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RESIDUAL BIOMASS OF *Lactobacillus* IMMOBILIZED IN ALGINATE FOR ORANGE 16 DYE RETENTION FROM AQUEOUS MEDIUM

BY

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Abstract. The biosorption abilities of residual *Lactobacillus* biomass immobilized in alginate towards reactive organic dyes have been studied. Batch biosorption studies were carried out for the retention of reactive Orange 16 dye. Studies have shown that the biosorption process is influenced by the size and amount of biosorbent, dye solution pH, initial dye concentration, temperature and phases contact time. Preliminary results have suggested that the studied residual biomass immobilized in sodium alginate may be an effective biosorbent for retaining dyes from dye-containing wastewater.

Keywords: biosorption; immobilized biomass; reactive Orange 16 dye; wastewater.

1. Introduction

Out of the desire and the need to find cheap, ecological and, of course, very efficient treatment for wastewater, the research still considers “adsorption” as a process variant that allows the most interesting adaptations (Rashid *et al.*,

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2021; Yousef *et al.*, 2020; Zaharia and Suteu, 2012). A major advantage of this method, after the fact that it is relatively cheap and easy to make it practically, is the fact that it allows the use of a very large and diversified number of materials with adsorptive properties (Elgarahy *et al.*, 2021; Crini *et al.*, 2019; Zaharia and Suteu, 2012).

Among many materials with adsorbent properties, whether chemical synthetized (ion exchangers, inorganic or organic composite materials, zeolites) or natural (algae, vegetable waste, food industry or agricultural waste), research is conducted concerning microbial biosorbents obtained by processing the residual biomass resulted in various food processes or pharmaceutical biosynthesis (Blaga *et al.*, 2021, 2020; Elgarahy *et al.*, 2021; Agarwal *et al.*, 2020; Crini *et al.*, 2019; Zaharia and Suteu, 2013; Suteu *et al.*, 2012). The processing of this biomass aimed at preserving or improving the adsorbent properties in parallel with improving the handling system of these materials.

Improving the performance of the biosorption process is also possible by using a new type of biosorption material, resulting from immobilization (adsorption, covalent bonding, crosslinking, clinging, encapsulation) microorganisms or residual microbial biomass. Several active or inert microorganisms have demonstrated relative high biosorption properties compared to clasical materials with adsorptive capacities (polymeric ion exchanger, activated carbon, other polymeric materials) (Blaga *et al.*, 2021; Ayele *et al.*, 2021; Yaashikaa *et al.*, 2021; Todorova *et al.*, 2019; Suteu *et al.*, 2012, 2013). Nevertheless, their widespread use is hindered by some physical problems, which can be solved by using preparation techniques that allow obtaining particles with much improved physico-chemical stability (higher mechanical strength). In this direction, the research was oriented towards immobilization (through entrapment and encapsulation) in different support polymeric materials that improved properties, but especially ease in handling and carrying out the biosorption process (Blaga *et al.*, 2020; Blaga *et al.*, 2021). Some aspects need to be considered when using an immobilization technique, such as the need for additional costs, diffusion of the contaminant through the suport which increases time and decreases process efficiency and also decreases biosorbent capacity due to the interaction between suport and biosorbent active sites (Blaga *et al.*, 2020).

The aim of this paper is to study the effectiveness of the residual biomass of *Lactobacillus* immobilized in sodium alginate, material with biosorptive properties in removal Orange 16 - an reactive anionic dyes from aqueous medium. Batch biosorption experiments were carried out in order to establish the influence of solution pH, biosorbent dose and size, initial dye concentration and temperature on the biosorption of dye onto *Lactobacillus sp.* immobilized in sodium alginate.

2. Experimental Methodology

2.1. Materials

Biosorbent. Lactic acid bacteria are industrially important microorganisms used in production of fermented vegetable products, wine and dairy industry, comprising spherical (cocci) or rod-shaped (bacilli), gram-positive bacteria, with the following characteristics: catalase-negative, immobile, non-sporulating and anaerobic (Capozzi *et al*., 2021).

The residual biomass from lactic acid production (*Lactobacillus plantarum* and *Lactobacillus casei*) used in the experiment, was separated at the end of the biosynthetic process by centrifugation at 8000 rpm and dried at 80°C (to increase its sorption properties) and immobilized by cell inclusion into sodium alginate. For the immobilization, 1% sodium alginate solution, prepared in distilled water at 70°C, was mixed with the residual biomass 5% dry matter concentration and dripped after complete homogenization into 1% calcium chloride (prepared in distilled water at 5°C), through different capillaries, thus obtaining spherical beads with Φ1 = 0.5 mm, Φ2 = 1.0 mm and Φ3 =1.5 mm diameter.

Fig. 1 – Residual *Lactobacilus* biomass immobilized in sodium alginate.

Adsorbate. A reactive dye, such as Orange 16 (O16- C.I. 18097) (MW = 617.54 g/mol, λ_{max} = 495 nm) with chemical structure showed in Fig. 2, was selected as model of chemical pollutant in the aqueous medium for this study. It was prepared a stock solution (with stock concentration of 724 mg dye/L) using a commercial salty form of the dye with analytical reagent grade, and distilled water. Working solutions were prepared starting from the stock solution by appropriate dilution with distilled water.

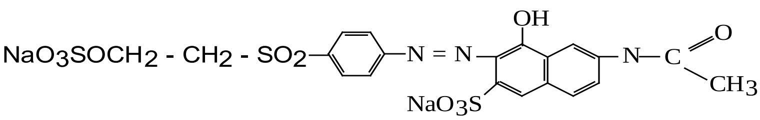


Fig. 2 – Reactive Orange 16 dye (C.I. 18097).

All other the chemicals required in the experimental part were of analytical purity and were used without further purification.

2.2. Batch Biosorption Equilibrium Studies

The batch biosorption studies were performed using 50 mL Erlenmeyer flask in which it was introduced, as a function of diameter size of particles, different g of biosorbent in contact each one with 25 mL of dye solution with different initial dye concentrations (in range of 28.96 – 231.68 mg/L) and pH values adjusted with 1N HCl solution, at a constant desired temperature (in a thermostatic bath). The experiments were performed at three different temperatures (5°, 20°, 40°C) with a contact time of phases about 24 hours. After reaching the equilibrium times, the dye content in supernatant was determined spectrophotometrically using a JK-VS-721N VIS Spectrophotometer at maximum dye wavelength of 495 nm (the dye was adsorbed onto the residual immobilized biomass as in Fig. 3 which illustrates the colored immobilized biosorbent granules after biosorption and separation from aqueous solution).

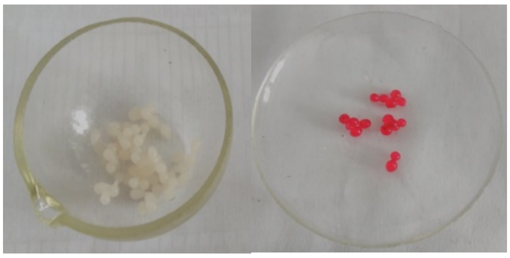


Fig. 3 – Immobilized residual biomass, before and after dye biosorption.

The biosorption capacity of the biosorbent was estimated using the amount of biosorbed dye (q, mg of dye/g of biosorbent) calculated as follows:

$$q = \frac{C_0 - C}{G} \cdot V \tag{1}$$

and the percent of dye removal, R%:

$$R = \frac{C_0 - C}{C_0} \cdot 100 \tag{2}$$

where C_0 and C are the initial and equilibrium (residual) concentration of dye in aqueous solution (mg/L), G is the amount of biosorbent (dry matter (dw) from alginate granule) (g) and V is the volume of solution (L).

3. Results and Discussions

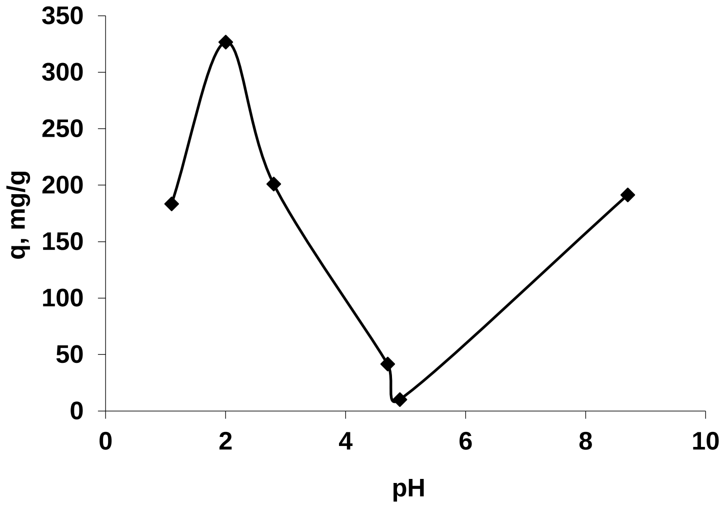
The evaluation of biosorptive capacity of the selected immobilized residual biomass was initially done by studying the influence of different operating factors on the biosorption balance, such as: pH, temperature, biosorbent dose, initial dye concentration, biosorption time, biosorbent particle size. The variables/operating factors studied and their limits of variation are presented in Table 3.

Table 3

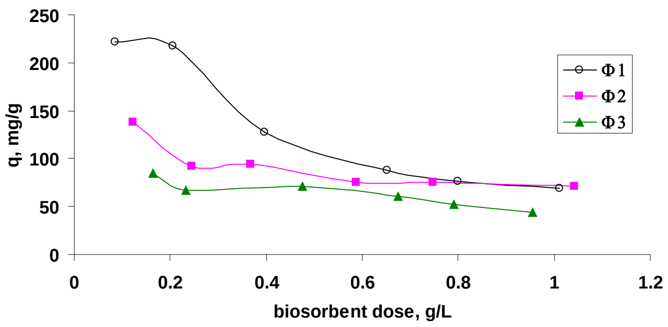
Physical-Chemical Parameters that Influence the Biosorption of Orange 16 Dye onto Biosorbent Based on Residual Immobilized Lactobacillus in Sodium Alginate

Parameters	Studied limits of variation
pH	1-9
T, °C	5, 20 and 40
t, min	0-1440
Biosorbent dose (g) or g/L, with g of active dry matter / L	0.05, 0.065 and 0.085 g of biosorbent (2.0, 2.6 and 3.4 g/L) with 4.728 % dry matter (dw)
Biosorbent particle diameter, mm	Φ1 = 0.5; Φ2 = 1.0; Φ2 = 1.5
Initial dye concentration in solution, mg/L	28.96 – 231.68

The obtained results were systematized in Figs. 4(*a*-*f*).

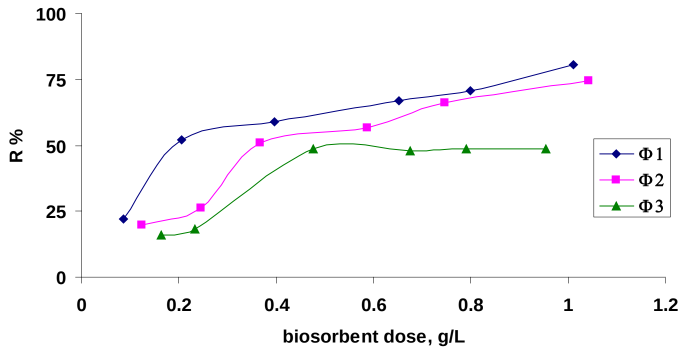


(*a*) for Φ1



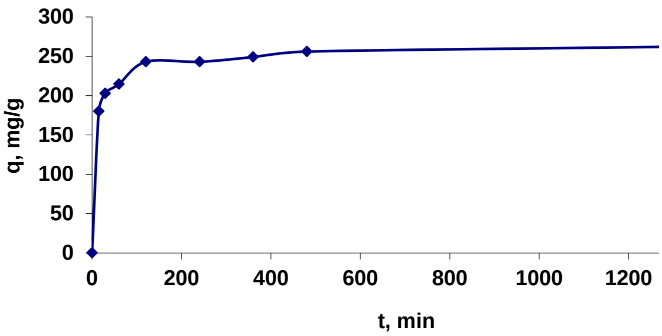
250
200
150
100
50
0
q, mg/g
0
0.2
0.4
0.6
0.8
1
1.2
biosorbent dose, g/L
Φ1
Φ2
Φ3

(*b*)



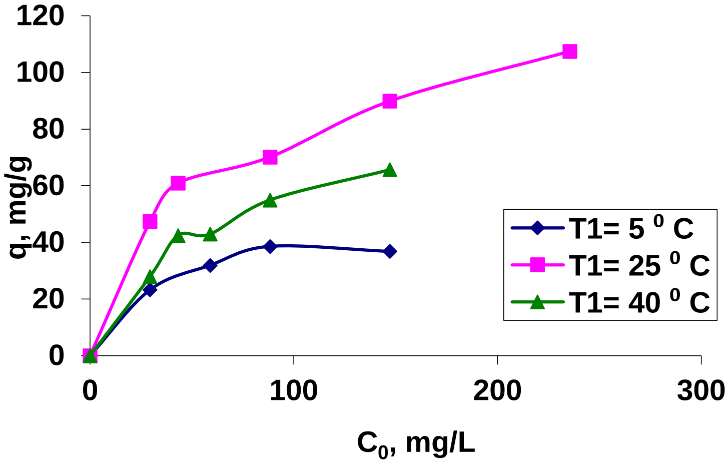
100
75
50
25
0
R %
0
0.2
0.4
0.6
0.8
1
1.2
biosorbent dose, g/L
Φ1
Φ2
Φ3

(*c*)

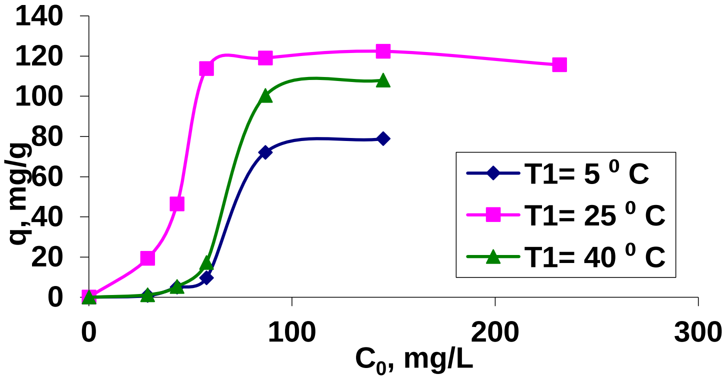


300
250
200
150
100
50
0
q, mg/g
0
200
400
600
800
1000
1200
t, min

(*d*) for Φ1



(*e*) for Φ3



(*f*) for Φ2

Fig. 4 – Operating factors influencing the biosorption of reactive Orange 16 dye onto residual biomass of *Lactobacillus* immobilized in sodium alginate (biosorption capacity or biosorption efficiency): (*a*) pH influence (size Φ1 = 0.5 mm); (*b-c*) biosorbent dose and diameter size; (*d*) contact time between phases Φ1 = 0.5 mm; (*e*) initial dye concentration and temperature for Φ3 = 1.5 mm; (*f*) initial dye concentration and temperature for Φ2 = 1.0 mm.

The analysis of the experimental data provided by Figs. 4(*a-f*) leads to the following conclusions:

• Biosorption process is dependent of the pH value (Fig. 4*a*) of the aqueous Orange 16 dye solution, because their value is important for the charge of the biosorbent surface (by the ionic form of the functional groups) and the ionic form in which the dye is present in the aqueous solution (dissociated sulphonic groups). Thus, a maximum biosorption capacity is observed at the pH value of a strongly acidic media (pH 2.0).

• Figure 4*b* shows a decrease of the biosorption capacity as the amount of biosorbent increases for all three biosorbent particle sizes. The highest value of the biosorption capacity for the reactive Orange dye was obtained in the case of biosorbent particles with a diameter of Φ1, of 221.77 mg/g, and the lowest in the case of biosorbent particles with a diameter of Φ3, respectively 43.63 mg/g. Also, it can be seen in Fig. 3*c* that the percentage of dye removal, R%, increases with the increasing of the biosorbent doses, due to higher number of available biosorption sites. The highest retention percentage for reactive Orange 16 dye in residual immobilized biomass was obtained in the case of particles of diameter Φ1, of 80.76%, and the lowest in the case of particles of diameter Φ3, respectively 16.11%.

• From Figs. 4(*b*-*c*) it can also observed that the maximum and minimum values reached by the biosorption capacity, respectively the percent of dye removed depends on the size of the biosorbent granules. Thus, it was observed that the best behavior both in the case of biosorption capacity and in the percentage of dye retained was recorded in the case of biosorbent with granules of the smallest diameter. This behavior can be associated with the fact that the small diameter of the biosorbent particles ensures a much larger contact surface than the larger granules, which facilitates the contact between the dye molecules and the functional groups on the biosorbent surface.

• Figure 4*d* shows an increase in biosorption capacity with phase contact time, an increase that is faster in the first 100 minutes after which the growth is much slower until equilibrium is reached, which can be approximately 500 minutes (8 hours).

• Figure 4*e*, *f* shows the behavior of the studied biosorption system in function of temperature variation. The highest values of biosorption capacity are recorded at temperatures of 20^0C. At low temperature, of 5^0C, the process is inhibited and at a temperature of 40^0C it also registered lower values probably due to the beginning of the biosorbent denaturation process.

• Figure 4*e*, *f* shows also, that an increase in initial concentration of anionic dye from 28 to 230 mg/L (Φ2 and Φ3) causes an increase in the biosorption capacity until reaching an upper limit value after which it remains constant, a sign that the maximum sorption capacity of the respective biosorbent has been reached.

• The maximum biosorption capacity depends on the initial dye concentration and biosorbent granules' size as shown in Figs. 4(*e*-*f*) better results being achieved at the lowest value of the biosorbent granules' diameter and highest initial dye concentration.

3. Conclusions

The removal study of reactive Orange 16 dye from aqueous solution using as biosorbent the residual biomass of *Lactobacillus* immobilized in sodium alginate, led to the conclusion that this biomass-based material manifests adsorbent properties. Biosorption of this dye on the immobilized residual biomass of *Lactobacillus* depends on the pH of the initial solution (favourable pH range of 1.5-2.9), the dose of biosorbent (0.8-1.2 g/L), the concentration of the Orange 16 dye (80-220 mg/L dye), the contact time of the phases (> 500 min) and the temperature (20°-25°C). In these conditions, it is of interest to continue and deeply investigate the biosorption balance for further extention of residual biomass-based biosorbents application to real industrial treatment systems.

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BIOMASA REZIDUALĂ DE *LACTOBACILLUS* IMOBILIZATĂ ÎN ALGINAT PENTRU REŢINEREA COLORANTULUI ORANGE 16 DIN MEDII APOASE

(Rezumat)

Au fost studiate abilităţile de biosorbţie ale biomasei reziduale de *Lactobacillus sp.* imobilizate în alginat de sodiu faţă de coloranţi organici reactivi. În acest sens, s-au efectuat studii de biosorbţie în regim static pentru reţinerea colorantului reactiv Orange 16. Studiile au arătat că procesul de biosorbţie este influenţat de dimensiunea şi cantitatea de biosorbent, pH-ul soluţiei de colorant, concentraţia iniţială de colorant, temperatura şi timpul de contact al fazelor. Rezultatele preliminare au sugerat că biomasa reziduală studiată imobilizată în alginat de sodiu poate fi un biosorbent eficient pentru reţinerea coloranţilor din apele reziduale.