Ultrasonic Radiation Influence on the Bioadsorbent Characteristics of Citrus *(Citrus x Lemon) & (Citrus x sinensis)*

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Abstract— *Of all the materials used as adsorbents, activated charcoal is the most popular as it is excellent for reducing/eliminating a wide variety of contaminants. This capacity is mainly due to its structural characteristics that give it a large surface area. However, it has several disadvantages. The most important are that the adsorbent material is quite expensive as is its regeneration. For this reason, more economical absorbents have been sought, highlighting the study of adsorption phenomena in some types of biomass as adsorbents.*

Bioadsorption is considered a viable alternative to the physical-chemical methods currently used for the recovery or removal of heavy metals dissolved in liquid effluents. Its main attraction, from an industrial point of view, is its low cost due to the great abundance, easy to obtain and low price of the bioadsorbent material. Bioadsorption is very effective in treating metal concentrations below 100 mg/L, where the application of physical-chemical methods is neither technically nor economically feasible.

One of these materials of interest is citrus peels, because, due to their abundance as a waste product of the food industries, they are hardly reused and currently have little economic value. However, these residues have a low adsorption capacity, so both physical and chemical modifications are required to increase their adsorption properties.

This study compares the characteristics of orange and lemon peels undergoing a physical-chemical treatment with ultrasonic radiation assistance and the optimization of experimental conditions to obtain useful bioadsorbents in discontinuous processes (batch).

Keywords*— *bioadsorption, orange peel, lemon peel, ultrasonic radiation, heavy metal removal.

I. INTRODUCTION

Conventional methods for the treatment of wastewater with low concentrations of heavy metals in the ion state are extremely costly. For this reason, adsorption techniques have gained acceptance due to their effectiveness in removing pollutants that are too stable for conventional methods, resulting in high-quality effluents (Basso et al, 2002). Beyond research with living plants (Seki et al, 1988), the use of plant waste material for the recovery of heavy metals has been addressed. An adsorbent can be considered inexpensive if it requires little processing, is abundant in nature, or is a by-product or waste from another industry. (Bailey et al, 1999).

Generally, the bioadsorbents studied come from waste that has problems for reuse and which do not represent any economic value, a clear example of this is waste from industry and agricultural area (Crini, 2006). They have adequate adsorption capacity: pine bark (Al-Asheh et al, 2000), conifers (Aoyoma et al, 2000), rice husk (Feng et al, 2004), etc. In this sense, the easiest and most accessible bioproduct is plant biomass (Mani & Kumar, 2014).

The cell walls of bioadsorbent materials contain polysaccharides, proteins and lipids. Therefore, they contain numerous functional groups capable of linking heavy metals on the surface of these (Wei et al, 2010). Among the functional groups present are the amino, carboxylic, hydroxyl, phosphate and thiol groups that differ in their affinity and specificity with respect to susceptibility to bind to the different metal ions (Ghimire et al, 2003). However, it should be noted that the content in functional groups in the bioadsorbent material may be different depending on the species, the season, the geographical area, etc. The most commonly consumed fruit peels are apple (Mallampati & Valiyaveettil 2013), banana (Memon et al, 2009) orange (Feng et al, 2011), lemon (Tejada et al, 2015), and mango (Iqbal et al,2009). All of them should be included among widely used bioadsorbents.

Citrus fruits are a diverse group of species native to tropical and subtropical regions of Asia that are cultivated in the world. The fruits of these species, in particular oranges, mandarins, lemons, limes and grapefruits, play an important role in the feeding of millions of people, whether as fresh fruit, concentrate, drink or in culinary preparations. The two largest producers are Brazil and the United States, with 21.4% and 14.5% of world production respectively. China, Mexico, Spain and India follow in importance, representing together 27.6% of the global total (FAO,2017).

Citrus essential oils are obtained from fruit bark (flavedo) (Tranchida et al., 2011). Different extraction techniques are employed, including direct methods such as shell compression and indirect methods such as steam stripping distillation and microwave radiation-assisted water (HDMO) distillation. The best method to extract them is steam distillation due to the variety of volatile molecules extracted, such as terpenes and terpenoids, phenol-derived aromatic components and aliphatic components (Palazzolo et al., 2013). However, this process requires several hours, high energy consumption, conventional heating and hot water agitation (Durán & Villa, 2014). HDMO is a very fast and relatively inexpensive process and the essential oils obtained are free of thermal decomposition products and contaminants. Recently, the extraction of microwaves without the addition of solvent has been introduced and using only water that is removed in situ from the tissues (Ferhat et al., 2006) obtaining higher yields and better quality of the oil in a shorter time.

Citrus pectin, a thickener commonly used in the food industry, is obtained from the extraction of albedo (white layer between the shell and pulp). However, in most processes, the production of pectins is linked with the obtaining of essential oil (Cerón-Salazar & Cardona-Alzate, 2011). Alternatively, high methoxyl pectins have been obtained from orange peels by performing an acid extraction to the orange albedo by a low-pressure steam injection (15 psi) (Fishman et al, 2003).

The industrial production of citrus pectin has the following stages: in the first, the peel must be washed to remove the greatest amount of soluble solids and impurities, as these components hinder the purification process. The peels are then subjected to a drying process, which inactivates the pectinaesterase enzyme and decreases the moisture content, increasing the stabilization of the peel for storage and reducing the cost of transport (Martí et al, 2014). Subsequently, the dry matter suspended in hot water with the necessary amount of a strong acid, starting the hydrolysis process.

During this process, starting from the macromolecular structure formed by cellulose, hemicellulose and pectins, hemicellulose begins its degradation to glucose, galactose and fructose; cellulose to glucose and pectin to pectin monomer through a depolymerization process (Chen et al, 2015). After a while, by filtration, the liquid phase is separated from the solid phase. Then the liquid phase is mixed with alcohol. As a consequence, the polymer that precipitates rapidly in the form of pectin is recovered. The precipitate is extracted and purified by washing it with more alcohol. Finally, it is dried and ground (Claus, 2002).

Depending on the source, pectins may vary in molecular size, degrees of acetylation and methylation, galacturonic acid content and neutral sugar residues. Therefore, pectins exhibit versatile gelling properties and are able to form complexes with other natural compounds and, as a result, are useful for designing food products (Gawkowska et al., 2018).

The resulting material from the extraction of pectin is a poor dietary supplement for animals due to its low protein content and high in sugars (Siles et al, 2016). However, it presents the optimal conditions for further treatment as a bioadsorbent (Masmoudi et al, 2008).

Acid-soluble pectin extraction is necessary for its ability to absorb large amounts of water and the formation of colloids. If not extracted, the final product would not have a sufficient degree of consistency to be used as a bioadsorbent. Along with pectin, acidic hydrolysis involves the solubilization and degradation of carbohydrates, especially xylan and hemicellulose, since glucomanane is relatively stable in acid medium (Van Buren, 1991).

Accepted the acid attack as the first phase to increase the adsorption capacity of the citrus shell, the second phase consists of an attack in alkaline medium, intended for the saponification of ester groups that have not been extracted during the acid attack and subsequent crosslinking with Ca(II) (Cardona Gutiérrez et al, 2013). Usually this process is carried out with NaOH, which is followed by crosslinking with $CaCl_2$ to increase the activation sites. However, it is proven (Arjona et al., 2018) that both processes can be carried out in a single stage with the use of a $Ca(OH)_2$ 0.2 M solution.

Process intensification is currently one of the areas with the greatest development potential in the food industry. Through more sustainable technologies, the aim is to increase the productive performance, quality and safety of the processed product, as well as reduce the size of equipment, waste and energy needs (Benali & Kudra, 2010). Among the different techniques that can be used for the intensification of processes (microwave, infrared, electrical pulses) it is worth highlighting the application of acoustic energy (Knorr et al, 2004). One of the main characteristics of power ultrasound is its ability to improve matter transfer processes (Cárcel et al., 2011).

In recent years there has been increased interest in the use of ultrasound to intensify the pectin extraction process as they not only increase the performance of the operation, but also reduce the extraction time compared to the conventional process (Bagherian et al., 2011; Minjares-Fuentes et al., 2014; Maran & Priya, 2015; Wang et al., 2015; Sundararaman et al., 2016; Freitas de Oliveira et al, 2016; Grassino et al., 2016).

II. MATERIAL AND METHOD

The transformation of citrus peels as cationic exchangers requires a physical-chemical treatment.

2.1 Treatment of citrus peels

This process begins with the collection and cleaning of the peels. It is important to select peels in good condition, i.e. without fungi, worms or decomposing parts. The edible part (endocarp) is then separated so that the peel (flavedo and albedo) is free from the pulp residues and is cleaned with detergent. In this way, waxes are extracted that, superficially, are incorporated to improve the appearance of these fruits in the commercial circuit. Next, the citrus peels are dried with forced air to constant weight and they are ground and sieved.

Physical modifications involve proper sizing of the peels by cutting or crushing, complemented by heat treatments such as reflux, microwave or ultrasonic irradiation. Chemical modifications include treatment with different types of chemical agents, which are used to increase binding groups in the final bioadsorbent, eliminate inhibitory groups and increase their surface area (Patel, 2012).

The chemical treatment begins with a process of acidification of the peels by hydrochloric acid, aimed at the extraction of pectin. Although the obtaining of pectin from orange peels has been extensively studied (Msebahi et al.,2005; Liu et al., 2006; Yeoh et al.,2008), from previous experiences (Arjona et al., 2018) the chemical attack is performed with ultrasonic radiation assistance (US), using a US Elmasonic bath model LC 30 H with a fixed frequency of 37.5kHz and time and temperature regulation, for a period of 45 minutes.

The acid treatment is repeated until obtaining a solid free of sugars and of the pectin and hemicellulose fractions soluble in these experimental conditions. One repeat is usually sufficient for the reducing sugar test (Fehling method) to be negative.

Once the pectin, hemicellulose and reducing sugars soluble in acid medium have been extracted, a treatment is carried out with distilled water to remove the excess hydrochloric acid used initially.

The resulting solid is then treated in an alkaline medium. In this way, saponification of non-soluble pectin in an acid medium is achieved, as well as solubilization of the soluble fraction of hemicellulose in an alkaline medium (Grace et al., 1996). The saponification and crosslinking process is carried out in a single stage with the use of a $Ca(OH)_2$ solution in the US Elmasonic bath model LC 30 H over a period of 45 minutes. After this treatment, excess Ca (II) is removed by washing with distilled water. The bioadsorbent obtained is dried at 110ºC and ground until the final product is obtained, which is kept in a hermetically closed container.

2.2 Behavior of citrus peels before chemical treatment

Orange peels and lemon peels have a different water absorption capacity, before undergoing chemical treatment. The experimental check was carried out as follows: 5g samples of orange and lemon peel were placed in a beaker, distilled water was added, drop by drop, with the help of a burette and the mixture was homogenized by means of a magnetic stirrer at a set speed of 400 rpm.

Progressively, a compact mass formed, to the point of reaching saturation. To perform its verification, the beaker was gently flipped. When a drop of liquid was released, the volume of water that had been poured into the glass from the burette was recorded (Figure 1).

FIGURE 1: Detachment of a drop of liquid from the water-saturated solid

Table 1 shows the mean values (3 repetitions) of the behavior of both types of citrus peel as a function of grain size. It was found that:

- Lemon peel presented a double adsorption to the adsorption of orange peel in all cases
- Adsorption decreased as particle size decreased

This second behavior was attributed to the possibility of caking increases when there is more contact between the particles.

In general, the smaller and less uniform they are, the greater the probability of caking (Pietsch 2002). The powdery particles become wet, sticky and compact, finally reaching the liquefaction phase (figure 2). In view of this behavior, it was decided to discard the particle size of less than 250 µm.

TABLE 1
WATER ADSORPTION ON SAMPLES OF 5 GRAM CITRUS PEELS OF DIFFERENT PARTICLE SIZES AT ROOM TEMPERATURE

Orange	Particle size	mL H_20 Average	mL/g
	1mm>X>500 µm	37,8 ± 0,35	7,6
	500 µm>X> 250 µm	28,2 ± 0,5	5,6
	X<250 µm	23,1 ± 0,8	4,6
Lemon	1mm>X>500 µm	71,5 ± 0,85	14,3
	500 µm>X> 250 µm	59,5 ± 1,25	11,9
	X<250 µm	52,6 ± 0,45	10,5

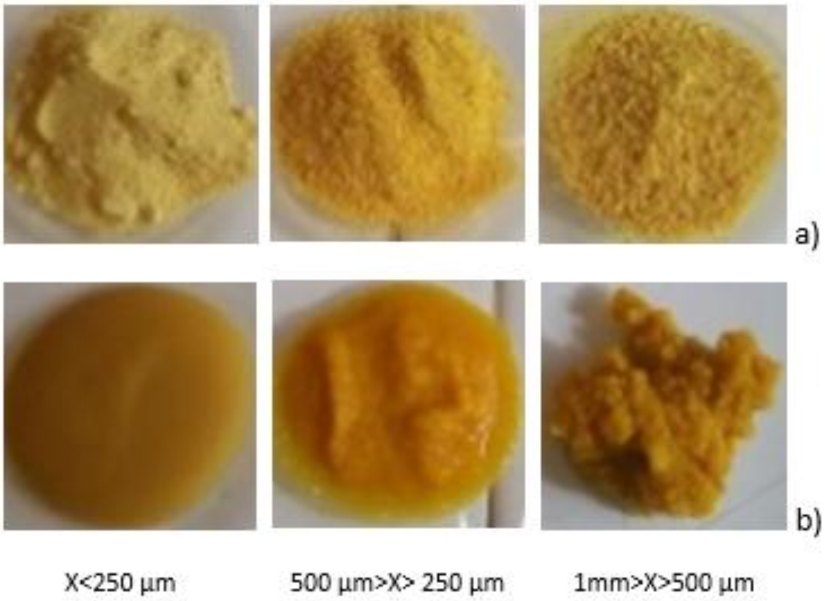

FIGURE 2: Appearance of orange peel vs particle size a) dry b) saturated with water

2.3 Influence of chemical treatment

The effectiveness of the various stages of chemical treatment was tested through the extracted organic matter content. To this end, the organic matter eliminated in acidic treatments and alkaline treatment was determined by the action of KMnO4 in sulphuric medium (permanganimetry) and expressed as mg O2/L. Table 2 compares the results obtained when citrus peels with a particle size between 1000 µm - 500 µm were used, showing the mean values of 3 repetitions.

TABLE 2
ORGANIC MATTER EXTRACTED IN EACH ATTACK (mg/L O_2)

Particle size 1000µm>X>500 µm	First acid attack	Second acid attack	Alkaline attack
Orange	103,7 ± 1,4	68,8 ± 2,1	34,4 ± 4,0
Lemon	155,5 ± 4,5	128,6 ± 5,8	53,6 ± 5,6

As expected, in both orange and lemon peel, the first acid attack was more effective than the second, with significantly higher extraction of organic matter observed in the lemon peel in both attacks. This behavior was associated with the higher lignin content in orange peel, whose three-dimensional structure with amorphous ordering, acts as a protective agent of cellulose conferring resistance and impermeability to the biomass in which it is located and, simultaneously, to the higher cellulose content in lemon peel (Ververis et al, 2007). The alkaline attack, responsible for the saponification and crosslinking of the pectin not soluble in acid medium, presented the same behavior, although the absolute value of the extracted organic matter was significantly lower.

On the other hand, in acid hydrolysis, polysaccharides experience hydrolysis (Sánchez-Orozco & Vázquez-Velázquez 2017). Through the Fehling test detected the presence of monosaccharides in the resulting liquid, after the attack of the citrus peel in hydrochloric medium with ultrasonic radiation (US) assistance. Figure 3 shows the evolution of the reducing sugar content at the various stages of chemical treatment on an orange peel until it is transformed into the final bioadsorbent.

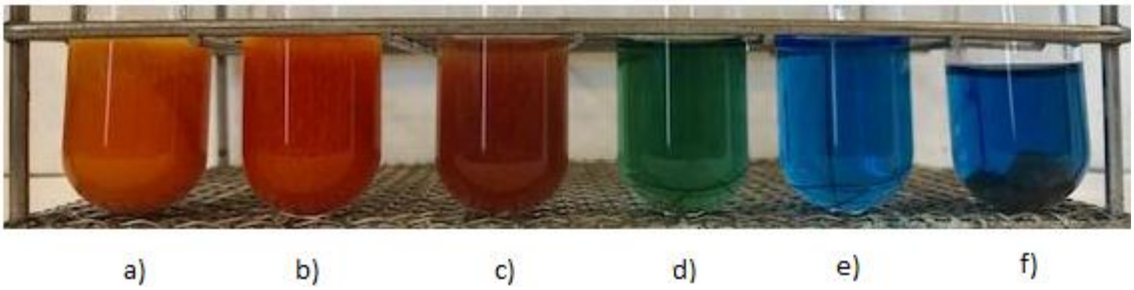

FIGURE 3. Fehling trial in the different liquid fractions of the chemical treatment of an orange peel: a) first acid attack; b) second acid attack; c) rinse with distilled water; d) alkaline attack; e) rinse with distilled water; f) final bioadsorbent

The effectiveness of the chemical treatment was checked by determining the infrared (IR) spectrum of the shells (initial bioadsorbents) and the products obtained through chemical treatment (final bioadsorbents).

Figure 4 shows the IR spectra before and after the chemical treatment of an orange peel, analogous to those obtained with lemon peel, performed with a Perkin Elmer model Paragoni 500 spectrophotometer.in both spectra the presence of the functional groups is confirmed: OH, C=O, C-O-C and C-O, as well as CH and CH_2 before and after chemical treatment.

The peaks that appear centered at 1741cm^{-1} and 1633cm^{-1}, which appear in the orange peel spectrum before chemical treatment, are attributable to the carbonyl group (C=O) as indicators of free and esterified carboxylic groups. In fact, the disappearance of the peak at 1741cm^{-1} in the spectrum of the orange peel once subjected to chemical treatment indicates the disappearance of high methoxyl pectins (García Raurich et al, 2019).

On the other hand, the signal in the form of a broadband between (3600-3200 cm^{-1}) centered at 3369 cm^{-1} before the chemical treatment and centered at 3420 cm^{-1} after the chemical treatment, is attributable to the hydroxyl group. This group is found in cellulose, pectin, absorbed water, hemicellulose and lignin, components present in the citrus peel. The presence of cellulose as the main component of citrus peels means that the removal of hemicellulose is not, for practical purposes, observable.

Finally, the peaks centered around 1430 cm^{-1} justify the presence of aliphatic and aromatic bonds (C-H), and the peaks centered around 1060 cm^{-1} correspond to the group C-O, present in alcohols, carboxylic acids, esters and ethers. A study in which the IR spectra of pectin, cellulose, hemicellulose and lignin appear is found in (Arroyo Salas et al, 2008).

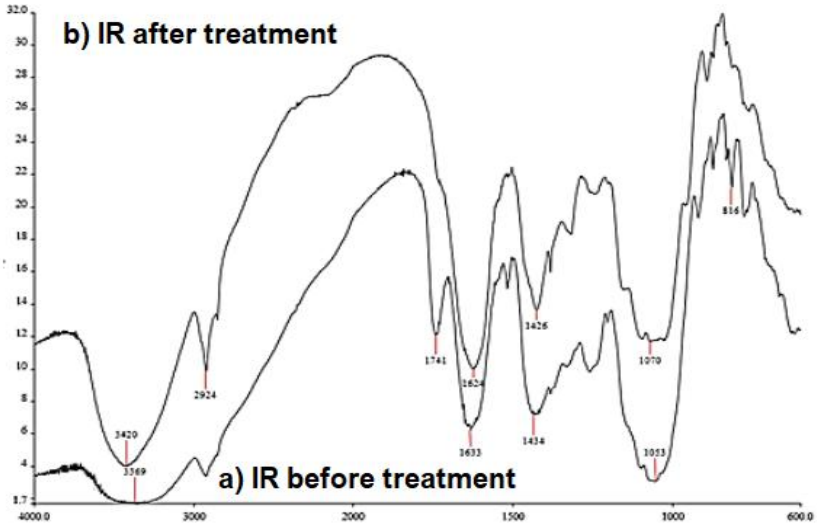

FIGURE 4. a) IR spectrum of orange peel before chemical treatment, b) IR spectrum of the final bioadsorbent

2.4 Increase in Ca(II) in final bioadsorbent

Ca(II) present in samples of orange and lemon peels that had only undergone physical treatment was determined and compared with the Ca(II) content in the final bioadsorbents. The particle size was set at 500-1000 μm and the samples were subjected to a calcination process at 525ºC inside a Hobersal JB 20 muffle for 4 hours.

Different proportions were used in the alkaline attack to check the influence of $Ca(OH)_2$ concentration: 1; 2,5 y 5 g de $Ca(OH)_2$.

The determination of Ca(II) and Mg(II) was made volumetrically, after solubilizing the corresponding ashes. EDTA 0.01M (Ethylenediaminetetraacetic acid) was used as a valuing agent and NET (Eriochrome Black T) was used as an indicator.

First, the total amount of Ca(II) and Mg (II) was determined. In another aliquot, Ca(II) with 0.05M oxalic acid was precipitated and then the complexometric titration was repeated, determining only the Mg(II) content, so that the amount of Ca(II) was quantified as the difference between the total and the amount of Mg(II).

After the alkaline attack, the content of Mg (II) disappeared into the final mass of the resulting bioadsorbents. In contrast, the content of Ca (II) experienced a significant increase. Table 4 shows the increment of Ca (II) as a percentage. These results are consistent with the four-stage adsorption mechanism: migration of metal ions from the solution to the surface of the adsorbent; diffusion through the surface layer of biomass, fixation in the active group and diffusion into the biomass (Horsfall & Abia, 2003).

TABLE 4
PERCENTAGE INCREASE IN Ca(II) IN FINAL BIOADSORBENT

Orange	g $Ca(OH)_2$	Δ Ca(II)
	1	150 %
	2,5	178 %
	5	248 %
Lemon	1	153 %
	2,5	294 %
	5	402 %

2.5 Determining the efficiency of final bioadsorbents

The characteristics of the bioadsorbents obtained were checked with a series of synthetic Cu (II) solutions prepared from CuSO4·5H2O, supplied by the company Panreac (Barcelona).

First, a qualitative trial was conducted to visually determine the degree of bioadsorption of Cu(II). To do this, 0,5 were added; 1 or 2g of the final bioadsorbents obtained with different chemical treatments, at 100mL of synthetic solutions of 500 mg/L of Cu(II) contained in containers that closed tightly.

Bioadsorbents differed in particle size (250-500μm or 500-1000μm), the amount of calcium hydroxide added in the alkaline attack (2,5 or 5g) and the hydration time of the final bioadsorbent (1 or 3 days) before adding the copper solution.

The containers were stirred for 15 minutes in a roller mixer, supplied by the company Selecta (Barcelona). After this time, the bioadsorbents turned green. Then, from each container, a 5 mL aliquot was removed to which 5 mL of a 5% (w/v) solution of KI was added and then 5 mL of dichloromethane. The emergence of a pink coloration of the organic phase confirmed the formation of iodine in the experiences in which the exchange capacity of the bioadsorbent had been exceeded.

In all cases, it was observed that the best results were obtained when the highest amount of bioadsorbent, 2g, was added in the 100mL of synthetic Cu (II) solution and that one day of hydration was sufficient. Visually, neither the particle size of the bioadsorbent nor the amount of $Ca(OH)_2$ added in the alkaline attack did not significantly influence the amount of copper exchanged.

Quantitative determination of copper removal from synthetic Cu(II) solutions was performed using an AA-6300 Shimadzu atomic absorption spectrophotometer. The initial and final particle size of the bioadsorbents was taken into account, using both the 250-500μm and the 500-1000μm.

The final bioadsorbent from the orange peel (OB) was used with a 30 mg/L Cu(II) solution. 25 mL of this solution and 0,5 g of the different versions of (OB) were introduced into test tubes with screw caps. The copper removal results showed an average adsorption of 97.04%±3.83, regardless of the particle size used, both initial and final.

The particle size was set at 500-1000μm and the behavior of (OB) was determined in a wide range of concentrations. For this, it was decided to establish two groups: Cu(II) concentrations up to 250 mg/L and concentrations greater than 250 mg/L. A working concentration of 1.5 g of bioadsorbent/100 mL of Cu(II) solution was established. This amount of bioadsorbent, previously hydrated for 24 hours, was in contact with the corresponding Cu(II) solution inside a hermetically sealed container with stirring for 15 minutes.

In the group of experiences of Cu(II) concentrations less than 250 mg/L, the residual Cu(II) concentration by atomic absorption was determined. In the group of Cu(II) concentrations greater than 250 mg/L, the concentration of non-bioadsorbed Cu(II) was determined by a redox titration, using as a titrating agent a dissolution of sodium thiosulphate 0.1M prepared from $Na_2S_2O_3$-$5H_2O$ supplied by the company Panreac (Barcelona).

The results obtained by atomic absorption are shown in Table 5. It can be seen that, with the exception of the concentration of 5 mg / L, the percentage of bioadsorbed Cu (II) exceeds 90%.

On the other hand, the relationship -γ- between the initial concentration of Cu (II) with respect to the mg/L of Cu (II) bioadsorbed/gram of bioadsorbent, confirms that this cation exchange takes place through an equilibrium process.

TABLE 5
BEHAVIOR OF SOLUTIONS WITH A CONCENTRATION OF Cu(II) < 250 mg/L

$[Cu(II)]_o$ (mg/L) -α-	mg/L Cu(II) bioadsorbeds	% Cu(II) bioadsorbed	mg Cu(II) absolutes	mg Cu(II)/ g bioadsorbent -β-	γ=α/β
5	3,27	65,4	0.5	0.22	22.73
10	9,02	90,2	1	0.60	16.67
25	22,74	91,0	2.5	1.52	16.45
50	47,21	94,4	5	3.15	15.87
100	95,64	95,6	10	6.38	15.67
250	241,86	96,7	25	16.12	15.51

The results obtained volumetrically are shown in Table 6. It can be seen how the percentage of Cu(II) bioadsorbed decreases as the initial concentration of Cu(II) increases, showing a tendency to saturation of the orange bioadsorbent (OB).

TABLE 6
BEHAVIOR OF SOLUTIONS WITH A CONCENTRATION OF Cu(II) >250 mg/L

$[Cu(II)]_o$ (mg/L) -α-	mg/L Cu(II) bioadsorbeds	% Cu(II) bioadsorbed	mg Cu(II) absolutes	mg Cu(II)/ g bioadsorbent -β-	γ=α/β
500	475.0	95.00	50	31.70	15,77
750	562.5	75.00	75	37.50	20,00
1000	657.9	65.79	100	43,86	22,80
1500	810.0	54.00	150	54,00	27,78
2000	1027.8	51.39	200	68,52	29,19
2500	1175.0	47.00	250	78,30	31,93
3000	1200.0	40.00	300	80,00	37,50
4000	1437.9	35.95	400	95,86	41,72

When the final bioadsorbent from the lemon peel (LB) was used, 1.0 g of LB, previously hydrated for 24 hours, was established as a working concentration, which was contacted with 100 mL of the different Cu(II) solutions inside a set of hermetically closed containers. These containers were subjected, during a period of 15 minutes, to a stirring process on the same roller mixer.

From the results of table 4, it was decided to determine the influence of the concentration of Ca(II) on the LB, as well as the particle size. For this, the particle sizes of 250-500 µm and 500-1000 µm were used.

In an analogous way to the procedure with (OB), in experiences with concentrations greater than 250 mg/L, the content of Cu(II) was determined by redox titration, using as a titrating agent a 0.1M sodium thiosulphate solution prepared from $Na_2S_2O_3 \cdot 5H_2O$ and, in the experiences of concentrations below 250 mg/L, the concentration of non-adsorbed Cu(II) was determined by an atomic absorption spectrophotometer AA-6300 Shimadzu. Table 7 details the percentages of bioadsorbed Cu (II), in the series of experiences with concentrations greater than 250 mg/L Cu (II), by the final lemon bioadsorbent (LB). The initial particle size of the bioadsorbent before chemical treatment (LBo), the amount of $Ca(OH)_2$ used in the alkaline attack and the particle size of the final bioadsorbent (LB) were taken into account.

TABLE 7
INFLUENCE OF EXPERIMENTAL CONDITIONS IN OBTAINING (LB) ON THE BIOADSORPTION

[Cu(II)] mg/L	[%] Cu(II) bioadsorbed							
	LB$_o$ 500-1000 µm		LB$_o$ 250-500 µm		LB$_o$ 500-1000 µm		LB$_o$ 250-500 µm	
	Alkaline attack: 1g $Ca(OH)_2$				Alkaline attack: 5g $Ca(OH)_2$			
	µm LB 500-1000	µm LB 250-500	µm LB 500-1000	µm LB 250-500	µm LB 500-1000	µm LB 250-500	µm LB 500-1000	µm LB 250-500
500	30.77	46.15	30.77	46.15	61.54	76.92	61.54	84.62
750	23.53	29.41	23.53	35.29	41.17	52.94	47.06	58.82
1000	17.86	25.00	21.43	28.57	39.28	50.00	46.42	57.14
1500	13.89	16.67	13.89	16.67	33.33	38.89	36.11	41.67
2000	10.42	14.58	12.50	14.58	31.25	37.50	33.33	37.50
3000	5.63	7.04	7.04	9.86	19.72	23.94	23.94	23.94

Percentages of Cu(II) at concentrations greater than 250 mg/L

The influence of $Ca(OH)_2$ concentration and particle size of the initial bioadsorbent (LB_0) and the final bioadsorbent (BL) were revealed. The highest percentages of bioadsorption were determined at high concentrations of $Ca(OH)_2$ and particle size of 250-500 µm of both the initial bioadsorbent (BL_0) and the final bioadsorbent (BL).

Table 8 is analogous to Table 7. It details the results obtained in the experiences carried out with Cu(II) solutions with concentrations below 250 mg/L. Comparison of Cu (II) bioadsorption percentages from both tables shows a significant increase in values at concentrations below 250 mg/L compared to concentrations above 250 mg/L. In fact, the values in Table 7 show a trend towards bioadsorbent saturation.

TABLE 8
INFLUENCE OF EXPERIMENTAL CONDITIONS IN OBTAINING LB ON THE BIOADSORPTION PERCENTAGES OF Cu(II) AT CONCENTRATIONS BELOW 250 mg/L

[Cu(II)] mg/L	[%] Cu(II) bioadsorbed							
	LB$_0$ 500-1000 µm		LB$_0$ 250-500 µm		LB$_0$ 500-1000 µm		LB$_0$ 250-500 µm	
	Alkaline attack: 1g Ca(OH)$_2$				Alkaline attack : 5g Ca(OH)$_2$			
	µm LB 500-1000	µm LB 250-500	µm LB 500-1000	µm LB 250-500	µm LB 500-1000	µm LB 250-500	µm LB 500-1000	µm LB 250-500
5	83.60	85.40	78.00 *	85.40	86.40	89.40	87.60	90.60
10	86.80	90.40	85.20	91.50	92.30	93.40	92.20	93.90
25	78.48*	91.56	85.32	89.88	94.20	95.96	94.80	96.24
50	80.56	90.96	77.86*	85.92	92.44	95.76	95.28	95.94
100	84.24	88.19	83.14	87.79	92.87	95.43	95.61	96.26
250	93.23	93.39	93.23	93.60	93.80	95.45	93.96	96.40

outliers values

With the results of the experience carried out with a particle size of 250-500 µm with both (LB$_0$) and (LB), having used 5 grams of Ca (OH) 2 in the alkaline attack, table 9 was obtained.

TABLE 9
BEHAVIOR OF SOLUTIONS WITH A CONCENTRATION OF Cu(II) >250 mg/L

[Cu(II)]$_o$ (mg/L) -α-	mg/L Cu(II) bioadsorbeds	% Cu(II) bioadsorbed	mg Cu(II) absolutes	mg Cu(II)/ g bioadsorbent -β-	γ=α/β
5	4.53	90.60	0.5	0.45	11.11
10	9.39	93.90	1	0.94	10.64
25	24.06	96.24	2.5	2.41	10.37
50	47.97	95.94	5	4.80	10.42
100	96.26	96.26	10	9.63	10.38
250	241.01	96.40	25	24.10	10.37

Analogously to what was observed in the behavior of (OB), this time the relationship -γ- between the initial concentration of Cu (II) with respect to the mg of Cu (II) bioadsorbed/gram of bioadsorbent, also confirms that this exchange Cationic passes through a process of equilibrium.

Figure 5 shows, jointly, the values of mg of bioadsorbed Cu(II)/gram of bioadsorbent as a function of the initial concentration of Cu (II) in mg/L obtained both for concentrations below and above 250 mg/L.

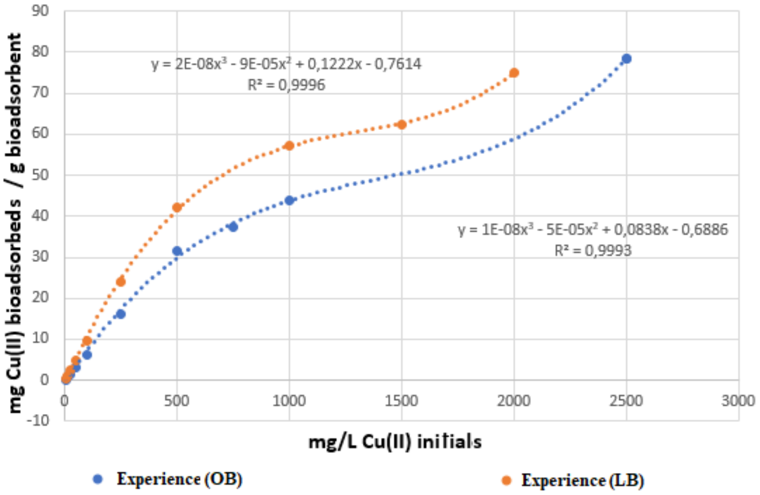

FIGURE 5. mg Cu(II) bioadsorbeds/ gram bioadsorbent vs [Cu(II)] initial (mg/L)

In the case of the lemon bioadsorbent, the values corresponding to the set of experiences carried out with a particle size between 250-500 µm for (LB_0), (LB) and 5 grams of $Ca(OH)_2$ in the alkaline attack have been represented.

In both cases, a polynomial trend line of order 3 is observed that can be adjusted to the BET equation (Brunauer et al, 1938). As a result, it can be admitted that adsorbate covers the adsorbent until a monolayer is formed and the process continues with adsorption in multilayers. However, if only values for concentrations below 250 mg/L of Cu(II) are considered, they conform to the Langmuir equation (Langmuir, 1916). Consequently, it is apparent that for high concentrations of Cu(II) the process of diffusion into the biomass is predominant, while at low concentrations surface fixation predominates (Horsfall & Abia, 2003).

Another parameter considered was the contact time, i.e., the time required to reach the equilibrium point, especially in discontinuous processes (batch). When this value is reached, no further bioadsorption occurs, even if the two phases (adsorbate and bioadsorbent) remain in contact.

In general, bioadsorption processes are rapid, between 15 and 30 minutes a considerable percentage of the cation present in the dissolution has already been eliminated. This fact seems to suggest that the bonding of metal ions with the active sites of the bioadsorbent probably takes place preferably on the surface of the bioadsorbent. (Yus&Mashitah, 2014).

It was confirmed that the period of time initially established, 15 minutes, both for the bioadsorbent obtained from the orange peel (OB) and that obtained from the lemon peel (LB) was sufficient in batch experiences. Using (LB), the bioadsorption percentage of three Cu(II) solutions of 10, 50 and 250 mg/L was compared at 15 and 120 minutes of contact. The results obtained shown in Table 10 correspond to a particle sizeof 250-500µm of both the initial bioadsorbent (LB_o) and the final bioadsorbent (LB).

TABLE 10
INFLUENCE OF STIRRING TIME

	% Cu(II) bioadsorbed	
$[Cu(II)]_0$ mg/L	15’	120’
10	93,90	94,60
50	95,94	96,48
250	96,40	96,50

Obtaining virtually identical results confirmed that a period of contact between adsorbate and 15-minute bioadsorbent is sufficient to establish the corresponding balance in discontinuous processes (batch).

III. DISCUSSION

Conventional adsorbents are those natural or synthetic materials that must be treated to activate, such as charcoal or clays, and that, after use, can be regenerated. Non-conventional adsorbents are alternative materials (biopolymers, plant parts, etc.) that do not necessarily need to be pretreated to activate. However, its activation improves its adsorption capacity (Valladares-Cisneros et al, 2018).

Bioadsorption is a particular type of adsorption process. It consists of the adsorption of chemical species by a biomass (living or dead). In wastewater treatment it is defined as “the ability of biological materials to accumulate heavy metals in wastewater through metabolic mediation or chemical capture means” (Volesky, 2003).

Lignocellulosic biomass obtained from fruit and vegetable waste is considered one of the cheapest sources for bioadsorbent production that plays an important role in the removal of traces of contaminating metals from wastewater (Basso et al, 2002). Citrus waste is one of the most important sources because it is rich in carbohydrates and has a low lignin content (Ververis et al, 2007).

Citrus waste includes shells, pulp and membrane residues, and seeds, which make up approximately 40-60% of the whole fruit. This amount exceeds 110-120 million tonnes per year worldwide (Mahato et al, 2020).

Among the fundamental characteristics that distinguish a good bioadsorbent are taken into account: its high porosity, its large contact surface and the specific adsorption sites (Valladares-Cisneros et al, 2018).

The biomaterials used in these bioadsorption processes act in short contact times and generate high quality effluents by different mechanisms (Sharma et al., 2007). In the presence of heavy metals, the mostly accepted mechanism is ion exchange, especially in divalent metal ions that are exchanged with polysaccharides' own ions present in biomass. Most sorbents contain Na^+, K^+, Ca^{2+} and Mg^{2+} salts in their structures. These cations can be exchanged for the metal ions being attached to the material. (Li et al., 2008). However, bioadsorption mechanisms depend on the metal and sorbent material in each case. However, due to the complex structure of the different bioadsorbents, it is considered that more than one mechanism may appear simultaneously in the bioadsorption of heavy metals (Villaescusa et al., 2004).

The set of physical and/or chemical processes that lead to the selective separation of the polymers that make up biomass are fractionation processes. Polysaccharides, cellulose and hemicellulose are hydrolyzable in acidic media obtaining sugary solutions. Hemicellulose is the most easily attackable polymer, while lignin is little affected by acids and remains as solid residue. Alkaline treatments or hot water treatments under moderate conditions dissolve only hemicellulose. Polysaccharides are difficult to oxidize, while lignin is easily degraded by oxidative methods. (Feng &Guo, 2012).

Citrus peel is mainly composed of cellulose, pectin (galacturonic acid), hemicellulose, lignin, chlorophyll pigments and other low molecular weight compounds, including limonene. These components contain several functional groups, such as carboxyl and hydroxyl, which make citrus peel a potential adsorbent material to remove metal ions from aqueous solutions (Titi& Bello 2015).

The use of citrus peel has been carried out under different conditions, such as:

a) Simply washing it with distilled water to remove the dirt attached, dried in acirculating air furnaceat 100-105ºC for 24 h and, after drying, grinding and sieving through a mesh size of 150-mesh and used as such (Ajmal et al, 2000).
b) Washing with deionized water, dried at 70ºC, ground, sifted and treated with NaOH (0.1M) for 24 hours. After decanting and filtration, the treated biomass was washed with deionized water until the solution reached a pH value of 7.0 (Khormaei et al, 2007).
c) Washing it with deionized water, dried in a convection furnace at 50 ° C for 72 h. It is then ground and sieved to a size of approximately 0.1 to 0.2 mm. The influence of different treatments was compared: washing with isopropyl alcohol, alkaline saponification, attack with citric acid and attack with citric acid followed by alkaline saponification (Li et al, 2007).

Traditional techniques used for solvent extraction of natural products are associated with longer extraction times and lower yields. The effects of ultrasound applied to liquid media are associated with the phenomenon of cavitation, which consists in the formation, growth and implosion of nano/microbubbles of gas within the liquid as a result of pressure fluctuations generated by the passage of ultrasonic waves (Adewuyi , 2005; Virot et al., 2010; Shirsath et al., 2012).

The use of ultrasound can be a very useful process for the cleaning treatment of impurities in citrus peels, since ultrasound waves can destroy bonds that hold compounds such as sugars, oils or low molecular weight polymers (Romero de Ávila et al., 2006).

Its use helps to achieve a final product with a degree of consistency useful to be used in both batch and continuous applications. In the latter case, the particle size must be taken into account, resulting in the 500-1000 µm being more effective than the between 250-500 µm (García Raurich et al, 2020).

IV. CONCLUSION

The incorporation of ultrasonic radiation into the chemical treatment allows pectins to be extracted more quickly than by conventional heat treatment.

The inherent physicochemical properties of bioadsorbents significantly influence their adsorption capacity before being chemically treated.

After chemical treatment, the behavior of the bioadsorbent obtained from the lemon peel (LB) is determined by the particle size and by the concentration of $Ca(OH)_2$ used in the alkaline attack, contrary to what was observed in the bioadsorbent obtained of the orange peel (OB).

In batch applications, both the 250-500 µm and 500-1000 µm particle sizes can be used. The choice of particle size is affected by the nature of the adsorbate (metal ion, organic molecule).

In continuous applications, the particle size 500-1000 µm has a better response, since it presents a degree of compaction that allows the homogeneous advance of the eluent at atmospheric pressure.

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