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Bioflocculant pectin activity extracted from the orange peel (Citrus sinensis (L.) Osbeck) for wastewater treatment

Names: Daniel Victor Santos Silva and Iago Martins Felipe

Country: Brazil

Advisor teacher: Dr. Alexandre de Jesus Barros

School: ETEC Irmã Agostina

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SUMMARY

The main flocculant agent used in the water and wastewater treatment process is the polyacrylamide, of which monomers are highly toxic. The purpose of this project is to solve this problem by evaluating the behavior of the bioflocculant pectin extracted from the orange peel. The pectin extraction was made by the citric acid and characterized the obtained material with the degree of esterification (DE) and galacturonic acid content (GAC) with neutralization volumetric method. Flocculation tests were made in synthetic residual water considering 3 values of initial pH: 3.3; 7.8 and 10.6. In pH 10.6, all the samples presented flocculation activities better than 90%, that is, is the most favorable to the pectin flocculation process.

LIST OF ABBREVIATIONS AND ACRONYMS

Af^{550nm} - absorbance measure in the aliquot after the treatment

Ai^{550nm} - absorbance measure in the control

BRL - Brazilian Real (Brazilian currency)

CI - Confidence Interval

CP - Citric commercial pectin

DE - Degree of esterification

et al - et alii (and others, male)/et aliae (and others, female/et alia (and others, neutral plural)

FA - flocculant activity

FAO - Food and Agriculture Organization of the United Nations

GAC - Galacturonic acid content

HMC - High methoxylation Content

LMC - Low methoxylation Content

MNaOH - standard NaOH (mol L-1) solution concentration

min. - minutes

N - Replicates/samples Number

pH - potential for hydrogen

P11/P21/P31 - Protocol 1 conditions

P21/ P22/P32 - Protocol 2 conditions

rpm - revolutions per minute

s - standard deviation

α – alpha

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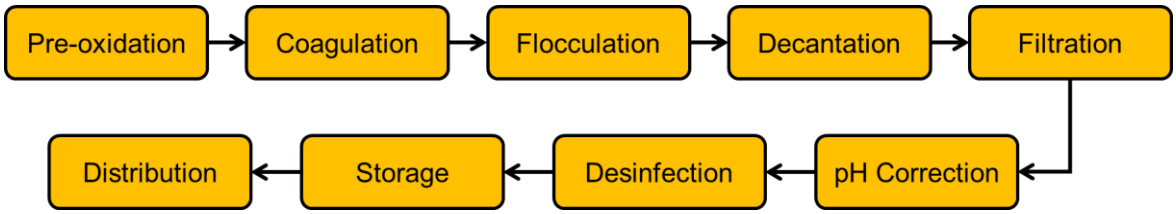
1. INTRODUCTION

Water is the most important resource for humanity and its treatment is essential. Nowadays, flocculation is one of the most used processes wastewater treatment (Figure 1). Together with coagulation, it results in a lower water's turbidity level, an important physical-chemical parameter in water quality control analysis. Both processes succeed because of an interface phenomenon which destabilizes and aggregates a part of the suspended solids present in the effluent and colloidal particles, both of them cause turbidity. Such material has, predominantly, a negative surface electrical charge in aqueous solution, creating resistance in the particles for the spontaneous clustering and settlement, due to the mutual repulsion forces against each other (PAVANELLI, 2001; JÚNIOR; ABREU, 2018).

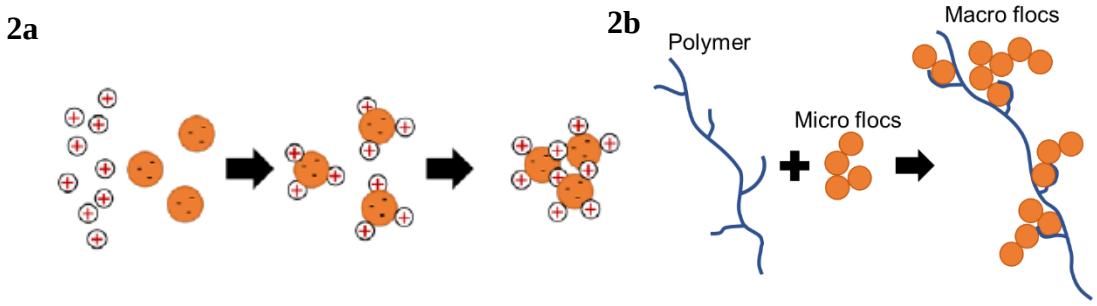
Figure 1: Scheme of the traditional water treatment system



(Source: adapted from Júnior and Abreu, 2018)

The first step of Coagulation process occurs when the negative charges are neutralized by addition of metallic inorganic salts to the water - commonly aluminum sulfate ($Al_2(SO_4)_3$) or iron (III) chloride ($FeCl_3$). Those salts, when undergoing many hydrolysis reactions, produce cationic species in solution, capable of reducing the repulsion between the particles and favoring the creation of "micro flocs" (Figure 2a). At the flocculation process, high molecular mass polymers are used - they can be cationic, anionic, amphoteric or non-ionic; by agglomerating the micro flocs in their chain, they promote a larger, denser and more resistant to disruption “macro flocs” (Figure 2b), facilitating the removal in the decantation and filtration phase (LEE; ROBINSON; CHONG, 2014).

Figure 2: The coagulation process by neutralized negative charges (a) and flocculation process by agglomeration of the micro flocs in their polymer chain (b).



(Source: adapted from Suopajärvi, 2015 and Process Principles, 2019)

A persistent problem is that the polymer used on the flocculation step, the polyacrylamide, presents environmental disadvantages since it is non-biodegradable and capable of generating residual acrylamide monomers, a neurotoxic and probably carcinogenic agent in humans according to the International Agency for Research on Cancer (JÚNIOR; ABREU, 2018). In order to address these issues, the research for new bioflocculants, flocculants from natural biodegradable raw material, has gained attention in research and development centers of this field (Ho *et al*, 2010).

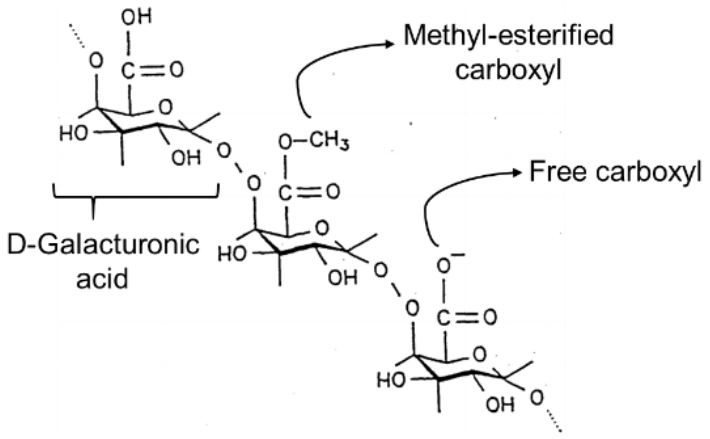
Combining this issue, after the orange processing at the beverage industry, from 40% to 60% of the fruit weight is considered a waste. Usually, only part of them is used, as animal feed or organic fertilizer. The abundant amount of solid waste – mostly orange peel –, could become a soil pollutant. Also, it is an extra cost for the industry to dispose it (LICANDRO; ODIO, 2002; FILHO; FRANCO, 2015).

The residue contains substances with meaningful commercial value, standing out the citric pulp bran, essential oils, flavonoids, adsorbent material, and pectin. Seed-producing land plants usually produce pectin, a hydrocolloid polysaccharide, which combined with another polysaccharide as cellulose and hemicellulose, helps the adhesion between vegetable cells and ensuring its wall mechanical resistance (THAKUR; SINGH; HANDA, 1997; ZANELLA, 2013).

The linear structured pectin is a homopolymer D-Galacturonic acid, connected by a glycosidic bond at α(1→4) (Figure 3), able to interrupt the principal chain for Rhamnose units at α(1→2) which inserts side chains of neutral sugars in the molecule. The pectin has different methyl-esterification carboxylic groups, it has the proportion between the esterified carboxylase and totals carboxylase, both can be used for classifying pectin in two types: pectin high methoxyl pectin (HTM), representing 50% above of esterification, and low methoxyl pectin LTM, that represent 50% below. According to The Food and Agriculture Organization of the United

Nations (FAO), the pectin has to contain a minimum of 65% of d-Galacturonic acid to be considered high quality (CANTERI; WOSIACKI; SCHEER, 2011; ZANELLA, 2013).

Figure 3: Linear fragment of the pectin chain.



(Source: adapted from Thakur, Singh and Handa, 1997)

The purpose of this research is to extract, characterize and observe the behaviour of bioflocculant pectin extracted from the orange peel (*Citrus sinensis* (L.) Osbeck) on suspended kaolin ($Al_2O_3.2SiO_2.2H_2O$), with the aim to check the applicability as a biopolymer flocculant for water treatment. The methodology was: extracting pectin from the orange peel by following up two extraction protocols; characterizing the pectin sample by neutralization volumetric; executing flocculation trial in the sample; analyzing the pectin characteristic and the pH necessary for flocculation process.

2. METHODOLOGY

2.1. Sample selection and pectin extraction

To guarantee random samples, the orange fruit was bought in 3 different markets in São Paulo, Brazil - avoiding any possibility of being from the same tree. The first step of the extraction was washing the selected fruits, using water and neutral dish soap, to remove the dirtiness. Next step, with a stainless steel knife, the oranges were divided into 8 equal parts, for splitting by hand the pulp - juice vesicle and the seeds - from the orange peel (albedo e flavedo). Those orange peels were exposed to heat treatment for enzymatic inactivation by immersion in boiling water for 3 minutes (CANTERI *et al*, 2010). The cut pieces were dried in the drying oven at 50°C for 2 days, then blended it until being an orange peel flour. This material was ground in a common sieve and the process was repeated with the part left in the sieve. The flour resulted in this process was weighed and stored in a plastic bag, in a refrigerator at (≈ 8°C) until extraction was done. With

this flour, pectin was extracted based on the methodology of Zanella (2013), Canteri *et al* (2010) and El-Nawawi e Shehata (1987), with some adjustments. The factors adopted a controlled extraction (temperature, pH, flour: water, and extraction time) are in Table 1.

Table 1. Extraction conditions

Parameters	**Adopted level**	
	Protocol 1	**Protocol 2**
Temperature °C	80	80
pH	1.7	2.5
Flour:water	1:70	1:70
Time (min.)	60	120

Around 5 g of orange peel flour was suspended in 175 mL of water. At the same time, a citric acid solution ($C_6H_8O_7$) was prepared with the same volume, and with correct mass so the final mixture reaches a desirable pH of 1.7 or 2.5. Both bottles were placed in a heating plate until achieve 80 °C (the temperature was controlled by a thermometer), then the acid solution was shed in the bottle with the orange peel flour. At this moment, it started to mix and timed the extraction. At the end of the reaction, the system was put in an ice bath until it reaches room temperature. After that, the extract was filtered in a fabric filter, the retained part was disposed and the liquid part was put in an amber bottle, that was refrigerated until the precipitation occurred. From that point, the extracted volume was measured and then added in small parts under constant stirring, two volumes of ethyl alcohol 96% to pectin precipitation. The system was mixed and then rested per 30 minutes, before filtered in a fabric filter through two washes: the first with ethyl alcohol 70% and the second in ethyl alcohol 96%. The pectin was pressed manually and put in a drying oven at 50°C for 2 days. The dried material was weighted, crushed with mortar and pestle and stored in a plastic bag, and put in a refrigerator.

The extraction yield was calculated by the percentage ratio between the dried pectin mass and the weighted flour mass. The procedure was duplicated and followed for the 3 orange batches. At the end of the extraction, both of the samples duplicates were stored in the same bottle, obtaining 6 pectin samples based on Table 1: pectins extracted in the protocol 1 conditions (P11, P21 and P31) and the pectin extracted in the protocol 2 conditions (P12, P22 and P32).

2.2. Pectin characterization

The resulted pectin was characterized to determine its degree of esterification (DE) and its galacturonic acid content (GAC) using the neutralization volume technique, based on the methodology proposed by Fertonani (2006). Approximately 100 mg of dried pectin were

weighted and wet in 2 ml of ethyl alcohol 70%. After that, 100 mL of water was added to the mixture and it was constantly stirred to dissolve the polymer. Following up first the titration of the free carboxyl with NaOH mol L^{-1} and after the neutralization was added 10 mL of NaOH mol L^{-1}, followed by 30 minutes of constant stirring to the de-esterification of the carboxyl groups esterified. This procedure was added 10 mL de HCl 0,1 mol L^{-1} the second titration was from the solution of NaOH 0.1 mol L^{-1}. The DE and GAC determination were performed by the equations 1 and 2 (FERTONANI, 2006), where the M_{NaOH} is the standard NaOH (mol L^{-1}) solution concentration, m is the pectin mass-weighted (mg), V_1 is the titrant volume spent in the first titration(mL) and V_2 is the volume spent in the second one (mL).

$$DE = 100\ x\ \frac{V_2}{V_1 + V_2} \quad (1) \qquad GAC = 100\ x\ \frac{M_{NaOH}\,(176V_1 + 207V_2)}{m} \quad (2)$$

2.3. Flocculation tests

The flocculation tests were based on the tests proposed by Ho et. al. (2010) e Yokoi et. al. (2002), with some adjustments. As the synthetic wastewater, it was used a kaolin stock suspension with equivalent concentration at 0.4 g L^{-1} (Figure 4). The Al^{3+} coagulant concentration was fixed in 0.62 mmol L^{-1} and the flocculant concentration in 20 mg L^{-1}. All the tests were made in a room temperature (≈ 25 °C).

Figure 4: Aspects of the kaolin stock suspension

In a beaker 100 mL was collected 50 mL of the stock suspension previously homogenized and the pH was adjusted to the desired value with HCl 0.1 mol L^{-1} or NaOH 0.1 mol L^{-1}. In this project, it was studied the flocculant activity of pectin in 3 initial pH values, based in previous tests: an acid (3.3); a neutral (7.8), and an alkaline (10.6). After adjusting the initial pH it was added the cation Al^{3+}, followed by a fast stirring (≈ 300 rpm) with a glass stick for 3 minutes, and after that, it was added a pectin sample stirring slowly (≈ 25 rpm) for 15 minutes. The system rested 5 minutes to the collection of an aliquot of the supernatant that was tested in the

spectrophotometer to read the absorbance in the 500 nm wavelength. Also, the absorbance of an aliquot of the stock suspension was tested before the treatment and used as a control. The flocculant activity (FA), in percent, was calculated according to the Equation 3, where Ai^{550nm} is the absorbance measure in the control and Af^{550nm} is the absorbance measure in the aliquot after the treatment (Ho *et al*, 2010).

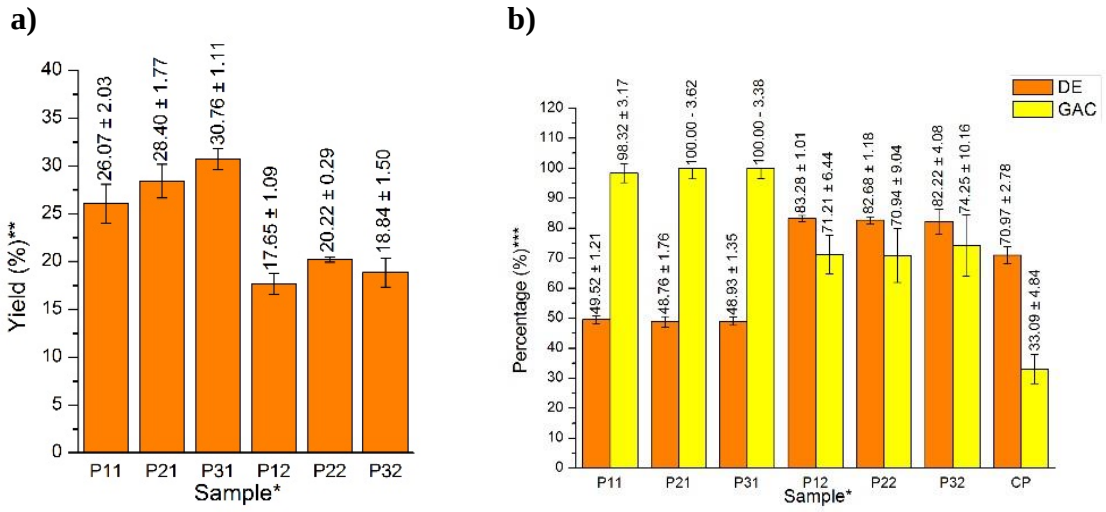
$$FA = 100\ x\ \frac{A_i^{550\ nm} - A_f^{550\ nm}}{A_i^{550\ nm}} \quad (3)$$

3. RESULTS

3.1. Extraction yield and characterization results

Figure 5 presents the values of pectin extraction yield, the esterification degree of esterification (DE), and the galacturonic acid content (GAC) of the pectin extracted samples and a citric commercial pectin (CP), that was used to compare.

Figure 5: Obtained yields for the pectin extraction (a) and the sample characterization results (b).



*P11, P21 e P31 = pectins extracted using protocol 1; P12, P22 e P32 = pectins extracted using protocol 2 e CP = citric commercial pectin.
**Values expressed as x = average ± s (N = 2).
*** Values expressed as x = average ± CI (CI = 95% e N = 3)

Analyzing the extraction yield data was possible to confirm, with 95%, that the average yield obtained inside the same protocol has no statistical difference among them and, also, that the average extraction yield using protocol 1 was greater than by protocol 2. the fact that the

extraction that occurs in lower pH present a greater yield, agrees with the researches Tiwari *et al* (2017), that observed a linear decrease of orange pectin extraction yield when pH increase, in the range of 1 to 2.5. Therefore, it is possible to certify the results of the pectin extraction yield with the literature.

The characterization data analysis allowed it to conclude, with 95% of reliability, that the results of DE and GAC didn't have statistical differences inside the same extraction protocol. However, it differs between protocols, it is possible to observe that the pectins extracted following protocol 1 have lower DE and greater GAC in comparison with the ones extracted following protocol 2. The pectins extracted by protocol 1 can be classified as pectins LTM (low degree of carboxyl groups esterified), while the ones extracted by protocol 2 are classified as HTM (DE>50%). Both of them exceed the limit of 65% of D-Galacturonic acid in its composition, allowing in this way to certify its quality and purity (CALLIARI, 2004).

According to Paiva, Lima, and Paixão (2009), the extraction pH reduction value speed up the pectin degradation and de-esterification, in accord with the data obtained in this project, as the pectins extracted in a lower pH besides classified as LTM, in other words, experience de-esterification, also had great values of GAC, meaning that side-chains that contain neutral sugars were removed/degraded. Facing this scenario, it is possible to determine that the results from pectin characterization are consistent with the literature.

3.2. Flocculation test

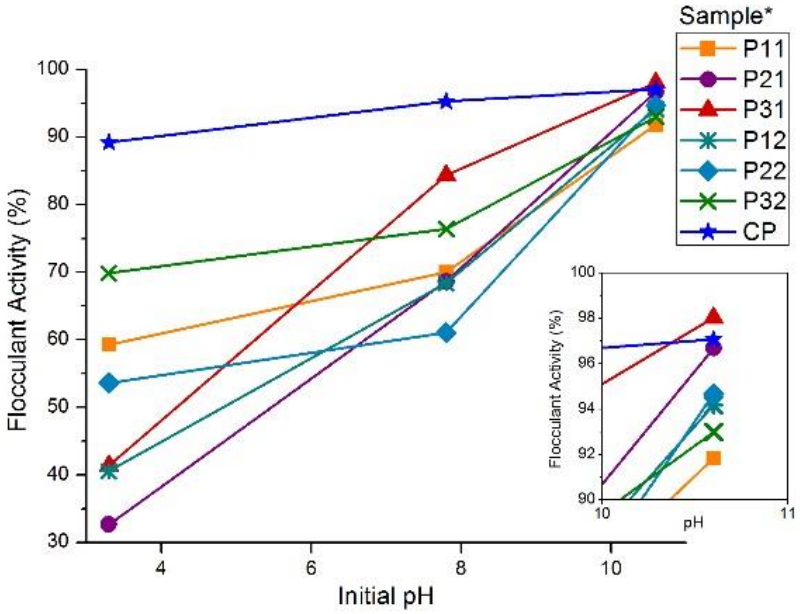
Table 2 and Figure 6 present the values of pectin extracted samples flocculant activity and of a citric commercial pectin (CP), that was used to compare.

Table 2: Flocculant activity of the pectin samples

Initial pH	Flocculant activity (%)*						
	Sample**						
	P11	**P21**	**P31**	**P12**	**P22**	**P32**	**CP**
3.3	59.30^{a} ± 10.84	32.68^{b} ± 11.51	$41.39^{b.c}$ ± 15.53	$40.58^{b.c}$ ± 13.24	$53.65^{a.c}$ ± 17.45	69.87^{a} ± 18.66	89.21^{d} ± 10.95
7.8	$70.03^{a.c}$ ± 18.50	$68.69^{a.c}$ ± 15.32	84.38^{b} ± 10.52	$68.41^{a.c}$ ± 17.65	61.06^{a} ± 18.66	$76.41^{b.c}$ ± 6.54	95.31^{d} ± 4.32
10.6	91.84^{a} ± 8.03	$96.69^{a.b}$ ± 11.21	98.03^{b} ± 2.42	94.18^{a} ± 4.20	$94.68^{a.b}$ ± 9.09	$92.99^{a.b}$ ± 11.51	$97.09^{a.b}$ ± 3.37

*Values expressed as x = avarege ± CI (CL = 95% e N = 3); averages with same letters in the same line didn't differ between then in the 95% reliability level.
**P11, P21 e P31 = pectins extracted by protocol 1; P12, P22 e P32 = pectins extracted by protocol 2 e PC = citric commercial pectin.

Figure 6: Average values of the samples flocculant activity according to the initial pH



*P11, P21 e P31 = pectins extracted by protocol 1; P12, P22 e P32 = pectins extracted by protocol 2 e PC = citric commercial pectin.

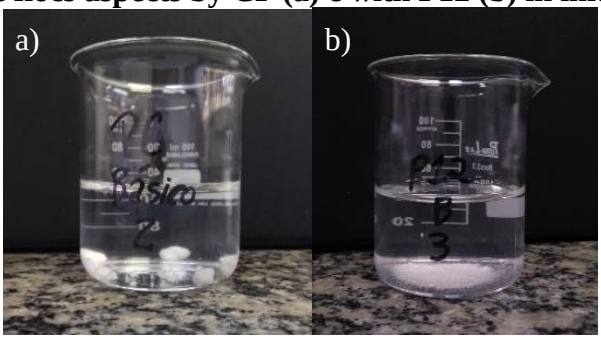
Considering the results it is possible to conclude that, apparently, to the N sample studied, the pectin flocculant activity extracted by both protocols did not present a meaningful difference between them, mainly considering the high-reliability levels observed in the analysis. In other words, the characteristics differences between the protocols observed in the characterization phase were not enough to impose a difference in the flocculant activity between protocols.

Realizing that high values in the reliability range are commonly related to the lower values of flocculant activity, that occur in the majority in the flocculation tests with pectins extracted in initial pH 3.3 or 7.8. This phenomenon could be explained by the inappropriate spectrophotometric technique to analyze turbid dispersions because many variations occurred in the quantity of the suspended particles that were reached by the light beam sent by the equipment. Thus, as the tests result in lower flocculant activity, reflecting that the final aliquot has great turbidity, it is coherent that those are followed by a greater reliability level.

The fact that citric commercial pectin samples had, typically, a great flocculant activity in comparison with the extracted pectins. It should be related to a possible difference in the molecular mass among the samples. Ho et. al., in studies published in 2009 and 2010, obtained values of 163 kg mol^{-1} e 25,3 kg mol^{-1} to the average molecular masses to the citric commercial pectin sample and for pectin extraction from the orange peel in an acidic medium and warm

environment, respectively, indicating a tendency in the commercial pectin, on average, a greater molecular mass when compared to the extracted ones. According to Wu e Ye (2007), the molecular mass of a polymer is an important factor in its flocculant activity, since high molecular mass polymers are capable to have more colloidal particle interaction and thus, create bigger clusters, and also achieve a greater flocculant activity. This phenomenon could be observed during the flocculation tests, where the clusters formed in the tests using commercial pectin (Figure 7a) reveal more fibrous and bigger than those formed by the pectin extracted (Figure 7b), mainly in the initial pH of 10.6.

Figure 7: The flocs aspects by CP (a) e with P12 (b) in initial pH - 10,6.



The increase in the flocculant activity can be explained by the increase in the initial pH considering the role of the free carboxyl group, present in the pectin molecule in the flocculation process. Those groups, when the conditions are more alkaline, are deprotonated and negatively charged (COO^-), exerting electrostatic repelling forces among the same molecule, causing the enlargement of pectic chain and the increase of the contact surface, that will be more susceptible to interact with the kaolin particles and built the cluster (WU; YE, 2007).

The average flocculant activity values obtained in the initial pH of 10.6 present above 90% to all the pectin samples studied, which shows an optimal region to flocculation. However, Ho, et. al. (2009) reached even greater values (above 99%) to the citrus pectin flocculant activity in the process optimized conditions, that were pH3, Al^{+3} concentration of 0,5 mmol L^{-1} and pectin concentration of 20 mg L^{-1}. Considering the information above, it would be interesting the execution of optimization tests, capable of certifying the bioflocculant feasibility, extracted from the orange peel, in the wastewater treatment.

4. CONCLUSION AND RECOMMENDATION

At this project was possible to analyze the potential of pectin use by extracted the orange peel as a bioflocculant in the water and wastewater treatment, to replace the polyacrylamide, a common flocculant agent used in the water treatment plants that, it has great efficiency and low cost, however, has environmental and toxicological disadvantages. The orange peel pectin has the same flocculation mechanism, but generate biodegradable subproducts.

The costs from the study considered the spends with electricity and reagents used to the extraction, the average cost was 6.19 BRL/g of pectin extracted by protocol 1 and 8.78 BRL/g pectin extracted by protocol 2. Using these values it was calculated the average cost to treat 1L of water, which was 0.12 BRL to pectins extracted by protocol 1 and 0.18 to pectin extracted from protocol 2.

Thus, the results from the flocculation test showed that the differences between pectins extracted by the protocol 1 and 2 did not have a significant influence in the polymer activity, meaning that in an implementation phase, the extraction by protocol 1 would be more cost-benefit.

Also, the study presented a way to use an amount of residue from orange peel produced by the industry that uses orange as a primary material to its products, stimulating the sustainability and reducing disposal costs. Additionally, it could be a reverse logistics for the company.

As a recommendation, considering the obtained results, it would be interesting to use the right equipment to the turbidity measure (turbidimeter), to improve the flocculation tests precision and to compare the final aliquot turbidity with a reference value, which has great importance to the method effectiveness; collect the orange peel diary in the industry to confirm the material capacity in the pectin production; studying the influence of pectin molecular mass in flocculation activity, and performing optimization tests to pH 10.6 to reach out the best results to the process with pectin.

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