Immobilizing Different Carbon Sources in Alginate Beads for Melanoidins Removal from Yeast Effluents

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Abstract: Presently work describes a new method for melanoidins removal encountered in yeast industry effluents. Three different kinds of carbon sources were inmobilized in alginate beads and include rubber tire pyrolysis, activated carbon and multiwalled nanotubes. The yeast effluent was obtained through aerobic fermentation with 40 g/L of molasses. The effluent was separated through filtration. The alginate beads consisted in 3 g of alginate and 4 g of the carbon sources, which were dissolved in one liter of distilled water. The last was added drop by drop into a solution of $CaCl_2$ (15 g/L). The alginate beads were used in different proportions (w/v) in the effluent (1:6, 1:3.5, 1:2.6 and 1:1). The melanoidins amount adsorbed was determined through a spectrophotometer UV vis (600 nm). At 1:1, the concentration of melanoidins at the equilibrium (qe) for rubber pyrolysis was 3.5 mg/g, for the activated carbon was 5.0 mg/g, for multiwalled nanotubes qe was 5.3 mg/g and when the alginate beads probed alone qe was only 1.5 mg/g. In order to predict the adsorption capacity in a continuous stirred tank we assessed the saturation constant (Ks) in the batch treatments. The continuous fermenter was simulated from 0.01 to 1.0 h^{-1} dilution rates. At the lowest proportion (w/v) 1:6 the maximum sorbtion capacity was at 0.427 g/L.h obtained with the rubber pyrolysis at 0.26 h^{-1}. When we used a proportions (w/v) of 1:3.5, 1:2.6 and 1:1, the maximum adsorption capacity were 0.77, 1.04 and 1.13 g/L.h, respectively and these values were obtained with multiwalled nanotubes.

Keywords: Carbon Sources, Alginate Beads, Melanoidins, Removal, Continuous Treatment, Batch Treatment, Simulations

1. Introduction

The brewing industry produces great amount of polluted effluents that are not easy to remove, mainly to the presence of melanoidins compounds, which are part of the organic matter. These can be assessed by determination of the chemical oxygen demand (COD). Furthermore, COD must be considered the biochemical oxygen demand (BOD) and sulfate concentration [1]. Production of brewing yeast from molasses results in high values of DQO and DBO and these can reach values from 50 to 100 g/L [2] and high nitrogen concentrations, around 1.5 g/L [3]. These effluents are characterized by the pH values from 4 to 5, have a strong

odor and black color [4].

The black color is a characteristic factor of the presence of melanoidins pigments. These organic molecules are composed mainly of high molecular weight polymers that are formed at high temperatures through Maillard reactions and a low water activity. These pigments avoid the penetration of light in the biological wastewater treatment, making the process less efficient, therefore are able to resist the biodegradation [5]. As a result, the removal of these melanoidins pigments is a priority to the effective treatment of effluents of the brewing industry [2]. In spite of the numerous methods reported for the wastewater treatment, the yeast effluent still deals for effective treatment due to the high costs and low encouraging results when their feasibility is performed. Furthermore, the effluent treatment is hard mainly due their volumes and melanoidins concentrations.

Today, the wastewater effluents of the yeast industry are treated by biological systems which consist of several anaerobic and aerobic steps. The biological wastewater treatment is a conventional process and this is effective for the removal of chemical demand of oxygen. However, the black color remains in the water effluents and this is due mainly to the pigment re-polymerization [2]. Therefore, there are other methods reported in the literature for the effluent yeast treatments: oxidation process, electrochemical, coagulation, flocculation, membrane process, adsorption methods or Fenton. But unfortunately, still far from solving the problem due to the effluent volume to treat.

In this work, we propose the use of alginate, this co polymer is composed of mannuronic and guluronic acids, which is used Food, Pharmaceutical industries, mainly the sodium alginate. Many of these applications of the alginate are based on their capacity to form gels through the join of cations, mainly in the guluronic acid [6]. Furthermore, the alginate is an excellent polymer that has been used widely in the waste water systems. The aim purpose of this current research is the design of alginate beads for the removal of melanoidins, this new method consists of alginate beads where activated charcoal, rubber pyrolysis and multiwalled nanotubes were immobilized. The alginate beads designed were proposed by the ability of the sodium alginate to interact with calcium, these interactions cause the formation of "egg box" and this model predict their ability for englobe substances into their vicinity. Therefore, the immobilization technique has the ability to adsorb melanoidins from the yeast effluents.

2. Methodology

2.1. Yeast Fermentation

In order to obtain yeast effluents, aerobic fermentations were performed. For this we used the strain *Sacharomyces cerevisiae* (commercial strain). The medium culture compositions consisted (g/L): molasses (40), ammonium sulfate (6), KH_2PO_4 (1.2), $(NH_4)_2HPO_4$ (1.2), ferrum sulfate (3.0), $MgSO_4.7H_2O$ (0.3), $MnSO_4.4H_2O$ (0.03). The

fermentations were performed at 37°C. In order to separate the yeast, we filtered with a Watman filter and the effluent was used, subsequently for removing the melanoidins. The inoculums were carried out in an orbital shaker (Thermo Scientific, Mod Max Q) through 30 hours at 150 rpm, 500 mL of nominal volume with 200 mL of working volume. The batch fermentations were performed in a stirred tank fermenter with at an aeration rate of 0.8 VVMs, agitation speed of 30 rpm, pH 6.8 and 37°C. In order to separate the yeast, we filtered with a Watman filter and the effluent was used in order to remove the melanoidins.

2.2. Carbon Sources

Black carbon was obtained from scrap tires pyrolysis, the tires were crushed and placed in a pyrolysis tank of 3.0 kg capacity treated at 500°C for 20 minutes [7]. We also used a commercial active charcoal and multi-walled carbon nanotubes "length 6-9 nm and width 5 μm" (Sigma, USA).

2.3. Carbon Sources Treatment

The carbon sources were washed with deionized water in test tubes for about 10 minutes. The carbons left to settle and dry at room temperature.

2.4. Design of the Alginate Beads

For this we used two solutions: a) we used 3.0 g of sodium alginate (Food grade) from marine brown algae and was dissolved in a 1.0 liter of distilled water. The mixing was dissolved at 37°C in a stirred plate. Later, was dissolved 4.0 g of the carbon sources. b) The second solution contains 15 g of $CaCl_2$ dissolved in a 1.0 liter of distilled water. In order to immobilize the carbon sources, solution a) was taken with a pipette and this left drop by drop into the solution b). Finally, the alginate beads were filtered and were ready to use in order to remove melanoidins obtained from the yeast fermentation.

2.5. Melanoidins Removal Treatments

We used 25 ml of effluent obtained from yeast fermentation and 5, 10, 15 and 25 ml (1:6, 1:3.5, 1:2.6, 1:1 w/v) of alginate beads was used. The removal treatments were performed in a shake flask of 250 ml agitated in an orbital shake (Thermolab Scientific, USA) and agitated at 150 rpm through 6 hrs at room temperature (around 18°C).

2.6. Melanoidins Concentration Determination

The measures of color removal were performed in a spectrophotometer UV vis, the wavelength used was 600 nm. The choice of wavelength was due at the highest light amount adsorption of the melanoidins [8].

$$q = \frac{(C_o - C_f)xV}{m} \qquad (1)$$

Where m is the fresh weight of the alginate beads (g), Co is the initial concentration of melanoidins and C_f is the final

concentration. The percentage of removing melanoidins in solution was calculated as previously reported by Liakos and Lazaridis [8].

$$R = \frac{(C_o - C_t) x 100}{Co} \qquad (2)$$

2.7. Mathematical Modeling for Melanoidins Removal

In a batch mode, for estimate the color disappearance (sorption) we used the next expression.

$$V \frac{d(qt)}{dt} = qtV \qquad (3)$$

The specific melanoidins sorption rate (qt) was estimated as follow [9]:

$$qt = \frac{k_2 * (q_e^2 t)}{1 + (k_2 q_e) t} \qquad (4)$$

Where, q_e: adsorption amount (g/g) at equilibrium; qt: adsorption amount (g/g) at time t (min); k_2: rate constant of pseudo-second order (g/g min).

The slope and intercept of the plot t/qt Vs t were used to calculated values of q_e and K_2.

In continuous single stage removal we used the constant obtained in shake flasks treatment as follow:

$$D = \frac{F}{V} \qquad (5)$$

The amount of melanoidins in the effluent depends on the alginate beads added to a continuous fermenter.

$$[Mel] = \frac{DKs}{Ads_{max} - D} \qquad (6)$$

The rate of melanoidins adsorbed (qt) by the alginate beads was estimated as follow:

$$qt = \left[[Mel_{in}] - \frac{DKs}{Ads_{max} - D} \right] * qe \qquad (7)$$

We assumed that the effuent (Mel_{in}) contains an homogenous distribution of alginated beads at different proportions (w/v) that consider the adsorption amount (g/g) of melanoidins at equilibrium (q_e). Ads_{max} is the maximum velocity of adsorption (h^{-1}).

3. Results and Discussion

3.1. Experimental Melanoidins Removal in Shake Flasks

The first part of this work was the design of the immobilization system for activated carbon, rubber pyrolysis and multiwalled nanotubes in order to remove melanoidins. Furthermore, the treatment trials include a control group that consists only of alginate beads. The alginate beads are commonly used in Biotechnology in order to inmobilize enzymes and cells. The alginate beads had an average diameter of 3.75 mm (Figure 1).
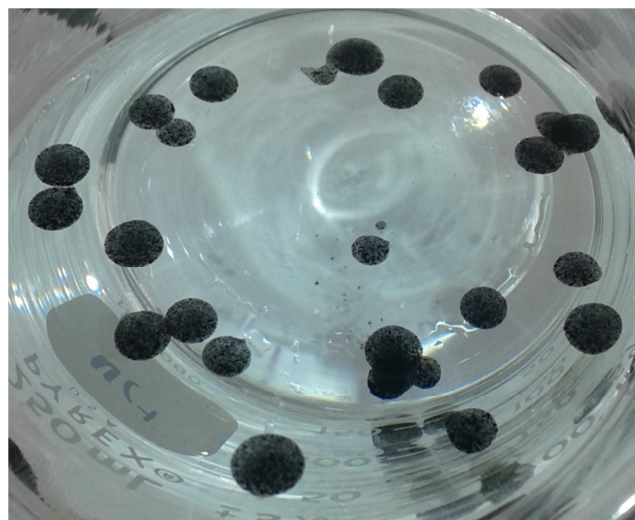

Figure 1. *Immobilization of active charcoal into alginate beads.*

Once the immobilization was achieved for the several sources of carbon into the alginate beads, we proceeded to the sorption experiments of melanoidins in a mode batch, using several relationships weight/volumen (alginate beads/effluent):1:6, 1:3.5, 1:2.6 and 1:1. The experiments were performed in shake flask environments and the Figure 2 shows the effectiveness of sorbtion of melanoidins after about 6 hours of treatment. The sorbtion capacity was dependent on the carbon source immobilized, for example, when we used only alginate beads at 1:6 (w/v) the color intensity was reduced around 5%, while with the rubber pyrolysis, activated carbon and multiwalled nanotubes; the reduction percentage color was reduced 20, 21 and 21.5%, respectively.

As increased the relationship w/v in the treatments, the color reduction was evident (Figure 2). The best results of the melanoidins removal were obtained when the relationship w/v was higher (1:1) where the rubber pyrolysis reduced the color at values by 36%, the activated carbon at 47% and multiwalled nanotubes at 47.5%. The Figure 3 shows the results with the activated carbon, multiwalled nanotubes and rubber pyrolysis in 1:3.5, 1:2.6 and 1:1 w/v.
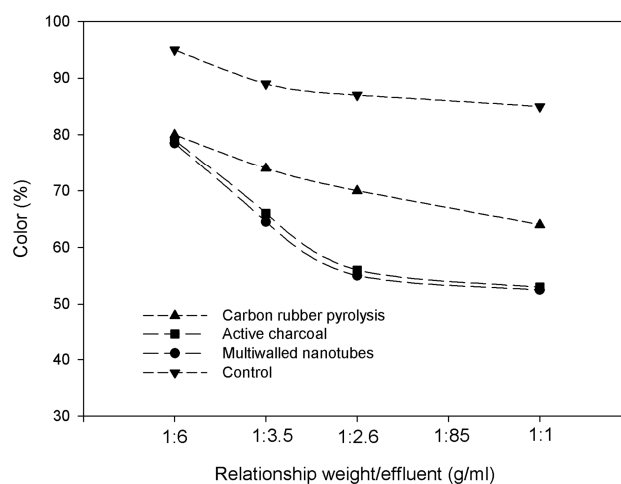

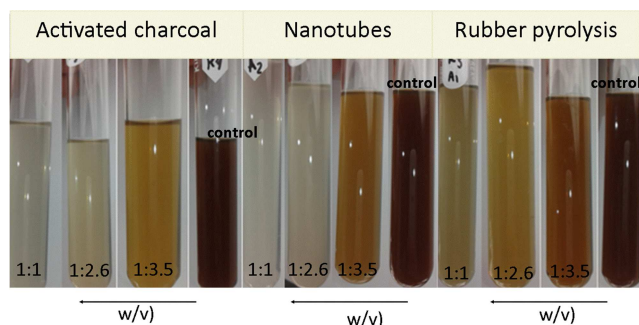**Figure 2.** *Melanoidins removal by different immobilized carbon sources.*

Figure 3. Melanoidins removal treatments: a) activated charcoal, b) multiwalled nanotubes and rubber pyrolysis (After of two cycles of batch removal).

3.2. Batch Treatment Modeling Removal

The experimental determination of melanoidins sorbtion was performed in the shake flasks. The amount of melanoidins adsorbed was dependent on the carbon source immobilized and the amount of dye adsorbed (q_t) is dependent on the time. The profile sorption was a pseudo-second order behavior where the experimental values of q_t were very near to the obtained by the modeling in each treatment (Figure 4 and Figure 5). The amount of melanoidins adsorbed by the different carbon sources was dependent of the relationship w/v in each treatment. For example, when the w/v was lower (1:6), the maximum mount of melanoidins adsorbed was around 2.03 mg/g, but when the relationship w/v was increased (1:2.6), the amount of dye removes from the effluent was around 3.7 mg/g in this result was obtained with the multiwalled nanotubes (Figure 5). The maximum capacity of melanoidins removal was obtained at w/v 1: 1 (Figure 5) and the amount of melanoidins adsorbed was around 5.3 mg/g.

The Table 1 shows the effectiveness of melanoidins removal reported by some authors and these values is compared with the data obtained by us. For example, Ojijo et al [10] used fly ash coal and the authors reported values of qe of 179 mg/g, whereas Figaro et al [11] obtained values of qe of 232 mg/g and finally Liakos and Lizaridis [8] reported the highest values when the activated carbon was used for melanoidins removal (qe of 5607.9 mg/g).

The sorption constant of pseudo second order (k_2) was higher when the activated carbon was used. The values of qe for this study were relatively low (qe = 5.3 mg/g) when the multiwalled nanotubes were used (1:1 w/v). The capacity of absorption of melanoidins of the activated carbon could be due to the large sorption area in the alginate matrix, and the model egg box could explain better this capacity and this is caused by the presence of guluronic acid when interacts with calcium ions [6].
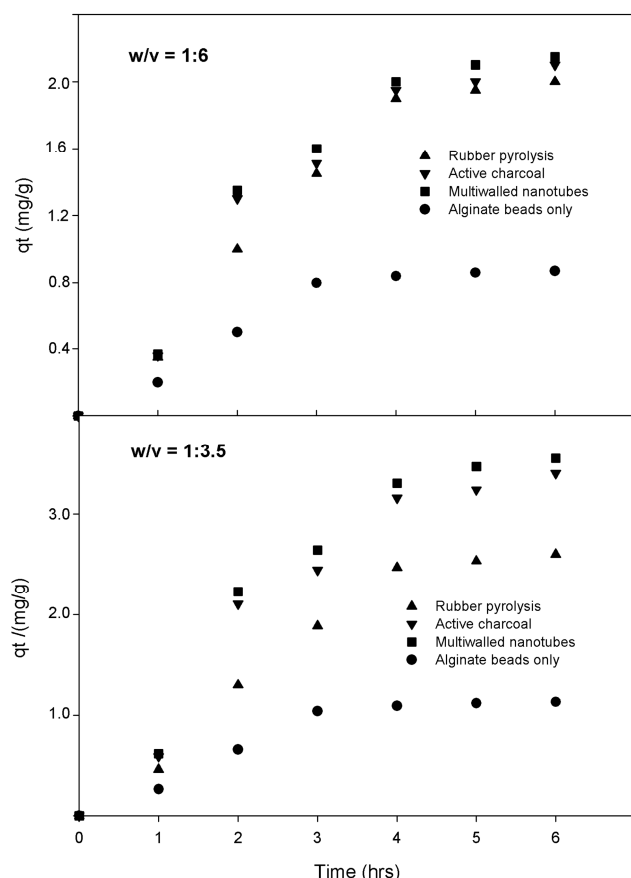

Figure 4. Melanoidins sorption in batch mode sorption: Relationship 1:6 and 1:3.5 (w/v).

Table 1. *Kinetic constant parameters of the pseudo-second order model report for de adsorption melanoidins.*

	qe (mg/g)	k_2 (g/mg/min)
Fly ash-clay [10]	170	9.29×10^{-5}
Activated carbón [11]	232	5.25×10^{-6}
Activated charcoal [8]	5607.9	1.835×10^{-4}
Alginate beads 1:1 (w/v)	1.5	1.835×10^{-6}
Pyrolysis 1:1 (w/v)	3.5	3.5×10^{-5}
Active charcoal 1:1 (w/v)	5.0	2.0×10^{-5}
Nanotubes 1:1 (w/v)	5.3	1.0×10^{-5}

3.3. Modeling Continuos Mode Operation

The wastewater treatment normally operated in a continuous mode mainly due at their high performance in the pollutant removal and includes biological or chemical wastewater treatment. This kind of mode operation is widely used for the production of metabolites of biotechnological importance and for the removal of pollutants when microorganisms are used [12]. In order to predict the removal capacity of melanoidins when the different carbon sources immobilized in alginate beads are used we simulated the continuous mode operation of the fermenter in order to predict the melanoidins adsorption based in the in the following assumption:
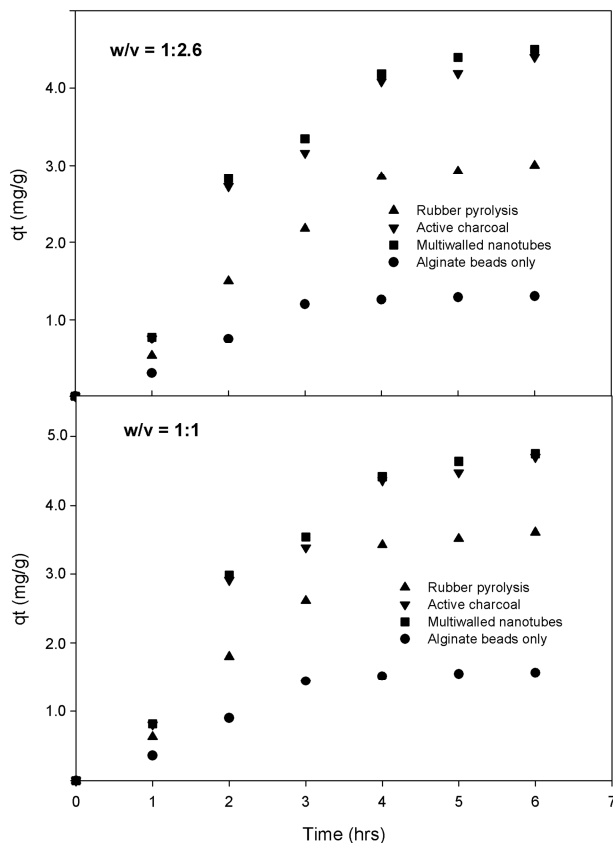
Figure 5. Melanoidins sorption in batch mode sorption: Relationship 1:2.6 and 1:1 (w/v).
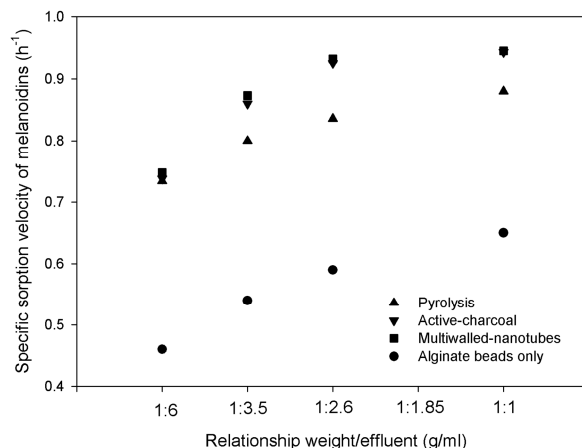

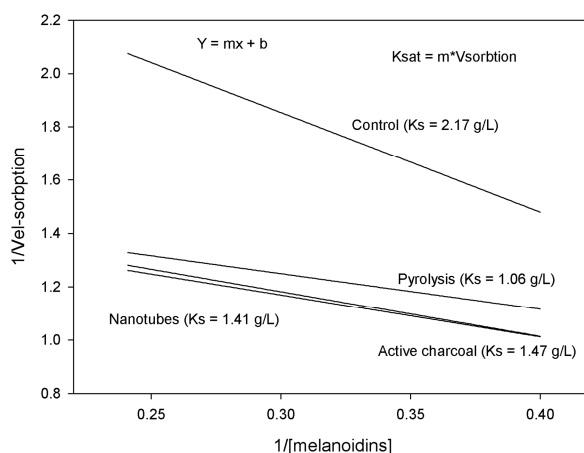*Figure 6. Estimation of the specific adsorbtion velocity of melanoidins at different alginate beads concentrations.*

Figure 7. Determination of the saturation constant of melanoidins in different designs of the alginate beads.

Due to the dynamic sorption of melanoidins from the effluent and their accumulation in the alginate beads this can be simulated because of their similarity with the biomass accumulation. The first step was the determination of the melanoidins rate sorption in each treatment used (Figure 6). The higher adsorption velocities were obtained with the multiwalled nanotubes and activated carbon, whilst the melanoidins sorption was lower when the alginate beads are exposed to the effluent without any immobilized carbon source. One of the main considerations of the continuous melanoidins removal is the determination of the value the saturation constant of the melanoidins into the alginate beads and this value is determinate when plotting 1/v Vs 1/M (M means melanoidins and could be considered as the substrate).

The Figure 7 shows that the value of Ks of the alginate beads without an immobilized carbon was 2.17 g/L, when the rubber pyrolysis is immobilized the Ks is around 1.06 g/L, for activated carbon and multiwalled nanotubes the Ks values were 1.17 and 1.41 g/L, respectively. The simulations of the melanoidins adsorption by the different kind of alginate beads were obtained at dilutions from 0.01 to 1.0 h^{-1}). The Figure 8 shows the results of simulations performed in a continuous fermenter for activated carbon immobilized in the alginate beads. When the relationship was 1:6 w/v, the sorption capacity of melanoidins was 0.367 g/L.h and these values was achieved at 0.22 h^{-1}, when the relationship w/v were increased at 1:3.6 the sorption capacity reached 0.70 g/L.h when the dilution was around 0.26 h^{-1}.

When the amount of alginate beads (w/v) was increased in the fermenter, the sorption capacity was higher, for example, at 1: 2.6 w/v, the sorption capacity was 0.98 g/L.h at 0.28 h^{-1}. Finally, when the higher relationship of p/v was used, the sorption capacity increased at 1.08 g/L.h reached at 1.29 h^{-1}. The complete results of the sorption capacity are shown in the Table 2 for the different immobilized carbon sources obtained for continuous fermenter at dilutions from 0.01 to 0.1 h^{-1}.

But if the objective is achieved lower concentrations of melanoidins in the effluent outlet of the fermenter operated in a continuous way is necessary operated the tank at lower dilutions it means values from 0.01 to 0.2 h^{-1} (Figure 8). Another way to reduce the melanoidins concentration in the effluent is the use of a second tank. The Figures 9 and 10 shows the predicted sorption of the melanoidins by the alginate beads when the different carbon source is used in a continuous fermenter. Our simulations showed the next results: at lower dilutions the concentration of melanoidins was reduced in the effluent, when the proportion w/v (alginate beads/effluent) is relatively low, the melanoidins concentration in the effluent is higher, when multiwalled nanotubes and activated carbon were used, the sorption capacity of the melanoidins were enhanced.
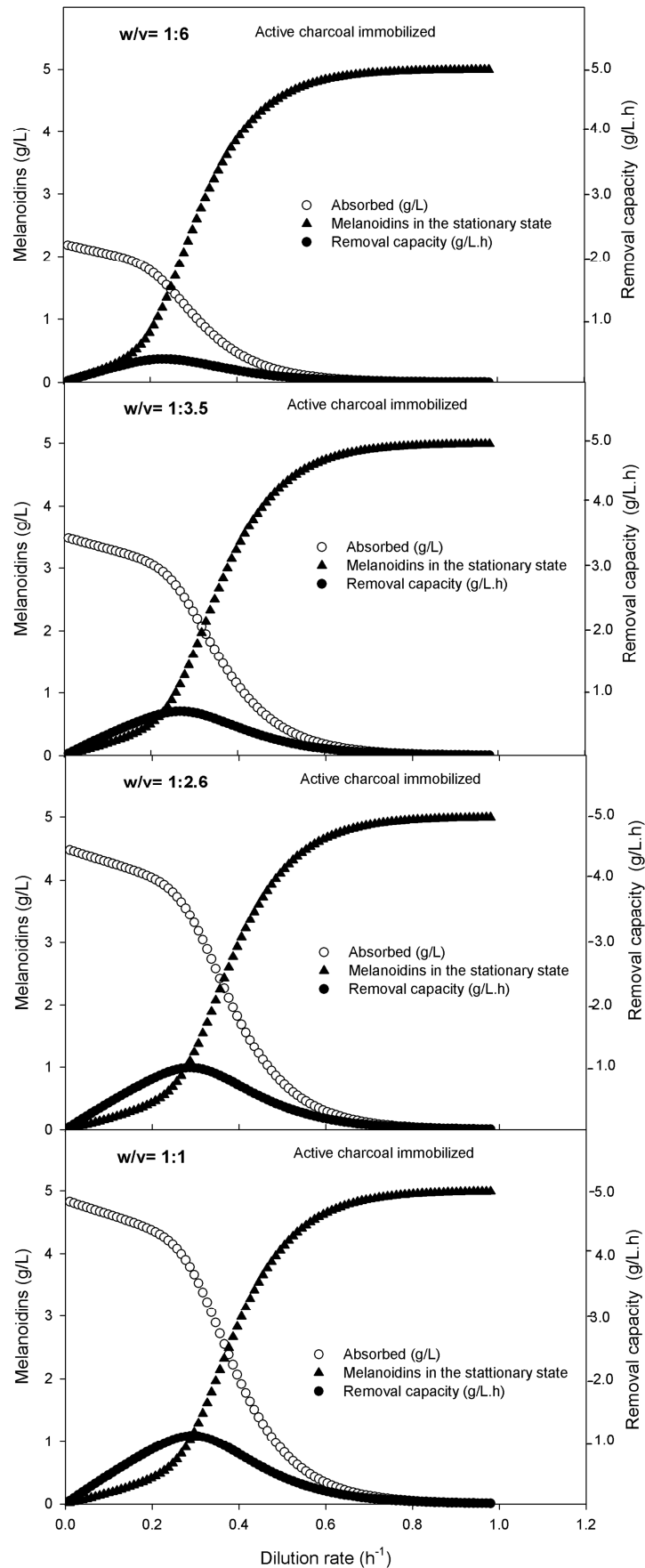
Figure 8. *Modeling sorbtion of melanoidins by active charcoal immobilized in alginate beads in a continuous mode operation.*

Table 2. Capacity of melanoidins sorption in contonuous fermenter operated in a continous mode.

Treatment	Sorption capacity (g/L.h)	Dilution (h⁻¹)
Control 1:6 (w/v)	0.03	0.18
Pyrolysis 1:6 (w/v)	0.42	0.26
Active carbón 1:6 (w/v)	0.36	0.22
Nanotubes 1:6 (w/v)	0.39	0.23
Control 1:3.5 (w/v)	0.06	0.18
Pyrolysis 1:3.5 (w/v)	0.60	0.28
Active carbon 1:3.5 (w/v)	0.70	0.26
Nanotubes 1:3.5 (w/v)	0.77	0.27
Control 1:2.6 (w/v)	0.08	0.19
Pyrolysis 2:2.6 (w/v)	0.73	0.30
Active carbón 1:2.6 (w/v)	0.98	0.28
Nanotubes 1:2.6 (w/v)	1.04	0.29
Control 1:1 (w/v)	0.11	0.21
Pyrolysis 1:1 (w/v)	0.93	0.31
Active carbón 1:1 (w/v)	1.08	0.29
Nanotubes 1:1 (w/v)	1.13	0.21
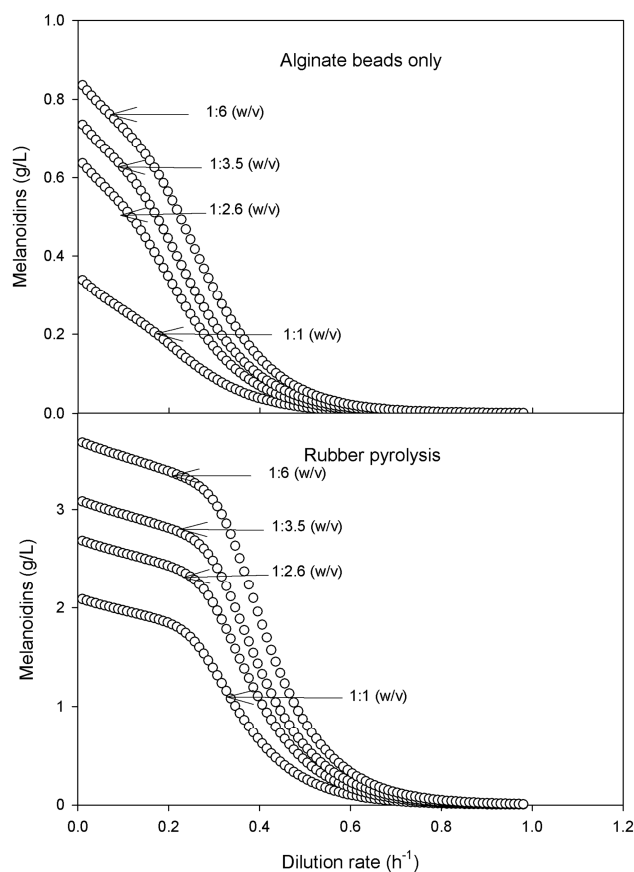
Figure 9. *Sorbtion of melanoidins by alginated beads and rubber pyrolisis inmobilized in alginated beads.*

Although there are several methods reported in the literature in order to remove or degrade melanoidins from the wastewater of the yeast industry, the absorption decolourization technique have the advantage of low cost. Furthermore, the melanoidins adsorbed by materials have proved the removal effectiveness of melanoidins, for example, the modified zeolite with surfactant demonstrated a high capacity of melanoidins removal [13]. In this context, the use of African coal fly ash was used as a removal melanoidins, the maximum removal capacity was around 53 mg/g (melanoidins/coal) [10]. Activated carbon is a well known adsorbent due to its extended surface area, microporous structure, high adsorption capacity and a high degree of reactivity [14]. The activated carbon almost removes completely the melanoidins that are present in the yeast effluents [8] and can be used is a successive cycle of adsorption-desorption of these water pollutants. The exact mechanism of melanoidins adsorption by the activated carbon was revealed by infrared spectroscopy [8] where the electrostatic interactions between the charged groups of the sorbent (rings of the activated carbon) and the sorbate (aromatic rings of the melanoidins) are the main forces involved in the color removal of the color of the yeast effluents. In contrast to these reports where the activated carbon was used alone and the porous characteristics were used for retaining the melanoidins, the experimental outfits reported a sorption time of 20 minutes, followed by a plateau phase and in this work we reported almost 6 hours for both (Figure 9). These differences in sorption rates could be to the diffusion of the melanoidins through the alginate matrix of the beads and maybe by the average diameter used and these problems could be reduced by the reduction of bead diameter (Figure 10).

The relevance of the technology presented here is based on the use of relatively low cost materials, such as the rubber pyrolysis and their effectiveness of melanoidins removal when is compared with the multiwalled nanotubes and the activated carbon. The sorption techniques are considered to be the most effective and proven technology [15], the main advantage of the use of the several carbon sources is their ability of desorption and the alginate beads in order to remove melanoidins can be recycled when will be used in the batch melanoidins removal of the waste water treatment or when used a mode continuous operation mode where the removal capacity could be increased. The main difference with the technologies previously reported and the technology reported here is the requirement of hydrodynamic conditions for the melanoidins removal. For example, the use of biofilms to absorb these pollutants required mixing when a stirred fermenter is used. In our case, the shake flask melanoidins removal required at least a Reynolds number of 1,200. Therefore, the hydrodynamics and mixing should be taken into account for the design a wastewater treatment and these hydrodynamic could be generated by the use of impellers of by diffuser bubbles in stirred tank fermenter or in a bubble column, respectively.

Finally, although the waste water technology designed and reported in this work was proven only in a lab conditions, without a temperature control or pH fit at the beginning of melanoidins removal has the potential to be applied of scale up in a real effluents and this was deduced by the simulations and modeling in a batch waste water process or in a continuous process.
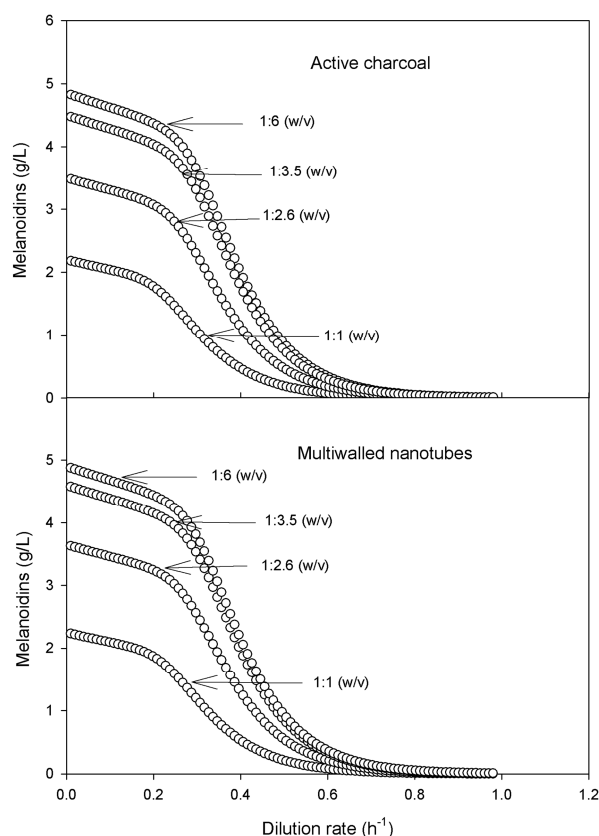
Figure 10. *Sorbtion of melanoidins by multiwalled nanotubes and active charcoal inmobilized in alginated beads.*

4. Conclusion

Our experimental results showed us the affectivity of melanoidins adsorption by the alginate beads designed. Although the removal capacity is low when is compared with activated carbon or fly ash-clay. Better result could be abtained if the amount of immobilized carbon is increased. The carbon nanotubes have shown the maximum removal capacity in probes performed in shake trials. The second part of the experiment were the modelling in batch mode and continuous cultures for simulate the melanoidins remotion capacity of the beads designed for at several concentrations of carbon sources, where the carbon nanotubes got the maximum removal capacity. Therefore, the results of this study could be used as a nascent technology in order to tackle this problem.

Acknowledgements

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