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ИСПОЛЬЗОВАНИЕ ПЕКТИНМЕТИЛЭСТЕРАЗЫ И ЩЕЛОЧНОГО ГИДРОЛИЗА ДЛЯ МОДИФИКАЦИИ ОСТАТКОВ ФРУКТА МАРАКУЙЯ ПРИ УДАЛЕНИИ ИОНОВ МЫШЬЯКА

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Нерастворимая фракция отходов при производстве сока маракуйи является важным источником волокон, которые можно использовать для получения с хорошим экономическим эффектом новых материалов. Показано, что при проведении простых химических и ферментативных модификаций отходов кожуры маракуйи и волокон можно получить материал для эффективного удаления ионов мышьяка. Остатки кожуры маракуйи содержат биополимеры, например, сплетенный пектин, которые были модифицированы химически или ферментативно с помощью пектинметилэстеразы. Этот фермент разрывает эфирные связи при метильных группах пектина нерастворимой фракции, что приводит к образованию деметоксированных пектинов. Материалы, полученные при гидролизе двумя методами (щелочной и ферментативный гидролиз), обладали примерно одинаковой адсорбционной способностью. Волокна, гидролизованные химическим методом, лучше адсорбировали ионы железа (III).

INTRODUCTION

Arsenic is classified as one of the most toxic and carcinogenic chemical element constituent, thereby posing epidemiological problems to human health [1]. Arsenic pollution has been seriously observed not only in various mineral and chemical processes but also in some ground water and/ or hot spring water over large areas in Laguna region of Coahuila State in Mexico, as well as in other countries such as Bangladesh, West Bengal in India, Inner Mongolia in China and Japan [2]. European Commission has recently revised the maximum concentration limit (MCL) for arsenic in drinking water, which has been reduced to 0.010 mg/L and by 2002 all drinking water supplies must comply with this new limit [3]. In Mexico this limit still has not been adopted, and the value of 0.050 mg/L is accepted.

Most common arsenic species found in aqueous media are anionic species of arsenate (As V) and arsenite (As III). Arsenate is the thermodynamically stable form of inorganic species and it generally predominates in surface waters. Arsenite is favored under reducing conditions, such as in anaerobic ground waters. Arsenite is far more toxic in biological systems than arsenate. Organic arsenic species occur in natural waters commonly due to organo-arsenical pesticides use or the biomethylation mechanisms of microorganisms. The toxicity of organo-arsenicals is generally lower than that of inorganic arsenic species [4]. The demand for effective and inexpensive adsorbents is

on the increase in response to the widespread recognition of the deleterious health effects of arsenic exposure through drinking water.

To date, arsenic has been removed by means of the different methods: co-precipitation with ferric sulfate, lime softening, alum precipitation and precipitation as the sulfide using either sodium sulfide or hydrogen sulfide. Other treatment techniques for arsenic removal are reverse osmosis, ion-exchange, flotation and adsorption on iron oxides or activated alumina [5–7]. These methods have been reported to be effective mainly for the removal of pentavalent arsenic. Therefore, a pre-oxidation step is usually required, in order to achieve efficient removal trivalent As. Although, among these methods, the iron coprecipitation method has been reported to be the most successful in lowering the arsenic content to drinking water standard level, it still suffers from a post treatment problem regarding the alkaline sludge produced after the treatment [8]. In view of this, an alternative adsorption gel prepared from cheap residues of maracuya juice has been explored in the present work. Effective use of fruit or plant wastes has become one of the promising fields of investigation due to the availability of the raw materials at almost no cost as well as due to their environmentally friendly nature [9], which can help to resolve the problem of sludge post treatment.

To reach this goal, we prepared an adsorption gel from fiber of maracuya juice residue by means of simple

chemical modification with hydrogen peroxide followed by alkaline or enzymatic hydrolysis, addition of iron (III) and examination of arsenic adsorption using the sample of ground water from Laguna region, Mexico. As enzyme the pectin-methyl esterase was applied. This enzyme breaks the ester bonds of methyl ester groups in the pectin resulting in demethoxylated pectins. A similar adsorption gel was also prepared from maracuya juice residue by means of the same treatment so as to compare its adsorption behavior for arsenic with that prepared from the maracuya fiber and in order to validate the application of the latter adsorption gel. The adsorption of arsenic by maracuya juice residue and maracuya fiber without iron (III) and hydrolysis treatment was also carried out.

MATERIALS AND METHODS

Samples of crude maracuya waste were provided by the maracuya juice factory at Puebla (Mexico). Applied reagents were the analytical grade and were provided from Jalmek (Mexico). Iethylthiocabamate silver salt was purchased from Sigma Chemical Company (USA) and Zinc from J. T. Baker (USA). Citric pectin-methyl esterase was kindly provided by Coyote Foods Inc. (Mexico).

Maracuya fiber obtaining

Maracuya fiber (MF) was obtained from crude fruit waste according to the method described previously [10]. Passion fruit shells were cut and washed with ethanol/acetone (1:1) to inactivate the natural enzymes that are present in the natural material. One part of the washed maracuya waste was used in the next assay, hereafter abbreviated as MW.

The other part of washed shells was treated by steam in an autoclave for 20 minutes. The remainders were washed with distilled water twice in a ratio of 1:2 (w/v). The soluble pectin was eliminated in this step. The solid material was dehydrated first by ethanol (2x) and then by acetone (1x). The obtained dehydrated material (MF) was powdered and packed in plastic containers.

Maracuya waste (MW) and fiber (MF) treatments

Before the hydrolysis, the shells treated in the previous step and obtained fiber were washed with distilled water (6 L per 20 g of material) to eliminate the low molecular weight compounds. The humid samples were treated twice with 300 ml of 3% hydrogen peroxide for 8 h followed by the vigorous washing with distilled water and finally obtained oxidized MW (OMW) and oxidized MF (OMF).

The alkaline hydrolysis of obtained materials (OMF and OMW) was carried out by addition of 15 ml of 1 M NaOH to each g of material and the mixture was incubated at room temperature for 24 h in batch reac-

tors with agitation of 100 rpm. After hydrolysis, the materials were washed with distilled water to neutral pH. The names of obtained materials were abbreviated as CHOMW (chemically hydrolyzed, oxidized maracuya waste) and CHOMF (chemically hydrolyzed, oxidized maracuya fiber). The process was stopped by vacuum filtration when there was no change in pH, and followed by washing with ethanol/acetone (1:1) mixture. The amount of initial and hydrolyzed H^+ exchangeable groups was calculated by titration in the presence of phenolphthalein [9].

The enzymatic hydrolysis was carried out at 40°C under similar agitation condition. The reactors contained 19 ml of distilled water at pH 7 and 0.5 ml of enzyme solution for each g of material (shells or fiber treated as described previously). The enzyme addition was performed only after 3 h of incubation of materials in water after verifying that the pH was stable at 7, in order to avoid unspecific pH changes. After enzyme addition, the pH was monitored and manually adjusted to 7.0 by using 0.02 M NaOH solution. The process was stopped by vacuum filtration when there was no change in pH, and followed by washing with ethanol/acetone (1:1) mixture. The quantity of hydrolyzed groups was calculated from the values of alkali used for maintaining the pH. The names of obtained materials were hereafter abbreviated as EHOMW (enzymatically hydrolyzed, oxidized maracuya waste) and EHOMF (enzymatically hydrolyzed, oxidized maracuya fiber). The enzyme activity was measured previously by means of monitoring the kinetics of the proton liberation during pectin hydrolysis process and pH was adjusted to 7.0 with 0.02 N NaOH [11]. One activity unit (U) was defined as the amount of enzyme that catalyzed the formation of 1 mmol of H^+ in 1 min under the experimental conditions. The activity was 2.49 ± 0.29 U/ml.

Adsorption of iron (III)

In this assay the waste and fiber treated with peroxide and chemically or enzymatically hydrolyzed (OMW and OMF, CHOMW and CHOMF, EHOMW and EHOMF) were used. The adsorption of iron (III) was carried out in batch process for 24 h, using initial concentration of Fe (III) at 0.3 M and the sample of maracuya gels at 0.1 g/ml for EHOMW and EHOMF, as well as for OMW and OMF, and 0.7 g/ml CHOMW and CHOMF. The time of assay was chosen based on the data reported previously by Ghimire et al. [9]. The gels obtained after treatment were washed with the distilled water three times by using on the same volume as was applied for a treatment. The initial and final concentrations of iron as well as the Fe (III) in water obtained after washing were determined by gravimetric method based on iron hydroxide precipitation by means of 10% NH_4OH solution [12].

Analytical method for arsenic detection

Arsenic detection in groundwater samples of Laguna region of Coahuila State (Mexico) before and after treatments with the test materials was carried out spectrophotometrically, according to a technique described in Official Mexican Norm as NOM-AA-46-1981 [13]. This method is based on application of diethyldithiocarbamate silver salt in pyridine for detection of AsH₃ formed during the reduction process of the arsenic species present on water. The detection limit of this method, according the calibration plots obtained at 525 nm by Párez Moreno et al., was 0.01 mg/ml [14]. The calibration plot was described by linear trend, characterized by equation $y = 16,236x + 0,0284$ ($R^2 = 0,982$).

Procedure of the As adsorption test

Adsorption tests were carried out under batch conditions. In the batch test, the defined quantity of dried material (0.1, 0.5, 1, or 5 g) was placed in a flasks with 100 ml aqueous sample containing the As ions. In the case of assays performed with iron (III)-loading and unloading materials 0.1 g and 1 g of samples per 100 ml of water were applied.

The pH of the water samples from Laguna region (Mexico) was adjusted to pH 3 by addition of small amounts of HCl, and the assay was also carried out without addition of acid. The original pH of groundwater sample was 8.4. The flasks were shaken vigorously in a shaker at room temperature for 24 h. The pH was measured before and after incubation by means of CON-UCTRONIC PC 45 pH meter. The As concentrations before and after adsorption as well as on solutions obtained after double washing of absorbent materials with 30 ml of distilled water, were measured spectrophotometrically by means of NOM-AA-46-1981 method [13–14].

RESULTS AND DISCUSSION

Maracuya waste (MW) and fiber (MF) treatments

MW contains cellulose, pectins, hemicellulose, yellow pigment and other low molecular weight compounds

[10]. Previous studies demonstrated that the maracuya shells contained 30–35% of pectins, and the 16% of them is insoluble in water. The maracuya fiber (MF) is free of soluble pectins, as they are removed during the fiber preparation. However, the pigments had some solubility in water, and they were removed by hydrogen peroxide treatment of shells (MW) and MF and thereby eliminated the coloration as well as the specific maracuya scent in the studied materials. It also was considered that peroxide treatment also helped to increase the quantity of oxidized groups that can bind with Fe (III) ions.

The active binding sites for metals are supposed to be the functional carboxylic groups of the pectins. We demonstrated previously that maracuya pectins are highly methoxyl polysaccharides; up to 80% of carboxylic groups are present as methyl-ester [10]. The content of carboxylic groups in the original MW was considered not to be sufficient to strongly load enough Fe (III) to adsorb arsenic.

The pectin content on OMW and OMF was demethoxylated in order to convert its abundant methylester (–OCOCH₃) groups into carboxylic groups with an affinity for ferric iron to enhance the loading capacity for iron.

The hydrolysis was carried out by means of chemical alkaline treatment and enzymatically in the presence of citric pectin-methyl esterase. The results (Table 1) demonstrated that alkaline hydrolysis increased the H⁺-exchangeable groups by 1.75 and 8 fold in OMW and OMF respectively. The greater quantity of hydrolyzed groups in OMF was due to pretreatment of MW, which eliminated considerable part of soluble material and concentrated the fiber polymeric fraction. Hence, the same weight of the MF contained higher polysaccharide content than the one in MW. The interpretation of results obtained by this chemical process was difficult due to the low specificity of the chemical hydrolysis. Many groups presented in the maracuya shell or fiber may be hydrolyzed by means of alkali and the residual concentration of was detected by titration and considered as

Table 1

Quantification of hydrolyzed groups based on the change of alkalinity after chemical hydrolysis measured by titration

Sample/process	Amount of initial H ⁺ exchange groups, mol	Amount of final H ⁺ exchange group, mol	Concentration of hydrolyzed groups per g of material, mmol/g
OMW/alkaline hydrolysis	0.0002	0.00035	6.8
OMF/alkaline hydrolysis	0.0002	0.0016	8.0

the H^+ -exchangeable groups. The amount of exchangeable cations for OMW and OMF was 6.8 and 8.0 mmol/g respectively and was significantly higher than the values reported for cellulose material and orange waste, which were 1.02 and 1.72 mmol/g respectively [9].

In the case of enzymatic process, alkali was used to neutralize the proton produced during the enzymatic reaction, which was specifically related to the increase in the content of free carboxylic groups (Table 2). To calculate the data presented in the Table 2, we assumed that one hydroxyl participated in the hydrolysis of one group of polysaccharides. The concentration of enzymatically hydrolyzed groups was considerably lower than one quantified after the chemical hydrolysis. However, these data provided real information about the amount of $-COOH$ groups. Higher concentration of carboxyl group was detected on OMW. It may be due to the reason that some part of pectin is dissolved [10] during the pretreatment of MW performed to obtain the MF and the OMF was purer in pectin than OMW.

In both cases, the evidence of the hydrolysis was obtained and the amounts as well as concentrations of hydrolyzed groups were calculated (Tables 1 and 2). It was observed that the greater quantity of hydrolyzed groups per g of applied materials was obtained with chemical hydrolysis.

Adsorption of iron (III)

The hydrolyzed and un-hydrolyzed samples were further loaded with iron (III) in order to create a suitable chelating environment for arsenic removal. Table 3 showed the adsorption of Fe (III) on the different test materials used in this study. The balance of Fe after removal from initial solution was obtained by considering the presence of iron in solutions obtained after washing. Although in all cases the red gels (iron color) were obtained after the treatment, the low sensitivity of gravimetric measurements did not permit the quantification of adsorbed Fe (III) for the un-hydrolyzed or enzymatically hydrolyzed samples.

The initial pH of iron solution was 3. However, after the adsorption process the pH was decreased (Table 3). As mentioned previously, iron (III) was considered to be adsorbed on the carboxyl group of the materials according to a cation exchange mechanism. This was further supported by the fact that the pH of the aqueous iron solution had been decreased after adsorption, which could be attributed to the release of protons during the adsorption process. At the same time, it is well known that the chemical species of iron in the weakly acidic conditions involved in the adsorption of the hydrolyzed form of iron (III) [15].

The Table 3 shows that the adsorption of iron (III) was quantified only for chemically hydrolyzed samples (CHOMW and CHOMF), which was characterized by the presence of higher concentration of cation exchangeable groups. The results also correlated with their concentrations (Table 2): higher concentration and Fe (III) adsorption was detected on fiber sample (CHOMF) than on shell sample (CHOMW). The higher value of iron (III) uptake capacity by the chemically hydrolyzed samples might be due to the synergistic effect of additional carboxyl functionalities present within the polymer matrix which in turn could accommodate more metal ions.

The loading capacity for iron (III) was lower than one reported for cellulose material and orange waste, which were 1.21 mmol/g and 0.96 mmol/g respectively [9].

After determination of the maximum loading capacity of the corresponding materials, they were dried in order to investigate their adsorption capacity for As from groundwater sample.

As adsorption test

Removal tests of arsenic with the iron (III)-loaded and unloaded materials were carried out in batch conditions. The water sample from Laguna region, Coahuila, Mexico containing 0.032 mg/ml of As at pH 8.4 was applied. The results on the adsorption of arsenic with unmodified maracuya waste (MW) and maracuya fiber (MF) are given in Table 4. ue to the detection limit

Table 2

Quantification of hydrolyzed $-COOCH_3$ groups in enzymatic hydrolysis, based on evaluation of NaOH (0.02 M) applied to maintain pH 7

Sample/process	Volume of 0.02 M NaOH applied to maintain pH, ml	Quantity of OH^- groups, mol	Concentration of hydrolyzed groups per g of material, $\mu\text{mol/g}$
OMW/enzymatic hydrolysis	5.5	0.00011	55
OMF/enzymatic hydrolysis	13	0.00026	26

Table 3

Iron (III) adsorption* on different test materials

Test material	Final pH	Adsorbed Fe (III), mmol per g of material
OMW	0.948	ND- not detectable
OMF	1.108	ND
CHOMW	1.0	0.52 +/- 0.37
CHOMF	1.0	1.04 +/- 0.73
EHOMW	1.263	ND
EHOMF	1.117	ND

*Initial pH of iron solution was 1.51; ND: Not detectable.

of As (0.01 mg/ml), only the initial concentration of arsenic and after removal was determined (Table 4). It was observed that the arsenic removal was favored under acid condition for both MW and MF materials, however, the MF showed some removal capability (approximately at 20% of initial As concentration) even at pH 8.4 and only in the presence of 0.05 g/ml of MF. The best removal results as per the limit established by European Commission were obtained using 0.05 g/ml of both materials at pH 3.

It was observed that treatment with MF and MW increased the pH to approximately 7–8 from pH 3 (Table 4). This could be considered as an evidence for the presence of the ion-exchange process that play an important role in the removal of arsenic [7].

The results obtained on the As removal using the iron (III)-loaded and unloaded, enzymatically and chemically hydrolyzed materials of shell or fiber previously oxidized with hydrogen peroxide, are presented in Table 5 and Table 6. The concentration of applied iron (III)-loaded materials were 0.001 g/ml, i.e. 1 g/l, while the unloaded materials were used at 0.01 g/ml, i.e. 10 g/l. It was at 50 and 5 fold less than the concentration of MW and MF required for the removal of As in the previous assays. Tables 5 and 6 demonstrated that arsenic removal has been achieved to an undetectable level (for the technique employed in this study) for all the assays carried out in this study. It can be concluded that the iron (III) loaded and unloaded materials obtained by chemical and enzymatic modification of maracuya fiber and maracuya shell waste can be used for the removal of arsenic from contaminated wastewater.

Arsenic ions have been considered to be adsorbed on chemically and enzymatically modified materials by the mechanism of ligand exchange. The ligands involved in such an exchange process may be hydroxyl ions or neutral water molecules existing in the iron (III) coordination sphere. These assumptions can be supported by

Table 4

Removal of arsenic from the water samples of Laguna region at pH 3 and 8.4 by untreated maracuya materials applied at different concentration

Sample	Applied concentration, g/ml	Measured parameters			
		Initial pH	Final pH	Initial [As], mg/L	Final [As], mg/l
MW	0.001, 0.005 0.01, 0.05	8.4	8.3	0.032+/-0.01	0.031+/-0.01
MW	0.001, 0.005, 0.01	3	6.81	0.032+/-0.01	0.028+/-0.01
MW	0.05	3	7.11	0.032+/-0.01	< 0.01
MF	0.001, 0.005, 0.01	8.4	8.4	0.032+/-0.01	0.030+/-0.01
MF	0.05	8.4	8.3	0.032+/-0.01	0.024+/-0.01
MF	0.001, 0.005, 0.01	3	7.00	0.032+/-0.01	0.029+/-0.01
MF	0.05	3.0	8.33	0.032+/-0.01	< 0.01

Table 5

Removal of arsenic from the water samples of Laguna region at pH 3 and 8.4 by iron (III)-loaded and unloaded maracuya waste and fiber treated enzymatically

Sample	Applied concentration, g/ml	Measured parameters			
		Initial pH	Final pH	Initial [As], mg/l	Final [As], mg/l
Iron (III)-loaded EHOMW	0.001	8.4	8.0	0.032+/-0.001	< 0.01
Iron (III)-loaded EHOMW	0.001	3	4.3	0.032+/-0.001	< 0.01
EHOMW	0.01	8.4	7.96	0.032+/-0.01	< 0.01
EHOMW	0.01	3	3.43	0.032+/-0.01	< 0.01
Iron (III)-loaded EHOMF	0.001	8.4	7.3	0.032+/-0.001	< 0.01
Iron (III)-loaded EHOMF	0.001	3	2.97	0.032+/-0.001	< 0.01
EHOMF	0.01	8.4	3.43	0.032+/-0.01	< 0.01
EHOMF	0.01	3.0	2.89	0.032+/-0.01	< 0.01

Table 6

Removal of arsenic from the water samples of Laguna region at pH 3 and 8.4 by iron (III)-loaded and unloaded maracuya waste and fiber treated chemically

Sample	Applied concentration, g/ml	Measured parameters			
		Initial pH	Final PH	Initial [As], mg/l	Final [As], mg/l
Iron (III)-loaded CHOMW	0.001	8.4	7.33	0.032+/-0.001	< 0.01
Iron (III)-loaded CHOMW	0.001	3	3.17	0.032+/-0.001	< 0.01
CHOMW	0.01	8.4	9.81	0.032+/-0.01	< 0.01
CHOMW	0.01	3	7.11	0.032+/-0.01	< 0.01
Iron (III)-loaded CHOMF	0.001	8.4	4.53	0.032+/-0.001	< 0.01
Iron (III)-loaded CHOMF	0.001	3	3.15	0.032+/-0.001	< 0.01
CHOMF	0.01	8.4	10.15	0.032+/-0.01	< 0.01
CHOMF	0.01	3.0	7.39	0.032+/-0.01	< 0.01

the fact that the pH of the solution increased or there was no change after arsenic adsorption in the present study. However, the increase in pH demonstrated the presence of ion-exchange processes.

The results of the present study demonstrated that effective materials for arsenic removal can be produced by making some simple essential chemical or enzymatical modifications of maracuya shell waste and fiber. The adsorption capacity was comparable for materials obtained by both hydrolysis techniques (alkaline and enzymatic hydrolysis). The fiber materials hydrolyzed chemically was superior for iron (III) adsorption.

The materials used in the present work as potential As sequestrers have a very low production cost compared

to the synthetic chelating resin, which is commonly applied. Their application does not require the use of oxidizing procedures, which is usual in traditional methods. The presence of some additional ions in the water did not strongly affect the arsenic removal. Since the materials used are environmental friendly, the residues after treatment can be easily burnt in an incineration plant, which is not possible with the use of synthetic chelating resins made of plastic. There will not be any post-treatment problem as encountered in conventional treatment processes. Hence, the biopolymer materials of maracuya juice residue could be used for the removal of arsenic species from aqueous medium.

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APPLICATION OF PECTIN-METHYL-LESTERASE AND ALKALINE HYDROLYSIS FOR THE MODIFICATION OF MARACUYA FRUIT RESIDUE TO SEQUESTER ARSENIC IONS

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The insoluble fraction of wastes from the maracuya juice industry is an important source of fiber, which can be used to obtain new materials with added economic value. The present study demonstrated that effective materials for arsenic removal have been produced by making some simple essential chemical or enzymatical modifications of maracuya shell waste and fiber. The residues of the shell of maracuya contain biopolymers (for example, interlaced pectin) that were modified chemically and enzymatically by means of the pectin-methyl esterase. This enzyme breaks the ester bonds of methyl ester groups in the pectin of the insoluble fraction resulting in demethoxylated pectins. The adsorption capacity was comparable for materials obtained by both hydrolysis techniques (alkaline and enzymatic hydrolysis). The chemically hydrolyzed fiber materials were superior on the adsorption of iron (III).