

The background features a blue-to-white gradient with faint molecular diagrams at the top. In the foreground, several test tubes are arranged in a row, and a pipette is shown dripping a drop of liquid into one of them.

O papel fundamental da

# QUÍMICA entre as CIÊNCIAS NATURAIS

Cleiseano Emanuel da Silva Paniagua  
(Organizador)



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O primeiro tema é constituído por doze capítulos que procuraram avaliar o processo de ressignificação e contextualização do ensino de química a partir: *i)* da percepção dos estudantes em relação ao consumo de água; *ii)* o ensino de química por meio de projetos; *iii)* a visão do aluno em relação ao processo de aprendizagem; *iv)* utilização de recursos tecnológicos e midiáticos como ferramentas facilitadoras no processo de aprendizagem; e *v)* utilização de materiais alternativos para a experimentação no ensino de química.

O segundo tema possui seis capítulos que procuraram avaliar o desempenho de novas substâncias químicas com inúmeras propriedades biológicas, entre as quais: a redução do número de larvas do mosquito *Aedes Aegypti*, bem como propriedades anti-inflamatória, antimicrobiana entre outras de interesse biológica. O terceiro tema é constituído por três capítulos que investigaram a síntese de nanopartículas de polianilina para composição de tintas utilizadas na impressão e do mineral hidroxiapatita. Por fim, o último tema é composto por seis capítulos que investigaram a remediação ambiental que se utilizou de resíduos de biomassa para remoção de metais pesados, a síntese de nanopartículas de sílica para a remoção de  $Ba^{2+}$  em matrizes aquosas, remediação de efluente contaminado com cádmio e chumbo e a aplicação de diferentes Processos Oxidativos Avançados para remoção de contaminantes.

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Cleiseano Emanuel da Silva Paniagua




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## REMOÇÃO DE METAL PESADO POR BIOMASSA OBTIDA A PARTIR DO PROCESSO DE PRODUÇÃO DE BIOETANOL

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**RESUMO:** A lignina contida no licor negro gerado durante a deslignificação alcalina da celulose para produção de bioetanol foi precipitada com  $H_2SO_4$  e avaliada como biossorvente de íons  $Pb(II)$  em solução aquosa. O planejamento experimental e a metodologia de superfície de resposta foram realizados com o objetivo de reduzir o número de experimentos e obter as melhores condições para o procedimento de biossorção. Três fatores em três níveis foram estudados: pH inicial (3; 4 e 5), massa biossorvente (50; 100 e 150 mg) e concentração inicial de  $Pb(II)$  (40; 60 e 80  $mg L^{-1}$ ). Os resultados indicaram que quanto maior a concentração do metal, menor a quantidade necessária de biossorvente na remoção do  $Pb(II)$ . A quantidade de  $Pb(II)$  adsorvida pela lignina foi de 60,4  $mg g^{-1}$  e correspondeu à remoção de 75,5%.

**PALAVRAS-CHAVE:** licor negro, desenho experimental, lignina, adsorção de  $Pb(II)$ , bagaço de cana-de-açúcar

### REMOVAL OF HEAVY METAL BY BIOMASS FROM BIOETHANOL PRODUCTION PROCESS

**ABSTRACT:** Lignin contained in the black liquor generated during alkaline delignification of cellulose for bioethanol production was precipitated with  $H_2SO_4$  and evaluated as biosorbent of  $Pb(II)$  ions in aqueous solution. Experimental design (DOE) and response surface methodology (RSM) were carried out in order to reduce the number of experiments and achieve the best conditions for biosorption procedure. Three factors at three levels were studied: initial pH (3; 4 and 5), biosorbent mass (50; 100 and

150 mg), and initial Pb(II) concentration (40; 60 and 80 mg L<sup>-1</sup>). The results indicated that the higher the metal concentration, the lower was the required amount of biosorbent in Pb(II) removal. Pb(II) amount adsorbed by lignin was 60.4 mg g<sup>-1</sup> and corresponded to removal 75.5%.

**KEYWORDS:** black liquor, experimental design, lignin, Pb(II) adsorption, sugarcane bagasse

## 1 | INTRODUCTION

Most of heavy metals present in aquatic environments cause severe damage to human and aquatic life and can bioaccumulate in these organisms (Wang et al., 2015). They are considered not biodegradable and must be removed from polluted water streams in order to meet increasingly stringent environmental quality standards (Vasconcelos et al., 2009).

Lead is one of the most widespread toxic heavy metal contaminants in the environment and its contamination in drinking water is a major source of concern due to its detrimental effect on human health. It can also cause a range of negative effects, from behavioural problems and learning disabilities to death (Yadav et al., 2014). Such effects are mainly associated to their bioaccumulation in bones (half-time over 20 years) and enzymes inhibition, which results in a probable carcinogenic agent (Morosanu et al., 2017). The highest amount of pollution with Pb(II) comes from textile dyeing, ceramic and glass industries, as well as electroplating, metallurgy, petroleum refining, plastic and battery manufacture and mining operations (Farooq et al., 2010). Thus, it is extremely important to reduce Pb(II) concentration in industrial effluents before their discharge into the environment.

Several processes of heavy metals elimination, mainly lead, are used: precipitation, electro-precipitation, electro-coagulation, cementing and separation by membrane, solvent extraction and exchange of ions on resins (Naima et al., 2013). Since the conventional methods for heavy metals removal are either too expensive or create large amounts of toxic sludge, attention has been paid to biosorption, technology that use microorganisms (Golab et al., 1991) or cheap, abundant, organic waste for sequestering pollutants from contaminated environments (Sadeek et al., 2015). Recent studies showed that common agricultural waste products or natural polymers could be used as potential biosorbents to remove heavy metals from wastewater (Sadeek et al., 2015).

The use of lignocellulosic biomass is an interesting and innovative adsorption process. This fact happens due its major constituents, which offer a large variety and abundance of functional groups that can act out as active sites on the biomaterial surface (Morosanu et al., 2017).

Lignin is one of the world's most abundant natural polymeric material, just like cellulose and chitin. It is a polymer of aromatic subunits usually derived from phenylalanine, such as coniferyl alcohol and other monolignols. It serves as a matrix around polysaccharide components of some plant cell walls, providing additional rigidity and compressive strength



as well rendering the walls hydrophobic and water impermeable (Whetten and Sederoff, 1995).

Lignin-related studies have revealed that it is a promising material to be used as an adsorbent, consequently it can remove heavy metal ions from wastewater (Yao et al., 2014). Several processes including ion exchange, surface adsorption, and complexation have been suggested by several researchers to explain the mechanisms of metal adsorption by lignin. However, detailed studies on understanding the exact mechanism of adsorption are yet to be reported (Ahmed and Ahmaruzzaman, 2016). Chemical modification of lignin, including crosslinking and functionalization, has been necessary to improve its adsorptive properties (Yao et al., 2014; Yan and Li, 2016). However, most of the existing studies have utilized lignin directly as an adsorbent material (Yao et al., 2014).

Currently, lignin is mainly obtained as a by-product of the papermaking industry and often directly burned as a fuel for energy generation (Barana et al., 2016). Large amounts of lignin are expected to be produced in future biorefineries as a by-product of biofuel production, which is stimulating new emerging applications, mainly as sustainable alternatives to non-renewable products such as polyurethanes, thermoplastic polymers, epoxy and phenolic resins as well as corrosion inhibitors (Brosse et al., 2011).

Along the last few years, experimental design (DOE) and response surface methodology (RSM) have been employed to optimize heavy-metals biosorption. The factorial experimental design involves using independent variables such as pH, initial metal concentration and biosorbent dosage and varying them from one experiment to the next, while response surface methodology involves a collection of mathematical and statistical techniques useful for analysing the effects of several independent variables on the response (Martin-Lara et al., 2011). Therefore, besides this methodology demands a reduced number of experimental trials to evaluate multiple parameters and their interactions, it plays a key-role in the process design and optimization, as well as the improvement of existing design (Tabaraki et al., 2014).

Thus, the use of lignin recovered from delignification step of biomass during bioethanol production process as biosorbent for heavy metals may represent an important contribution to the industrial implementation of bioethanol, mainly regarding lignin valorisation, as well as to minimize residues and environmental impacts of these chemical bio-industries. Although lignin extracted from black liquor of paper and pulp-manufacturing industry has been investigated for heavy metals sequestration in aqueous solutions, there are no reports in the literature, until this date, on the characterization and use of bioethanol-derived-lignin for this purpose. Therefore, the present research aimed at investigating lignin resulting from alkaline delignification of sugarcane bagasse during ethanol production as biosorbent of Pb(II) ions in aqueous solutions. Experimental design and RSM were used to study the effect of pH, initial Pb(II) concentration and lignin mass on biosorption. The kinetic parameters were also evaluated using the biosorption measurements. FTIR analysis was

conducted to identify the functional group involved in the biosorption process.

## 2 | MATERIAL AND METHODS

### Preparation of Biosorbent

The lignin used as adsorbent came from a study on bioethanol production of sugarcane bagasse. Firstly, the bagasse was submitted to acid hydrolysis treatment, using 1%  $\text{H}_2\text{SO}_4$  (w/v), solid-liquid ratio of 1:10, at 121°C for 20 minutes. After separation of hemicellulosic hydrolysate by filtration using filter paper, the solid mass (cellulignin) was delignified using 1.5% (w/v) NaOH, 1:20 solid-liquid ratio, at 100°C for an hour. The obtained black liquor was separated from the solid mass (cellulose residue) by filtration using filter paper.

For the recovery of lignin from black liquor, different acids such as acetic, chloridric and sulfuric and pH values were tested, and the best result was obtained with  $\text{H}_2\text{SO}_4$  at pH 2. After acidification, lignin was separated by centrifugation in 50 mL tubes for 15 minutes at 2050 x *g* and the supernatant was discarded. The precipitated lignin was resuspended in distilled water, centrifuged again, and process was repeated for three times. The resulting lignin was oven dried at 60°C for 6 hours.

### Preparation of Pb(II) Solution

A stock solution of Pb(II) ( $1.0 \text{ g L}^{-1}$ ) was prepared by dilution of Titrisol (Merck) flasks with distilled water. The stock solution was further diluted to obtain the desired initial concentrations (40-80  $\text{mg L}^{-1}$ ). NaOH and HCl solutions of  $0.1 \text{ mol L}^{-1}$  were used to adjust initial pH to the required value of each test solution before mixing the biosorbent. All chemical reagents were of analytical grade.

### Batch Biosorption Experiments of Pb(II)

For biosorption experiments under batch operation, 125-mL Erlenmeyer flasks containing 50 mL of aqueous solution with biosorbent quantity and Pb(II) concentration ranging from 50 to 150 mg and from 40 to 80  $\text{mg L}^{-1}$ , respectively, were used. The initial pH varied from 3 to 5 and was adjusted with NaOH and HCl solutions, both of them at  $0.1 \text{ mol L}^{-1}$ . The experiments were carried out in a shaker incubator at 25 °C and constant stirring at 200 rpm for a pre-determined time interval. The pH was not controlled during the experiments. All tests were prepared in triplicate to check reproducibility of the results and the average values were reported.

The experiments were carried out by suspended determined amount of biosorbent in Pb(II) solutions. The suspension was taken, filtered using filter membrane (0.45 mm) and analyses of residual Pb(II) concentrations were determined by flame atomic absorption spectrometry (FAAS), using Shimadzu AA-6300 equipment. The amount of adsorbed Pb(II)

ions at equilibrium per unit mass of adsorbent ( $q_e$ ) was calculated based on the difference between the initial and equilibrium concentrations of this metal in the aqueous solution volume.

## Experimental Design and Optimization

A three-factor ( $X_1$ ,  $X_2$ , and  $X_3$ ) and a three-level (-1, 0, and +1) experimental design (DOE) were used in this trial in order to achieve maximum information about Pb(II) biosorption process by lignin. The studied factors (independent variables) were initial pH ( $X_1$ ), biosorbent mass ( $X_2$ , g), and initial Pb(II) concentration ( $X_3$ , mg L<sup>-1</sup>) to evaluate lead biosorption response (mg g<sup>-1</sup>). Each variable was coded at one of three levels, -1, 0, and +1. The minimum and maximum levels (Table 1) given to each factor were chosen based on preliminary experiments. Each experiment was carried out in triplicate and the average values were taken as the response (Y).

Independent variable	Factor	Coded levels		
		-1	0	+1
Initial pH	$X_1$	3	4	5
Biosorbent mass, M (mg)	$X_2$	50	100	150
Initial Pb(II) concentration, $C_i$ (mg L <sup>-1</sup> )	$X_3$	40	60	80

Table 1. Coded and uncoded levels of the independent variables.

Experimental data were fitted to the first-order polynomial model and regression coefficients were obtained. The main effects and variables interactions, their respective coefficients for the mathematical model as well as the analysis of variance (ANOVA) were calculated to determine the model validity. Variable effects were described by the difference among the average response at the upper level and the average response at the lowest one. The optimal values concerning the selected variables were analysed by RSM. The adjustment quality of polynomial equation was evaluated by determining R<sup>2</sup> coefficient. The significances of all terms in the polynomial equation were analysed statistically by computing F-value at 0.05 probability (p).

## 3 | RESULTS AND DISCUSSION

### Biosorption Studies of Pb(II)

Table 2 shows the real and coded (in parentheses) values of the independent variables and the adsorbed amounts of Pb(II) by lignin achieved in each trial.

The results showed a variation in adsorption values that can be explained as a function of adsorptive capacity of the used biomass (chemical structure, functional groups)

and the availability of active sites, since contact time and temperature, which can also influence the results, were the same for all trials.

Trials*	Initial pH	M (mg)	$C_i$ (mg L <sup>-1</sup> )	$q_e$ (mg g <sup>-1</sup> ) <sup>§</sup>
1	3 (-1)	50 (-1)	40 (-1)	19.4 ± 1.2
2	5 (+1)	50 (-1)	40 (-1)	25.8 ± 2.0
3	3 (-1)	150 (+1)	40 (-1)	8.3 ± 1.6
4	5 (+1)	150 (+1)	40 (-1)	8.4 ± 2.1
5	3 (-1)	50 (-1)	80 (+1)	25.1 ± 1.8
6	5 (+1)	50 (-1)	80 (+1)	41.8 ± 1.1
7	3 (-1)	150 (+1)	80 (+1)	14.9 ± 1.0
8	5 (+1)	150 (+1)	80 (+1)	20.2 ± 1.7
9	4 (0)	100 (0)	60 (0)	21.6 ± 0.2
10	4 (0)	100 (0)	60 (0)	22.5 ± 1.0
11	4 (0)	100 (0)	60 (0)	23.3 ± 0.6

Table 2. Experimental design used in this study with results for lead uptake by lignin.

\* The number of each trial does not mean the order it was carried out.

§ Results are average of triplicate analysis for Pb biosorption ± standard deviation.

It was observed that overall average for Pb(II) biosorption by lignin was 21.0 mg g<sup>-1</sup>. The lowest obtained value corresponded to trial 3, whose value was 8.4 mg g<sup>-1</sup>. Trial 6 presented the highest value (41.8 mg g<sup>-1</sup>), indicating that the best adsorption condition for Pb(II) ion occurred at higher value of initial pH and metal concentration with a lower amount of biomass. For Martín-Lara et al. (2011), the lowest dosages of biosorbent are ideal for lead ions biosorption.

Guo et al. (2008), investigated the heavy metal adsorption of ions Pb(II), Cu (II), Cd (II), Zn (II) and Ni (II) on lignin isolated from black liquor from the paper industry. These authors observed better affinity for Pb(II), with an initial adsorption rate ( $v_0$ ) and amount of metal adsorbed on lignin ( $q_e$ ) of 162 mg (g min)<sup>-1</sup> and 63 mg g<sup>-1</sup>, respectively. Moreover, the adsorption mechanism involved two main types of acid sites attributed to carboxylic- and phenolic-type surface groups, the latter with higher affinity for metal ions than carboxylic sites.

In order to increase the adsorption capability to heavy metals by lignin, Ge et al. (2016), prepared a new type of lignin microspheres (LMS) from a lignin obtained from a pulp mill. LMS showed high adsorption capacity to Pb(II) of 33.9 mg g<sup>-1</sup>. However, this value was lower than the maximum  $q_e$  obtained in this work (41.8 mg g<sup>-1</sup>), showing the importance of a DOE planning in order to determine the best Pb(II) adsorption conditions by lignin and better exploit the potential of this biosorbent.

A green porous lignin-based sphere (PLS) manufactured by a feasible gelation-

solidification method from lignosulfonate cross-linked with sodium alginate and epichlorohydrin showed an excellent adsorption efficiency ( $95.6 \pm 3.5\%$ ) for lead ions at an initial concentration of  $25.0 \text{ mg L}^{-1}$ , that suggested the possibility of PLS to be applied for the continuous treatment of wastewater rich in heavy metals at the industry (Li et al., 2015).

Schafhauser et al. (2015), reported the use of a modified lignin from the corn residue as a lead ion biosorbent, whose removal capacity was 98.87% of an initial solution containing  $10 \text{ mg L}^{-1}$ . According to Zakzeski et al. (2010), lignin structure is highly functional and contains alcohol, aldehyde, ether or acid substituents groups. These monomeric compounds are susceptible to a wide range of transformations, mainly whether reductive in nature, forming simple hydrocarbons, or oxidative in nature, with an increase of aromatic compounds or specifically for the desired functionality.

The values obtained by the trials (9, 10 and 11) at the central points showed small variations, indicating a good reproducibility.

Pareto chart was drawn to analyse significant effects of independent variables and their interactions (Fig. 1a).

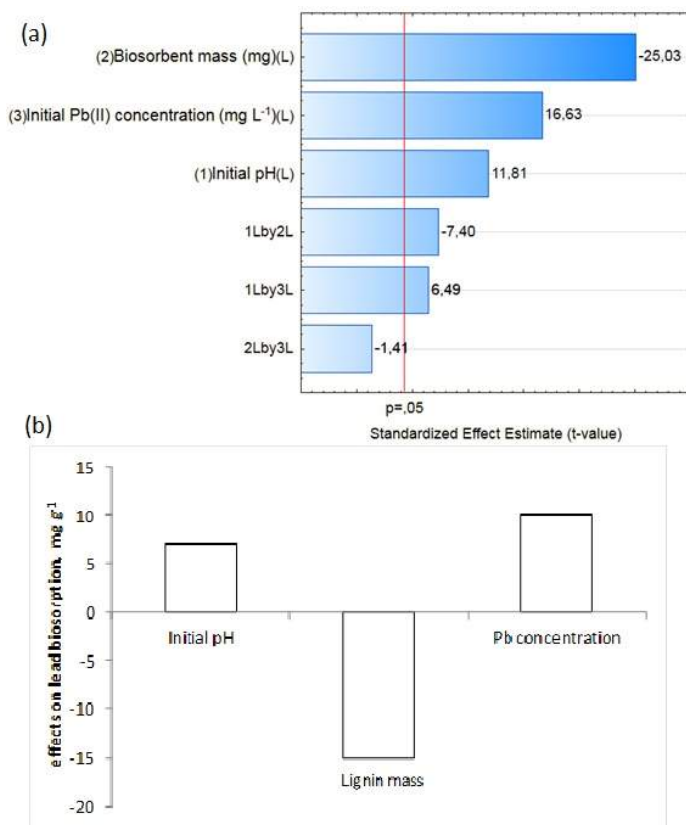


Figure 1. (a) Pareto chart of the standardized effects (t-value) for lead biosorption capacity ( $\text{mg g}^{-1}$ ),  $\alpha=0.05$  and (b) Linear effects (absolute values) of initial pH, lignin mass and lead concentration on biosorption capacity of lead ions.

When analysing the influence of each independent variable alone on Pb(II) ions adsorption, it was observed that the amount of adsorbent mass was statistically significant and showed a marked negative effect. That is, the larger the mass of adsorbent the lower the adsorption of this ion. The other two independent variables (initial pH and metal concentration) showed positive effects and were also statistically significant.

Thus, for the interaction between the variables, due to the simultaneous analysis of the effects of these interactions, the only positive and significant effect on Pb(II) ions adsorption was observed when the initial pH and metal concentration variables were combined, which means that the adsorption of this ion increased.

There was a negative and significant effect for the interaction between initial pH and adsorbent mass, which resulted in lower adsorption of Pb(II) ions. However, the effect of interaction between adsorbent mass and metal concentration did not show a significant answer.

Figure 1b shows the effects of initial pH, lignin mass and Pb(II) ions concentration on the ability of Pb(II) biosorption by lignin. It was observed a  $7.1 \text{ mg} \cdot \text{g}^{-1}$  increase in adsorption with an increase of initial pH from 3 to 5. This improvement in adsorption was possibly due to the reduction of  $\text{H}_3\text{O}^+$  ions concentration and, consequently, there was less competition between protons and Pb(II) for lignin binding sites. The increase in lignin mass from 50 mg to 150 mg provided a reduction of  $15.0 \text{ mg} \cdot \text{g}^{-1}$  in the adsorbed amount of Pb(II). This can be explained by the fact that the decrease of lignin mass induces a greater competition of Pb(II) ions for active sites present in lignin superfine available for cation binding (Chen et al., 2011). The increase in Pb(II) concentration from  $50 \text{ mg} \cdot \text{L}^{-1}$  to  $150 \text{ mg} \cdot \text{L}^{-1}$  increased  $10.0 \text{ mg} \cdot \text{g}^{-1}$  in the adsorbed amount of Pb(II) by lignin. This can be explained by the greater availability of Pb(II) ions present in solution to bind the lignin active sites.

Experimental values were predicted with empirical equations adjusted by analysis of variance in order to carry out an integral study of the independent variables influence on Pb(II) adsorption. Thus, the equation obtained to predict the response variables  $Y (\text{mg} \cdot \text{g}^{-1}) = 21.04 + 3.55X_1 - 7.53X_2 + 5.00X_3 - 2.23X_1X_2 + 1.95X_1X_3$  is presented as a function of coded terms of the independent variables, indicating the statistically significant values, at 95% confidence level. The empirical model to describe Pb(II) adsorption by lignin showed a value for determination coefficient ( $R^2$ ) equal to 0.98, which is considered of high quality because it indicates that only 0.02% of the response variability could not be explained by the model, confirming the quality of the adjustment.

Thus, a plot of normality residues was generated to check efficiency of Pb(II) removal in order to analyze the residual values distribution, defined as the differences between predicted and observed (experimental) models. The correlation validity, found out by the statistical analysis, which points out linearity and absence of trend in the studied data, revealed a model that does not lack a significant adjustment.

The graph representation of the response surface, corresponding to the parameter lead biosorption, is shown in Figure 2.

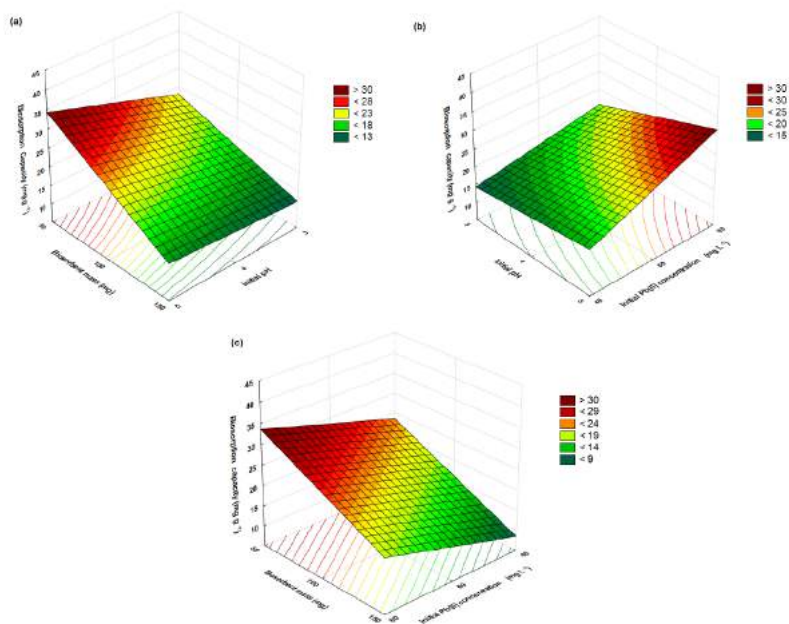


Figure 2. Response surface plots for the effect of **(a)** initial solution pH and lignin mass (mg); **(b)** initial solution pH and lead ion concentration ( $\text{mg.L}^{-1}$ ) and **(c)** lead ion concentration ( $\text{mg.L}^{-1}$ ) and lignin mass (mg) on the lead biosorption capacity ( $\text{mg.g}^{-1}$ ).

It can be observed that the use of lower biosorbent mass (50 mg) with a higher initial pH (5) resulted in higher adsorption (Fig. 2a). The same results can be observed in Figures 2b and 2c, respectively, with a higher initial pH and a higher concentration of Pb(II) ( $80 \text{ mg L}^{-1}$ ) and with lower biosorbent mass (50 mg) and a higher concentration of Pb(II).

The solution pH is one of the most important parameters in heavy metals biosorption from aqueous solutions, since it can influence on the nature of biomass binding sites and metal solubility and affect the solution chemistry of the metals, the activity of the functional groups in biomass and competition of metallic ions (Salman et al., 2014).

Adsorption studies with lignin isolated from black liquor of the paper industry revealed that by increasing pH, sorption edges were reached for Pb(II). When pH was higher than 6, more than 85% of metal ions were adsorbed on surface efficiently from a starting solution of  $41 \text{ mg L}^{-1}$ . Precipitation may also have occurred at higher pH (Guo et al., 2008).

## 4 | CONCLUSIONS

This study has concluded that lignin obtained from alkaline delignification step of bioethanol production from sugarcane bagasse can be used as Pb(II) ions biosorbent. The experimental design and RSM were important tools for determining the best Pb(II) biosorption conditions in aqueous solutions, revealing more efficiency with higher metal amounts and smaller adsorbent quantities.

Considering that this is the first study on heavy metal adsorption by lignin recovered from the bioethanol production, further studies are suggested to verify the removal of other metals.

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


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