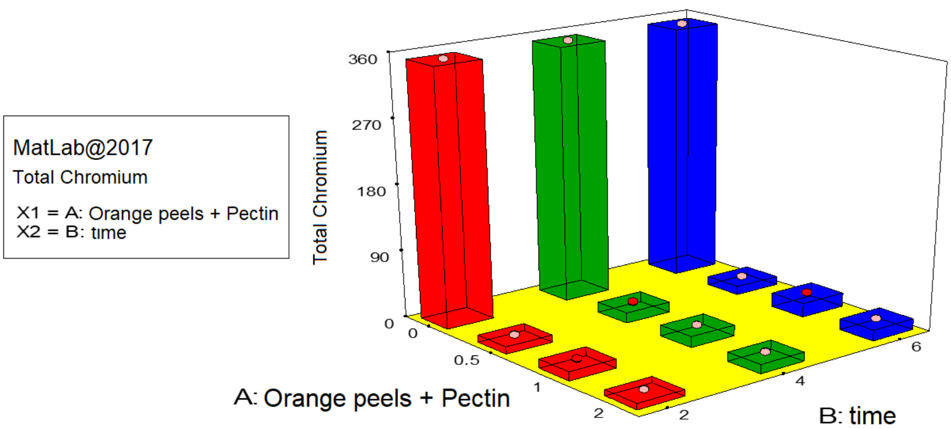

Figure 10. Interaction of orange peels plus pectin with respect to the times for Cr

Adsorption of Cl-

The highest adsorption potential was achieved in the time of 6h and size 0.5mm with a value of 2040 mg/L equivalent to 91.87% ($p < 0.0001$, CV = 0.31%), then the removal behavior it dropped to 87.01% for a size of 1.0mm in contact time of 4h (Figure 11). The adsorption that occurred between the time of 2h and 4h and the effect with the sizes of the particles presented a significant difference ($p < 0.0001$) without depending on the times in the removal [9].

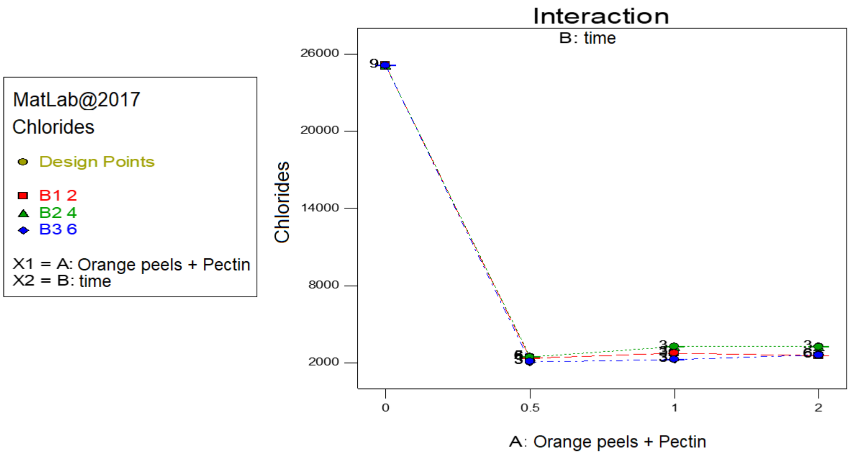

Figure 11. Interaction of orange peels plus pectin with respect to the times for Cl-

Conclusions

The experiments carried out serve as a complementary treatment of the traditional physicochemical processes that are applied to achieve compliance with environmental regulations. However, they can be used to reduce part of the polluting load generated by artisanal tanneries that mostly discharge without any treatment to water sources.
The tests that used microalgae indicated that the greatest chromium ions removal occurred with the species *Scenedesmus sp*. with 98.6% effectiveness, acting as a removal agent at a pH of 6.0, constant aeration, temperature of 28 ° C and a time from 48h.

The use of orange peels as an adsorbent of chromium ions in the residual water obtained a 94% removal for a particle size of 0.5mm, a pH of 5.0, a temperature of 28 ° C and a magnetic stirring of 150rpm a time of 2h.

The presence of Cl- ions affected the removal potential of Chromium ions because it acted as an inhibitory substance present in the bioremediation tests. Therefore the agents used had to remove in different proportions. However, the greater potential of ions of Cl- was presented in the trials of citrus pectin and orange peels was 91.87%

with particle size of 0.5mm, at a pH of 5.0, temperature of 28 ° C and magnetic stirring of 150rpm for a time 6h.

For a removal of Cr and part of Cl- to be carried out, adjusted to the regulations in force in Colombia in tannery water. It is recommended to carry out processes that obtain greater removal potential, that is, it is proposed to start the process with *Scenedesmus sp*, in a time 48h and finish with citrus pectin in a time of 2h or 6h.

Conflict of Interests. The authors declare that there is no conflict of interests regarding the publication of this paper.

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