Modeling of Sulfite Concentration, Particle Size, and Reaction Time in Lignosulfonate Production from Barley Straw Using Response Surface Methodology and Artificial Neural Network

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Barley straw is a lignocellulosic biomass that can be used to obtain value-added products for industrial applications. Barley straw hydrolysis with sodium sulfite facilitates the production of lignosulfonates. In this work, the delignification process of barley straw by solubilizing lignin through sulfite method was studied. Response surface methodology and artificial neural network were used to develop predictive models for simulation and optimization of delignification process of barley straw. The influence of parameters over sulfite concentration (1.0 to 10.0%), particle size (8 to 20), and reaction time (30 to 90 min) on total percentage of solubilized material was investigated through a three level three factor (3^3) full factorial central composite design with the help of Matlab® ver. 8.1. The results show that particle size and sulfite concentration have the most significant effect on delignification process. Both techniques, response surface methodology and artificial neural networks, predicted the lignosulfonate yield adequately, although the artificial neural network technique produced a better fit (R^2 = 0.9825) against the response surface methodology (R^2 = 0.9290). Based on these findings, this study can be used as a guide to forecast the potential production of lignosulfonates from barley straw using different experimental conditions.

Keywords: Delignification; Straw; Response surface methodology; Artificial neural networks

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INTRODUCTION

Lignocellulosic biomass is a main element in producing environmentally friendly raw materials (Zhu *et al.* 2013). Because of its high production volume and low use, biomass from agricultural waste, such as barley straw, is a good alternative as a carbohydrate source. Barley (*Hordeum vulgare*) is the second most important crop among the secondary cereals. Its annual global production is 180 million tons, and each kilogram of grain results in about 0.750 kg of straw (Singh *et al.* 2014). Barley straw has low nutritional value and is usually used as bedding for livestock. The straw is considered a waste, and pollution problems can result due to careless disposal.

The effective use of these residues as source of chemical compounds requires the separation of cellulose, hemicellulose, and lignin (Kahar *et al.* 2013) *via* physical (Iskalieva *et al.* 2012; Subhedar and Gogate 2014), chemical (Yan *et al.* 2010; Duque *et al.* 2013), and biological treatments (El-Zawawy *et al.* 2011; Wang *et al.* 2011). The removal of lignin of plant waste is perhaps the most difficult process. An alternative to remove lignin from plant biomass is used sulfite $(SO_3)^{2-}$, through the use of sodium, potassium, and calcium sulfite solutions (Meier *et al.* 1994; Chakrabarty *et al.* 2009). An advantage of this process is the production of soluble lignin (lignosulfonate) which has several industrial applications. The various functional groups of lignin provide many potential uses: dispersing agents in cement and plaster (Stráněl and Sebök 1997); emulsifiers and chelant (Weis and Bird 2001); polymer production (Effendi *et al.* 2008); soil conditioners (Deng *et al.* 2011); and vegetal growth promoters (Ertani *et al.* 2011). The use of sulfite for the removal of lignin in wood was a process widely used in the industry of paper pulp and had not been used for delignification of barley straw.

So far, it is unclear which are the most important variables to consider during a process of separation of the components of barley straw. Solubilization of lignin requires high temperatures and pressures to disarticulate and disaggregate cellulosic matter fibers. The particle size of barley straw biomass should provide the greatest amount of possible contact surface for reactions with all available material. Sulfite concentration must be high enough to completely saturate the material and remain in contact with it for enough time to attain suitably high delignification. These parameters have been studied in other chemical processes, using reagents such as sodium hydroxide, sodium chlorite (Rossberg *et al.* 2014), hydrogen peroxide (Sheikh *et al.* 2014), and formic/acetic acid (Vanderghem *et al.* 2012), demonstrating their influence on the hydrolysis of lignocellulosic raw. In this work, the process to obtain lignosulfonates and the interactions between the variables (particle size, sulfite concentration, and reaction time) were modeled. This will allow a better understanding of the possible interactions between the variables studied during the delignification of barley straw.

Currently, there are well-known adjustment data methodologies for modeling industrial processes. They predict how behaviors are caused by interactions between variables, which are analyzed with a small number of experiments. Response surface methodology (RSM) is extremely effective at exploring the relationship between controllable factors and response variables for the purpose of modeling experimental laboratory results (Box and Wilson 1951). In RSM, a mathematical model $\eta = F(x_1, x_2, \ldots, x_n)$ is developed to explain the real function, where x_1, x_2, …, x_n are factors influencing the response variable value (η) (Montgomery 2001). Another alternative is the artificial neural network (ANN), a computational technique for multifactorial analysis inspired by biological neural networks. An ANN consists of nodes distributed in layers interconnected by arcs assigned with certain weights. These nodes represent artificial neurons, which are processing units that execute a non-linear sum function (Dayhoff and DeLeo 2001).

In the present study the effects of combinations of variables as particle size, sulfite concentration, and reaction time are modeled using RSM and ANNs to determine relationships between the experimental variables in lignosulfonate solubilization. This is the first report comparing RSM and ANNs in delignification process modeling.

EXPERIMENTAL

Raw Material

The lignocellulosic material used in this study was barley straw harvested in Zempoala, Hidalgo, Mexico. The biomass was milled and sieved through three meshes: mesh number 8 [2.0 mm]; mesh number 12 [1.68 mm]; and mesh number 20 [0.84 mm]. Chemical hydrolysis was performed in a semi-industrial autoclave (AV-3580 Prendo®, SEV, Puebla, México), which was modified to reach 3 atm of pressure; sodium sulfite (Reproquifin®, Sigma-Aldrich, St. Louis, MO, USA) was applied in 450-mL glass screw-top flasks.

Characterization of barley straw was performed taking into account the Technical Association of the Pulp and Paper Industry (TAPPI) Standard (www.tappi.org). The measured components in barley straw were as follows: removable in solvent, removable in water, holocellulose, ashes and lignin. And their percentages were 3.5 % ± 0.43,11.2 % ± 0.37, 56.3 % ± 1.45, 10.34 % ± 0.12 y 19.2 % ± 0.05, respectively. 18.2% of lignin was not soluble in acid and 1% was found as soluble lignin in acid. The moisture content of the straw was 5.6%.

Experimental Method

Three samples (2 g) of ground straw from each particle size (mesh number 8, 12, and 20) were weighed and mixed with a 50 mL of sodium sulfite solution for each concentration (1, 5, and 10%). The digestion process was conducted in a closed system inside glass flasks, which were heated in an autoclave at 3 atm and 137 °C for 30-, 60-, and 90-min intervals to produce a cellulose pulp and solubilized material or liquor (Teschke and Demers 1996). The latter contained lignosulfonates, which were recovered in a vacuum with 1.6-µm pore filter paper. Range values for each parameter were chosen according to personal experience in previous experimental results. Finally, the solubilized material was quantified by weight difference (Ekeberg *et al.* 2006). The performance of solubilized material (P_{sol}) is calculated with Eq. 1,

$$P_{sol}(\%) = \frac{DW_s - (DW_f - A - W)}{DW_s} \times 100 \tag{1}$$

where DW_s is the initial dry weight, DW_f: is the final dry weight, A: are the residual ash content, and W is the sample without sulfite (only water).

Data Fitting Techniques

Response surface methodology

A three-level three factor (3^3) complete factorial design (CCD) was used to identify the relationship between the solubilized material (%) and process variables (particle size, heating time, and sulfite concentration). Hence, a total of 27 experiments were carried out in triplicate and the results were modeled by using RSM. Experimental values for the selected variables (with their units and notations) are presented in Table 1, and the corresponding CCD matrix is shown in Table 2. The data were fitted using the rstool command in the statistical toolbox of Matlab® ver. 8.1 (MathWorks®, Natick, MA, United States). The response variable P_{sol} (total percentage of solubilized material) is expressed as a formula that depends on the values of the factors influencing the process. The resulting formula is based on the response surface quadratic model (Eq. 2),

$$P_{sol}(\%) = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{j=2}^{k} \sum_{i=1}^{j-1} \beta_{ij}\, x_i x_j + \sum_{i=1}^{k} \beta_{ii} x^2 + e \qquad (2)$$

where β_0 is the equation constant, β_i is the linear term coefficient, β_{ij} is the variable interaction term coefficient, β_{ii} corresponds to the quadratic coefficient, x_i and x_j are independent variables, and e denotes the noise or observed error in the response model.

An analysis of variance (ANOVA) was used to validate the fitness of the developed response surface model and the statistical significance of the regression coefficients. The interaction among independent variables and response variables was analyzed using response surface contour graphs.

Table 1. Experimental Range and Levels of Independent Process Variables

Variable	Unit	Notation	Range and Levels (Coded)		
			-1	0	+1
Barley Straw Size		A	8	12	20
Heating Time	min	B	30	60	90
Concentration	%	C	1	5	10

Table 2. CCD for Three Independent Variables and the Observed Responses

Experiment No.	Coded Values			Real Values			P_{sol} (%)
	A	B	C	A	B	C	
1	+1	0	+1	20	60	10	20.17
2	+1	-1	+1	20	30	10	19.86
3	-1	-1	+1	8	30	10	19.86
4	-1	0	+1	8	60	10	16.84
5	-1	-1	-1	8	30	1	5.60
6	-1	-1	0	8	30	5	12.32
7	+1	-1	-1	20	30	1	7.71
8	+1	-1	0	20	30	5	20.22
9	-1	0	-1	8	60	1	2.76
10	-1	0	0	8	60	5	18.75
11	+1	0	-1	20	60	1	7.86
12	+1	0	0	20	60	5	22.56
13	-1	+1	-1	8	90	1	4.27
14	-1	+1	0	8	90	5	11.26
15	-1	+1	+1	8	90	10	10.07
16	+1	+1	-1	20	90	1	11.56
17	+1	+1	0	20	90	5	22.15
18	+1	+1	+1	20	90	10	20.94
19	0	-1	-1	12	30	1	6.86
20	0	-1	0	12	30	5	17.63
21	0	-1	+1	12	30	10	15.20
22	0	0	-1	12	60	1	7.68
23	0	0	0	12	60	5	20.49
24	0	0	+1	12	60	10	19.66
25	0	+1	-1	12	90	1	9.98
26	0	+1	0	12	90	5	19.42
27	0	+1	+1	12	90	10	17.68

Artificial neural network

An ANN was developed to replicate the effects of interactions among the three quantified factors (particle size, heating time, and sulfite concentration) on the solubilization of lignosulfonate. The ANN input layer was composed of three neurons representing the three independent process variables. The output layer contained a single neuron representing the percentage of solubilized material as the process response, as in the RSM model. ANN data were analyzed using the ANN toolbox in Matlab® ver. 8.1. The Hyperbolic tangent sigmoid transfer function was used as activation function for every hidden layer, and the Linear transfer function was applied in the output layer.

RESULTS AND DISCUSSION

Experimental Results

Different Na_2SO_3 concentrations, heating times, and particles sizes during barley straw delignification resulted in different yields of solubilized material (Table 2). The minimum percentage obtained was 2.76% (experiment no. 9), and the maximum was 22.56% (experiment no. 12), which showed the influence of the independent variables on the response variable.

Response Surface Methodology

The RSM model was developed using a quadratic model with three axes for the process variables, where *x* is particle size, *y* is heating time, and *z* is sulfite concentration. Using a 99% confidence level, the following quadratic equation, in terms of actual variables, was calculated,

$$\mathrm{P_{sol}(x,y,z)} = -20.3164 + 2.3312\,\mathrm{x} + 0.2032\,\mathrm{y} + 4.5859\,\mathrm{z} + 3.7856 \times 10^{-3}\,\mathrm{xy} + 2.7021 \times 10^{-2}\,\mathrm{xz} - 1.4610 \times 10^{-3}\,\mathrm{yz} - 7.6452 \times 10^{-2}\mathrm{x}^2 - 1.8724 \times 10^{-3}\,\mathrm{y}^2 - 0.3445\,\mathrm{z}^2 \tag{3}$$

where $P_{sol}(x,y,z)$ indicates the percentage of solubilized material.

ANOVA of the response surface quadratic model showed the influence of the three independent variables (*x*, *y*, *z*) on the response variable P_{sol} (Table 3), taking into account a Fisher's F value (24.717) and a very low probability value ($p < 0.0001$). The correlation coefficient (R^2) between the experimental data and those predicted by the response surface model was 0.9290, indicating that the experimental data were adequately adjusted (Fig. 1 A). The ANOVA analysis confirmed that the response surface model can be used to simulate solubilized material yield from barley straw because it produced values within the range of experimental values.

A regression analysis of the model equation (Table 4) showed that the main effects of the variables particle size and concentration were significant ($p < 0.05$), but time and square interaction had no effect on the treatment.

Three contour plots illustrated the interactions between the independent variables and their effects on the solubilized material. These graphs are three-dimensional representations of the response surface as a function of two independent variables, while the third variable is assigned a fixed value. The particle size 18, heating time 68 min and sulfite concentration 7% were selected as fixed values because their combination produces the maximum percentage of solubilized material. The relationships between heating time and sulfite concentration, sulfite concentration and particle size interaction, and particle size and heating time are presented in Fig. 1 B, C and D, respectively.

There was not a strong effect from the combination of variables; individually, particle size and sulfite concentration affected the response value. The percent yield of solubilized material was inversely proportional to particle size; this variable determines the amount of surface contact between lignin and the solution, which affects solubilization (Abud *et al.* 2013). At 5% sulfite, solubilization was favored because it brought enough sulfite into contact with the aqueous medium, leading to cation separation dependent on medium pH, ionic strength, and temperature. These conditions produced a dynamic chemical balance among species (sulfur dioxide, sulfurous acid, and sulfite and bisulfite anions), causing the formation of certain species in response to medium conditions (Chakrabarty 2009). The 3 atm pressure level also favored delignification. Depressurization of fibers at 5% sulfite ensured that all chemical species were in the necessary position for SO_3 ions to react with lignin and form lignosulfonates.

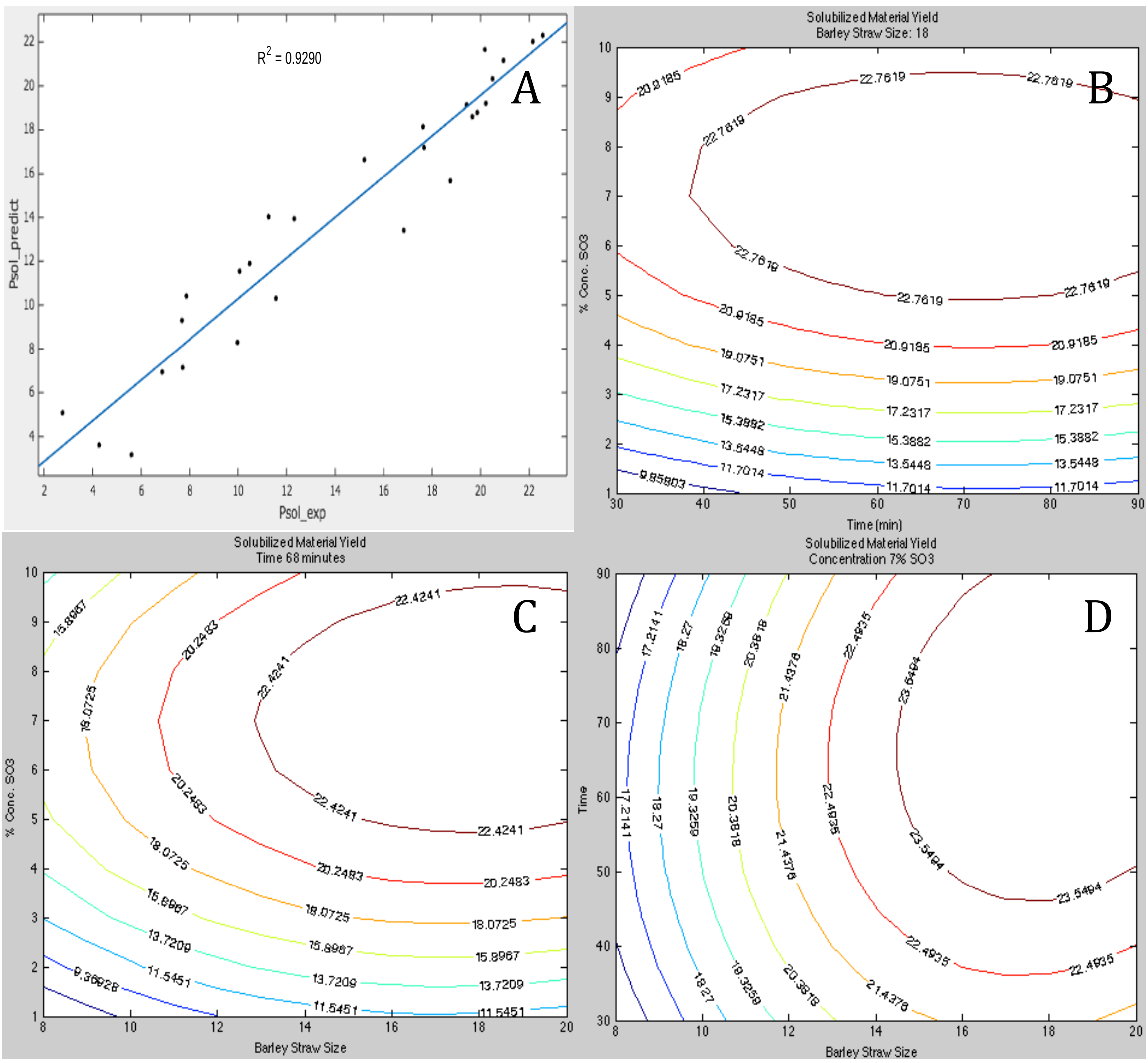

Fig. 1. Analysis of RSM model of delignification process of barley straw

Table 3. ANOVA of the RSM Quadratic Model of Barley Straw Delignification

Source	Sum of squares	Degree of freedom (df)	Mean square	*F*-value	*p*-value
Model	911.33	9	101.26	24.717	< 0.0001
Linear	563.14	3	187.71	45.821	< 0.0001
Nonlinear	348.19	6	58.032	14.66	< 0.0001
Residual	69.643	17	4.0967		
Total	980.97	26	37.73		

Lignin molecules contain approximately 4 to 8% sulfur (Glasser 1981), mainly in the form of sulfonate groups, which cause lignin to become soluble in water. Longer reaction times were expected to increase sulfite incorporation into lignin, but Fig. 1 D shows that time had no effect. This result was due to the creation of weak nucleophilic sites in the lignin aromatic ring in response to the pH of the solution. These sites compete with sulfite ions for the carbonyl ion intermediaries, forming condensed structures. Condensation reactions of this sort may explain why the increase in sulfite concentration and heating time decreased the amount of solubilized material (Deng *et al.* 2011).

Table 4. Regression Analysis using the 3^3 Factorial CCD

Model term	Coefficient estimate	Standard error	*F*-value	*p*-value
A	3.3711	0.4691	7.1862	< 0.0001
B	0.6357	0.4691	1.3552	0.1931
C	4.8118	0.4691	10.257	< 0.0001
AB	0.8057	0.5745	1.4023	0.1788
AC	0.7579	0.5745	1.3192	0.2046
BC	-0.2087	0.5745	-0.3632	0.7209
A^2	-1.3228	0.8125	-1.6280	0.1219
B^2	-1.6852	0.8125	-2.0740	0.0536
C^2	-6.3549	0.8125	-7.8214	< 0.0001

These results suggested that 9% SO_3 is optimum for creating a balance among the species in an aqueous solution (sulfur dioxide, sulfurous acid, and sulfite and bisulfite anions). This condition is also ideal for releasing the sulfite ions needed to attain bonding on the surface of 18-mesh size particles, obtaining lignin solubility.

Artificial Neural Network

The experimental response data was used to identify the most appropriate ANN model to represent this biotechnological process. It was also intended to identify the combination of input values that produced the optimum amount of solubilized material. A script was also implemented to find the ANN model with the lowest quadratic error. This script generated ANN models starting with a three-layer model in which the first (input) layer contained three neurons (particle size, heating time, and sulfite concentration). The number of hidden layers varied from one to ten, and the output layer consisted of a single neuron that indicated the percentage of solubilized material.

A random selection of 70% of the data (*i.e.*, 19 samples) was chosen to train the ANN, and another 15% (4 samples) was used to validate the model. Finally, 15% (4 samples) was considered to test it. The obtained ANNs were trained with a Levenberg-Marquardt backpropagation algorithm (Hagan *et al.* 2014). Each ANN contained from 1 to 10 hidden layers, and every layer was composed of 3 to 50 neurons. The network with the best performance contained four hidden layers and their corresponding neurons (9:5:9:3), where R^2 = 0.9825. Table 5 shows a comparison among ANN's with high performance and different number of hidden layers.

Table 5. R^2 and RMSE values for ANNs with different number of hidden layers.

Hidden layers	R^2	RMSE
1	0.9127	1.749
2	0.9515	1.395
3	0.9727	1.045
4	0.9825	0.8391

The goodness-of-fit between the experimental and the predicted response given by the ANN model had a high correlation value (R^2 = 0.9825) (Fig. 2 A). Thus, the developed ANN model accurately replicated the material solubilization process using the particle size, heating time, and sulfite concentration variables.

Comparison of RSM and ANN Models

To validate the RSM and ANN models, 9 experiments were performed with a combination of values for the three factors, whereas such values were not used to generate the models (Table 6). The comparison between RSM and ANN residuals are shown in Fig. 2 B, where the ANN model shows a lower deviation than the RSM model.

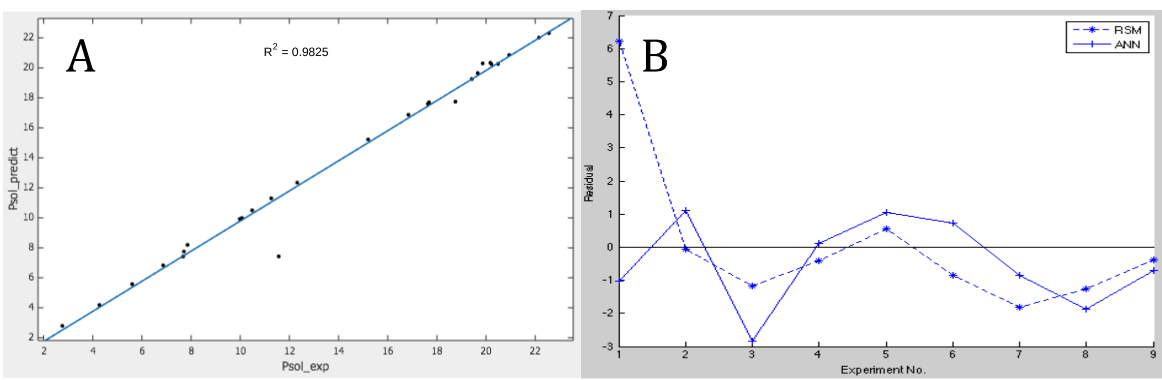

Fig. 2. Analysis of ANN model for delignification process of barley straw

Table 6. Validation Data Set

Run	Size (mesh number)	Time (min)	Sulfite Conc. (%)	P_{sol} (%)	RSM		ANN	
					Predicted	Residual	Predicted	Residual
1	8	45	3	5.0252	11.2645	6.2393	4.0049	-1.0202
2	8	45	5	15.2848	15.2255	-0.0593	16.3915	1.1067
3	8	45	10	14.2296	13.0714	-1.1582	11.396	-2.8336
4	12	45	3	15.8937	15.479	-0.4147	16.0132	0.1195
5	12	45	5	19.1167	19.6562	0.5394	20.1615	1.0448
6	12	45	10	18.8931	18.0425	-0.8506	19.631	0.7379
7	20	45	3	18.3713	16.5686	-1.8027	17.5181	-0.8531
8	20	45	5	22.4443	21.1781	-1.2662	20.5703	-1.874
9	20	45	10	21.0221	20.6452	-0.3769	20.3085	-0.7136

The performance for each model was statistically analyzed with the root mean square error (RMSE), coefficient (R^2) determination, and absolute average deviation (AAD), based on the following equations (Geyikci *et al.* 2012),

$$\mathrm{RMSE} = \left(\frac{1}{n}\sum_{i=1}^{n}(P_{sol,pred}\text{-}P_{sol,exp})^2\right)^{1/2} \tag{4}$$

$$R^2 = \frac{\left(\sum_{i=1}^{n}(P_{sol,exp}-\overline{P_{sol,exp}})(P_{sol,pred}-\overline{P_{sol,pred}})\right)^2}{\sum_{i=1}^{n}(P_{sol,exp}-\overline{P_{sol,exp}})^2(P_{sol,pred}-\overline{P_{sol,pred}})^2} \tag{5}$$

$$\mathrm{AAD} = \left(\frac{1}{n}\sum_{i=1}^{n}\left(\frac{P_{sol,pred}\text{-}P_{sol,exp}}{P_{sol,exp}}\right)\right) \times 100 \tag{6}$$

where n is the number of points, $P_{sol,pred}$ is the value from the RSM or ANN model, $P_{sol,exp}$ is the value from the experimental data, and the symbol $\overline{P_{sol,pred}}$ indicates the average of the equation beneath the symbol.

Values in both models were near to those of the experimental data, but the higher regression coefficient value of the ANN model indicated that it provided a better fit than the RSM model (Table 7).

Table 7. Comparison of RSM and ANN Models

	RSM	ANN
RMSE	2.27	1.36
R^2	0.78	0.92
ADD (%)	10.46	4.36

The RSM and ANN techniques were both used satisfactorily to model biotechnological processes. In one example, RSM was used to optimize the efficiency of Cr(IV) elimination in an aqueous medium, attaining a fit with 95% confidence (Jain *et al.* 2011). Another study used both RSM and ANN to model dye extraction from annatto seed with high resulting coefficients (RSM, R^2 = 0.89; ANN, R^2 = 0.95) (Sinha *et al.* 2013). In a third example, barley straw hydrolysis conditions were optimized using the variables of processing pressure, initial moisture content of wheat straw, and processing time (Maache-Rezzoug *et al.* 2011). RSM was then applied to 19 experimental results to produce a second-degree equation with a high coefficient (R^2 = 0.96), confirming that RSM was the most appropriate model for this process. Moreover, RSM and ANN have been applied to modeling and optimization for ultrasonic assisted adsorption of brilliant green and eosin B (Jamshidi *et al*. 2016), optimization of adsorption of Janus Green B from aqueous solution (Ghaedi *et al*., 2016), removal of methylene blue and Pb2+ ions (Mazaheri *et al*. 2015), ultrasound assisted ternary adsorption of dyes (Asfaram *et al*. 2015), and modeling of quaternary dyes adsorption (Alipanahpour *et al*. 2016).

CONCLUSIONS

1. Only particle size and sulfite concentration had a significant effect on the lignosulfonate yield. Neither time nor a combination of all three variables showed a significant influence on the treatment.
2. RSM and ANN techniques effectively fit experimental data from the solubilization process of barley straw in lignosulfonate production. Both techniques predicted the

lignosulfonate production in an adequate way, but the ANN technique fit the data more accurately.

3. Based on these findings, this study can be used as a guide to predict the solubilization of lignosulfonate from barley straw under different experimental conditions.

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