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Optimization with Response Surface Methodology of biosorption conditions of Hg(II) ions from aqueous media by *Polyporus Squamosus* fungi as a new biosorbent

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Abstract: Removal of mercury(II) (Hg(II)) from aqueous media by a new biosorbent was carried out. Natural *Polyporus squamosus* fungus, which according to the literature has not been used for the purpose of Hg(II) biosorption before, was utilized as a low-cost biosorbent, and the biosorption conditions were analyzed by response surface methodology (RSM). Medium parameters which were expected to affect the biosorption of Hg(II) were determined to be initial pH, initial Hg(II) concentration (C_o), temperature (T (°C)), and contact time (min). All experiments were carried out in a batch system using 250 mL flasks containing 100 mL solution with a magnetic stirrer. The Hg(II) concentrations remaining in filtration solutions after biosorption were analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Based on the RSM results, the optimal conditions were found to be 5.30, 47.39 mg/L, 20°C and 254.9 min for pH, C_o, T (°C), and contact time, respectively. Under these optimal conditions, the maximum biosorbed amount and the biosorption yield were calculated to be 3.54 mg/g and 35.37%, respectively. This result was confirmed by experiments. This result shows that *Polyporus squamosus* has a specific affinity for Hg ions. Under optimal conditions, by increasing the amount of *Polyporus squamosus* used, it can be concluded that all Hg ions will be removed.

Introduction

The pollution of water with heavy metal ions and organic pollutants is causing increasing environmental problems (Kumar and Min 2011). Specifically, heavy metal ions are widely found in wastewater from industries such as mining, metal plating, and electronics. Although some metals used in dentistry, medicine and industrial coatings, such as nickel (Ni), chromium (Cr), cobalt (Co), and molybdenum (Mo), are biocompatible, mercury, a remarkably toxic and non-biocompatible metal, is ubiquitous in the environment and derives from both natural sources and human activities. The presence of mercury in fish, wastewater, dental amalgams, vaccine preservatives, and in the atmosphere has made this particular toxic metal an increasing focus for health authorities and interest groups (Stephen Inbaraj et al. 2009, Zhang et al. 2005). Because of this, the removal of mercury from the aqueous environment has been a significant concern for public health.

For decades, traditional separation methods including chemical precipitation, chemical oxidation or reduction, filtration, ion exchange, and electrochemical processes have successfully been applied to the removal of heavy metals from industrial effluents. However, technical and economic constraints encountered in the application of these traditional methods have shown the need to search for new technological solutions for the removal of metals from waste streams. Most of these methods have several disadvantages and tend to be very expensive and time-consuming. However, among these methods, adsorption and biosorption are generally considered to be simple, relatively inexpensive and effective methods of removing heavy metals from wastewater (Hołda 2013, Ji et al. 2012, Wang et al. 2013). The use of microorganisms such as bacteria, fungi, yeast, and algae in treating wastewaters containing toxic heavy metals is gaining favor (Bhatti and Amin 2013, Bhatti and Hamid 2014, Hanif et al. 2015). Such microorganisms are able to remove heavy metals from aqueous solutions in rather substantial quantities, and their derivatives are abundant in nature and inexpensive (Kapoor and Viraraghavan 1997, 1998, Yan and Viraraghavan 2003). Fungal biosorption occurs as a result of ionic interactions and complex formation between metal ions and functional groups which are present on fungal cell surfaces. These functional groups include phosphate, carboxyl, amine and amide groups (Kapoor and Viraraghavan 1997, Yan and Viraraghavan 2003).

Traditionally, optimization has been performed by monitoring the influence of one factor at a time. While one parameter is changed, the others are held at a constant level. Its main disadvantages are that it does not account for the

interactive effects among the variables studied and that it does not indicate the complete effects of the parameter on the response. Furthermore, use of this technique requires an increased number of experiments, and additional time as well as expenses, in the consumption of reagents and materials (Bezerra et al. 2008).

In recent years, response surface methodology (RSM) has been widely used as a statistical method for rational experimental design and process optimization in the absence of mechanistic information, in contrast to traditional methods. It is a powerful optimization method which has recently been used for other optimization processes (Şahan et al. 2010). The reasons for its popularity are that it does not require additional consumption of chemicals for each parameter, nor is it especially time-consuming, costly or labor-intensive (Chi et al. 2012, Myers and Montgomery 2002, Öztürk and Şahan 2015, Şahan and Öztürk 2014). This method is based on the fit of mathematical models (linear, square polynomial functions, and others) to the experimental results generated from the designed experiment and confirmation of the model obtained via statistical techniques. The major aim of this method is to vary all significant parameters simultaneously and then fit the experimental data to a mathematical model. That is, all the parameters studied for optimization vary at the same time. As a result, it can be said that required experiment number for optimization using RSM is less than the conventional methods. Optimization via the RSM approach can be divided into six stages: (1) selection of independent variables and possible responses, (2) selection of the experimental design strategy, (3) performing the experiments and obtaining the results, (4) fitting the model equation to the experimental data, (5) obtaining response graphs and verification of the model (ANOVA), and (6) determination of optimal conditions (Krowiak et al. 2014).

The objective of this study was to optimize the most important biosorption parameters with the popular method, i.e., response surface methodology (RSM) and evaluate the use of *Polyporus squamosus* fungus as a biosorbent to remove Hg(II) ions from aqueous environments. The present study includes the investigation of the influence of biosorption parameters such as initial pH, initial Hg(II) concentration (C_o), temperature (T (°C)), and contact time (min) on treatment effectiveness. Hg(II) was selected as the model contaminant because it is the most toxic industrial pollutant and has been observed to exert negative effects at very low concentrations. In this study, central composite design (CCD) in RSM was successfully applied to optimize the biosorption conditions affecting Hg(II) biosorption onto natural *Polyporus squamosus* fungus.

Material and Methods

Biosorbent preparation and Hg(II) solutions

Natural *Polyporus squamosus* fungus was collected from Yüksekova Territory in Hakkari Province by the Yuzuncu Yil University Biology Department, Van, Turkey. Naturally obtained *Polyporus squamosus* fungus was ground with a mill and sieved to obtain the desired particle size (less than 150 µm) and then stored in desiccators for further utilization after oven drying at 40°C for 24 h. The specific surface area, pore volume, and pore radius were measured by using the Brunauer, Emmett and Teller (BET) method and found to be 1.08 m²/g, 0.007 cc/g, and 21.499 Å, respectively. The BET method has been used for nano, meso, and macro particle sizes for pore size distribution

analysis of powder and bulk samples. BET analysis provides precise evaluation of the specific surface area of materials by means of nitrogen multilayer adsorption measured as a function of relative pressure using a fully automated analyzer. The technique encompasses external area and pore area evaluations to determine the total specific surface area in m²/g, yielding important information for studying the effects of surface porosity and particle size in a variety of applications.

A stock solution of Hg(II) (100 mg/L) was prepared by dissolving weighed amount of Hg(NO$_3$)$_2$·1H$_2$O (purity 99%, Sigma-Aldrich) in 1000 mL of bidistilled water. Solutions in the range of required concentrations were prepared using a stock solution. The initial pH of each solution was adjusted to the required value with 0.1 M HNO$_3$ and 0.1 M NaOH solutions before mixing the biosorbent into the solution. The biosorbent was then added to the solution and biosorption experiment was performed. The pH values of biosorption solutions were not monitored throughout the experiments.

Batch biosorption experiments

All experiments were carried out in a batch system using 250 mL flasks containing 100 mL Hg(II) solution with a temperature--controlled magnetic stirrer. The Hg(II) concentrations remaining in filtration solutions after biosorption were analyzed using a Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). ICP-OES has more advantages than Atomic Absorption Spectroscopy (AAS) for the analysis of metals. It allows measurements to be made quickly, accurately, and with great sensitivity, for a large number of elements in both solid and liquid samples. This technique is thus extremely useful for studies requiring sensitivity in analysis. The amount of biosorbed Hg(II) was calculated according to the following equation (Şahan et al., 2010):

$$q_e = \frac{(C_O - C_e)V}{m} \tag{1}$$

where C_o and C_e are the initial and equilibrium concentrations (mg/L) of Hg(II) solution, respectively, V is the volume of the medium (L), and m is the mass (g) of the biosorbent, fixed at 5 g/L, which was used in the reaction mixture.

Experimental design and optimization

In RSM, CCD is the most popular choice for fitting a second--order model. The total number of experiments for four variables was *30 (2^k + 2k + 6)*, where k is the number of independent variables. Twenty four experiments were augmented with six replications at the center values (zero level) to evaluate the pure error. The behavior of the system is explained by the following empirical second-order polynomial model, Eq. (2) (Myers and Montgomery 2002, Şahan and Öztürk 2014):

$$\hat{y}_n = \beta_o + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_i^2 + \sum_{i=1}^{4} \sum_{j=i+1}^{4} \beta_{ij} x_i x_j \tag{2}$$

where, $\hat{y}_n$ is the response, is the constant coefficient, X$_i$ (i = 1–4) are non-coded variables, β_i is the linear, β_{ii} is the quadratic, and β_{ij} is the second-order interaction coefficient (Öztürk and Şahan, 2015).

Data were processed for Eq. (2) using Design-Expert 7.0 (trial version) program, including ANOVA, to obtain the interaction between the process variables and the response. The quality of the fit of polynomial model was expressed by the coefficient of determination (R^2), and statistical significance was checked by the F-test using the same program.

Results and discussions

CCD experiments

CCD experiments for optimization of significant parameters, such as initial pH, C_o (mg/L), T (°C), and contact time of the biosorbent with the metal solution (experiment time) (min.), were performed to locate the maximum removal of Hg(II) by Design Expert 7.0 (trial version). In CCD, values of the studied parameters are displayed at three coded levels by the program. The three coded levels of each parameter were designated -1, 0, +1. While the lowest and highest values of the parameters were -1 and +1, respectively, the middle of the lowest and highest values was marked as the center point (0). Together with six replications conducted at the center values to evaluate the pure error, twenty four experiments were completed for optimization. The experimental design of CCD and responses are shown in Table 1. The model equation for uncoded (real) values of the quadratic model fitting the experimental results is shown in Eq. 3:

$$
\begin{aligned}
\textit{Biosorbed Hg(II) amount (mg/g)} &= -1.90516 \\
&+ 0.96677\textit{(pH)} + 0.11877\textit{(C}_o) - 0.032360\textit{(°C)} \\
&+ 6.20735\text{E-}03\textit{(time)} - 2.99521\text{E-}03\textit{(pH)(C}_o) \\
&- 4.63028\text{E-}03\textit{(pH)(°C)} + 6.00575\text{E-}06\textit{(pH)(time)} \\
&- 6.32042\text{E-}04\textit{(C}_o)\textit{(°C)} + 7.60043\text{E-}05\textit{(C}_o)\textit{(time)} \\
&+ 4.92241\text{E-}05\textit{(°C)(time)} - 0.07165\textit{(pH)}^2 \\
&- 1.13188\text{E-}03\textit{(C}_o)^2 + 7.53988\text{E-}04\textit{(°C)}^2 \\
&- 2.04448\text{E-}05\textit{(time)}^2
\end{aligned}
\tag{3}
$$

Table 1. Experimental design matrix of four parameters in coded (-1, 0, +1) and natural units generated by Central Composite Design (CCD) in Response Surface Methodology (RSM) and biosorbed Hg(II) amount for each experiment as response

Run	Initial pH (X_1)	Initial Hg(II) conc. (C_o, mg/L) (X_2)	Temperature (°C) (X_3)	Contact time (min) (X_4)	Biosorbed Hg(II) amount (mg/g)
1	5 (0)	30 (0)	35 (0)	155 (0)	2.65
2	5 (0)	30 (0)	35 (0)	10 (-1)	1.01
3	2 (-1)	10 (-1)	20 (-1)	300 (+1)	0.75
4	8 (+1)	50 (+1)	50 (+1)	10 (-1)	0.30
5	2 (-1)	10 (-1)	20 (-1)	10 (-1)	0.45
6	2 (-1)	50 (+1)	20 (-1)	300 (+1)	2.46
7	5 (0)	30 (0)	35 (0)	300 (+1)	3.38
8	5 (0)	10 (-1)	35 (0)	155 (0)	1.41
9	8 (+1)	10 (-1)	20 (-1)	300 (+1)	1.33
10	2 (-1)	30 (0)	35 (0)	155 (0)	2.14
11	8 (+1)	10 (-1)	50 (+1)	300 (+1)	1.35
12	2 (-1)	10 (-1)	50 (+1)	300 (+1)	0.89
13	5 (0)	30 (0)	50 (+1)	155 (0)	2.91
14	8 (+1)	50 (+1)	20 (-1)	300 (+1)	3.16
15	2 (-1)	50 (+1)	50 (+1)	300 (+1)	2.94
16	5 (0)	30 (0)	35 (0)	155 (0)	2.64
17	5 (0)	30 (0)	35 (0)	155 (0)	2.77
18	5 (0)	50 (+1)	35 (0)	155 (0)	2.93
19	5 (0)	30 (0)	35 (0)	155 (0)	2.63
20	8 (+1)	50 (+1)	20 (-1)	10 (-1)	1.61
21	8 (+1)	30 (0)	35 (0)	155 (0)	1.83
22	8 (+1)	50 (+1)	50 (+1)	300 (+1)	1.36
23	2 (-1)	10 (-1)	50 (+1)	10 (-1)	0.37
24	8 (+1)	10 (-1)	20 (-1)	10 (-1)	0.97
25	5 (0)	30 (0)	35 (0)	155 (0)	2.70
26	2 (-1)	50 (+1)	20 (-1)	10 (-1)	1.61
27	8 (+1)	10 (-1)	50 (+1)	10 (-1)	0.39
28	2 (-1)	50 (+1)	50 (+1)	10 (-1)	0.72
29	5 (0)	30 (0)	35 (0)	155 (0)	2.60
30	5 (0)	30 (0)	20 (-1)	155 (0)	2.69

The statistical significance of the quadratic model was evaluated by the analysis of variance (ANOVA) as shown in Table 2. The value of the coefficient of determination ($R^2 = 0.924$) indicates that 92% of the variability in the response is explained by the model.

A plot showing observed removal of Hg(II) versus that obtained from Eq. 3 is shown in Fig. 1. The figure indicates that the predicted response from the empirical model is in good agreement with the observed data.

Fig. 2 shows normal percentage probability versus residuals. As the points on the plot follow a straight line, it can be concluded that the residuals are normally distributed and data transformation is not required. Therefore, the prediction

of the experimental data obtained from the quadratic model developed for the biosorption of Hg(II) onto *Polyporous squamosus* biosorbent is quite satisfactory.

It is usually necessary to check the fitted model to ensure that it provides an adequate approximation of the real system. Unless the model shows an adequate fit, proceeding with investigation and optimization of the fitted response surface will likely give poor or misleading results. The residuals play an important role in judging the adequacy of the model. Fig. 3 shows that the residuals were randomly scattered around ± 2. Based on this result, it can be concluded that the experimental data fit with the predicted data calculated from Eq. 3. (Myers and Montgomery 2002).

Table 2. Analysis of variance (ANOVA) for quadratic model obtained according to the experimental results of Central Composite Design (CCD)

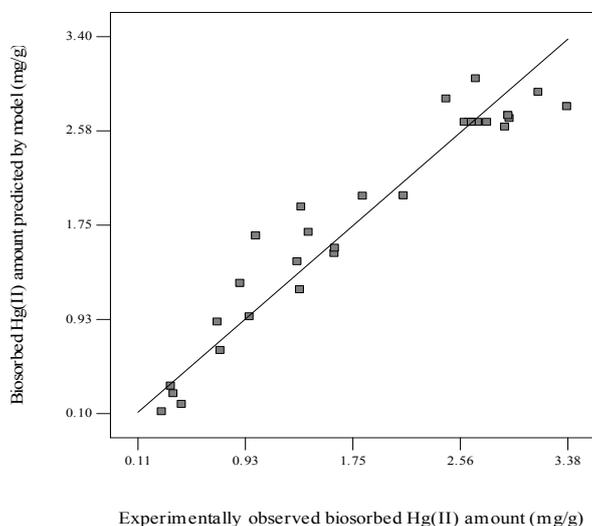| Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	25.26466	14	1.804619	13.02349	< 0.0001
X_1-Initial pH	8.28E-05	1	8.28E-05	0.000597	0.9808
X_2-Initial conc. (C_o)	4.696908	1	4.696908	33.89643	< 0.0001
X_3-Temperature (°C)	0.80104	1	0.80104	5.780911	0.0296
X_4-Contact time (min.)	5.763465	1	5.763465	41.5935	< 0.0001
$X_1 X_2$	0.516745	1	0.516745	3.729223	0.0726
$X_1 X_3$	0.694639	1	0.694639	5.013037	0.0407
$X_1 X_4$	0.000109	1	0.000109	0.000788	0.9780
$X_2 X_3$	0.575246	1	0.575246	4.151411	0.0596
$X_2 X_4$	0.777307	1	0.777307	5.609631	0.0317
$X_3 X_4$	0.183398	1	0.183398	1.323538	0.2680
X_1^2	1.07739	1	1.07739	7.775261	0.0138
X_2^2	0.531097	1	0.531097	3.832798	0.0691
X_3^2	0.074567	1	0.074567	0.538131	0.4745
X_4^2	0.478731	1	0.478731	3.454882	0.0828

Fig. 1. Hg(II) biosorption capacity predicted by Eq. 3 versus the experimentally observed Hg(II) biosorption capacity of biosorbent
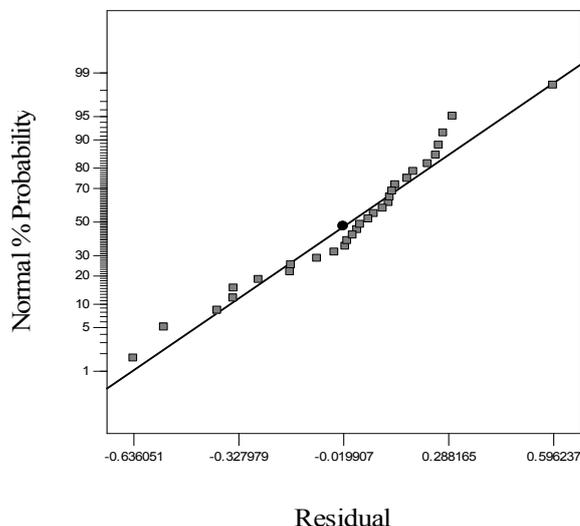

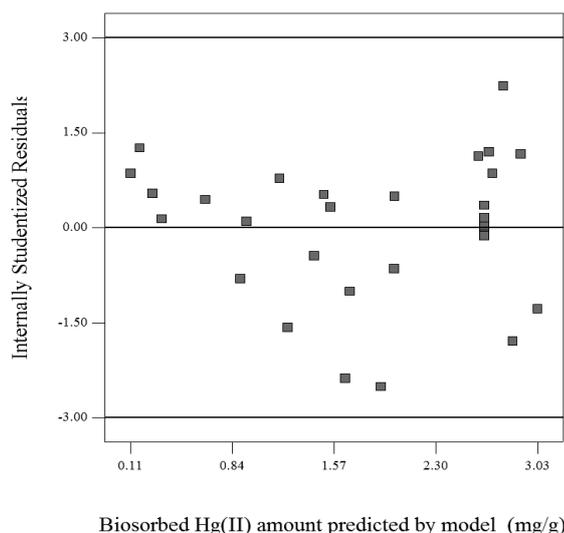**Fig. 2.** Validation of the prediction of Hg(II) biosorption residuals versus normal % probability

Fig. 3. Studentized residuals versus Hg(II) biosorption predicted by model

Fig. 4 shows 3-D simultaneous effects of initial pH and C_o on the Hg(II) biosorption capacity of *Polyporus squamosus* fungus. The initial pH of the biosorption solution is an important parameter affecting the biosorption of Hg(II) ions from aqueous solutions by different biosorbents. As shown in Fig. 4, biosorption capacity sharply increased with an increase from 2.0 to 5.30 in the initial pH of the Hg(II) solution. Above pH 5.3, the biosorption capacity decreased with increased initial pH. When the pH increases, the negative charge density on the biosorbent surface increases due to deprotonation of the metal binding sites. The biosorption of the positively charged Hg(II) ions on the negatively charged biosorbent surface increases due to electrostatic attraction forces. At lower pH values, the Hg(II) biosorption capacity of the biosorbent is decreased due to the competition between the excess hydronium (H_3O^+) ions and the positively charged Hg(II) ions for the active biosorption sites. At higher pH values, Hg(II) ions might precipitate as $Hg(OH)_2$ due to excessive concentration of OH^- ions in the biosorption solution. Therefore, we did not investigate pH values greater than 8. Similar results were found in previous studies (Singh et al. 2010, Wang et al. 2013). The surface charge density of a biosorbent is related to the pH of the media. The point of zero charge (PZC) is an important parameter to clarify the effect of pH on biosorption. At PZC, the charges from cations and anions are equal and the total charge of the biosorbent is zero. The pH_{pzc} for *Polyporus squamosus* biosorbent was calculated with experiments performed according to a method in the literature (Balistrieri and Murray 1981) as 4.8 (Fig. 5). At pH levels above the pH_{pzc} 4.8, because the biosorbent surface is mostly negatively charged with deprotonated surface sites, a high biosorption was observed. At pH levels below the pH_{pzc} 4.8, the low biosorption capacity is due to the increase in positive charge density on the surface sites, and thus, electrostatic repulsion occurs between the metal ions and surface sites (Öztürk and Şahan 2015).

Fig. 4 shows that the biosorbed Hg(II) amount rapidly increased with increasing C_o. With increasing metal ion concentration, there is an increase in the amount of biosorbed metal ions due to the increasing driving force of the metal ions toward the active sites on the biosorbent. Because it

provides an important driving force to overcome all mass transfer resistance of Hg(II) ions between solid and aqueous phases, biosorption capacity increases at higher initial Hg(II) concentrations in the biosorption medium. When C_o was between 40 and 50 mg/L, the metal uptake reached equilibrium and all sites were saturated with metals. This has also been reported in similar studies in the literature (Şahan et al. 2010).

As clearly seen in Fig. 6, temperature has a negative effect when increasing from 20 to 40°C. The decrease of Hg(II) biosorption with increasing temperature indicates that Hg(II) biosorption onto *Polyporus squamosus* in the 20 and 40°C temperature range is exothermic in nature. This result may be due to the weakening of biosorptive forces between the active sites of the biosorbents and Hg(II) ions, and also between the adjacent molecules of the biosorbed phase. In addition, the decrease in biosorption capacity at higher temperatures may be attributed to the damage caused by active binding sites on the biosorbent (Şahan et al. 2010). No change in Hg(II) biosorption by *Polyporus squamosus* fungus was seen with the increase in temperature from 40 to 50°C. As shown in Fig. 6, in order to determine the biosorption equilibrium
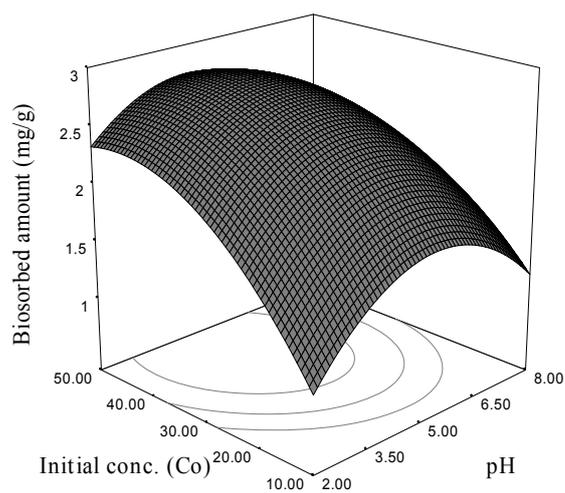

Fig. 4. 3-D response surface plot of simultaneous effects of initial pH and C_o at fixed temperature of 35°C and fixed contact time of 155 min
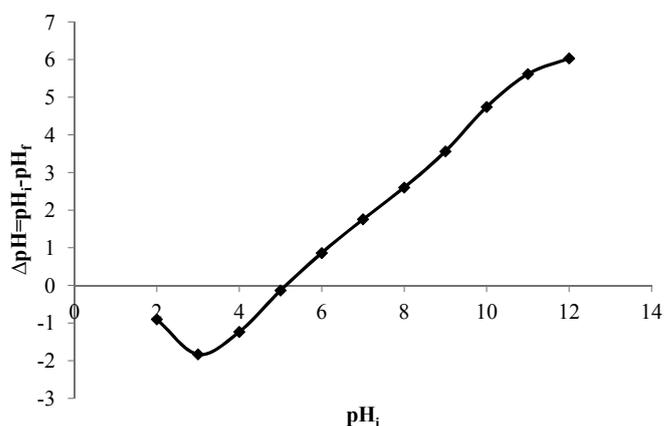

Fig. 5. Point of zero charge of *Polyporous squamosus* biosorbent

time for Hg(II) ions, the contact time was varied between 10 to 300 min. Most of the Hg(II) ions were rapidly biosorbed from aqueous solution within the first 160 min, then the biosorption rate became slower. Biosorption equilibrium was achieved after about 250 min. The rapid biosorption was observed at regular intervals from 10 to 160 min. After 160 min., occupation of the vacant sites remaining on the biosorbent will be impeded by the repulsive forces between the metal ions biosorbed on the biosorbent and in the aqueous phase (Tunalı Akar et al. 2009).

Determination of optimal biosorption conditions

The optimal biosorption conditions for removal of Hg(II) were found to be 5.30, 47.39 mg/L, 20°C and 254.9 min for initial pH, C_o, T (°C), and time (min.), respectively, based on a method used previously in the literature (Şahan and Öztürk 2014). Under these optimal conditions, the maximum biosorbed amount and removal yield were calculated by the quadratic model to be 3.54 mg/g and 37.35%, respectively. To confirm these values predicted by the model, several independent experiments for Hg(II) biosorption onto *Polyporus squamosus* were conducted under the optimal biosorption conditions. The

observed experimental results were very close to the predicted values. Thus, it can be concluded that RSM combined with an appropriate experimental approach can be effectively utilized to optimize the most important experimental conditions affecting the biosorption process.

Table 3 presents a comparison between the Hg(II) biosorption capacity of *Polyporus squamosus* and some biosorbents used in the literature. *Polyporus squamosus* is an inexpensive biosorbent and is abundant in nature. As can be seen from Table 3, we can conclude that *Polyporus squamosus* has a higher biosorption capacity for Hg(II) ions than some other natural biosorbents. Due to these properties, *Polyporus squamosus* has great potential for the removal of Hg(II) from aqueous environments.

This study presents an original contribution to the literature due to the biosorbent used and the optimization method for Hg(II) biosorption. Studies on the removal of heavy metals by biosorbents such as fungi are limited. In addition, *Polyporus squamosus* fungus is a natural material that does not produce toxic hazardous waste and is found abundantly in nature. Thus, it is considered to have potential for use in different areas. In addition, the metal ion mercury studied in the present work is highly toxic and its removal from waste and drink water has became increasingly important. Biosorption of Hg(II) from an aqueous environment by a new biosorbent, *Polyporus squamosus*, will make a significant contribution to the literature because it is a novel adsorbent-adsorbate combination.

The experimental method used in this study is the conventional batch method; the *Polyporus squamosus* fungi collected from Hakkari, Turkey were first used for Hg(II) removal from an aqueous environment. In addition, the statistical method of RSM, used to optimize the biosorption conditions, has many advantages in terms of cost and time. It provides more experimental data and graphic with very little experimentation necessary.

Conclusion

CCD in RSM was successfully used to optimize the biosorption conditions for Hg(II) biosorption onto *Polyporus squamosus* fungus. A quadratic model was improved in terms of initial pH, C_o, T(°C), and time to represent the maximum biosorbed Hg(II). With the obtained quadratic model, the
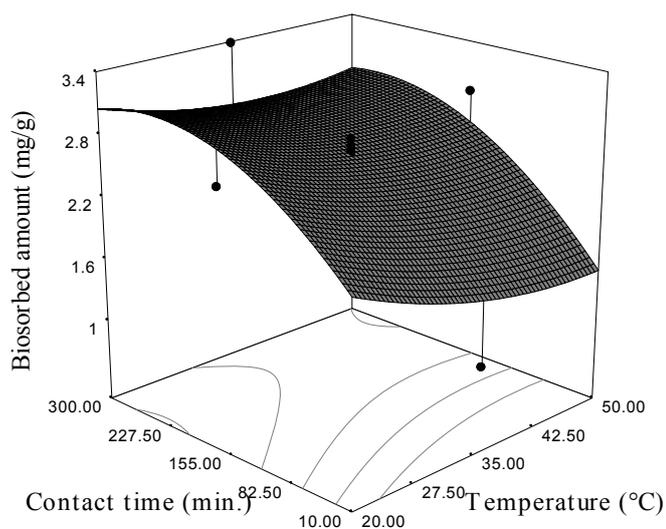

Fig. 6. 3-D response surface plot of simultaneous effects of temperature and contact time at fixed C_o of 30 mg/L and fixed initial pH of 5

Table 3. Comparison between *Polyporus squamosus* and other some biosorbents used in the literature

Biosorbent	Hg(II) Biosorption (mg/g)	References
Streptococcus pyogenes	4.8	(Tüzen et al. 2009)
Garlic (*Allium sativum* L.)	0.65	(Eom et al. 2011)
Guava bark	3.4	(Lohani et al. 2008)
Alkaline modified *Penicillium oxalicum var. armeniaca*	270	(Svecova et al. 2006)
Aspergillus niger	3.2	(Karunasagar et al. 2003)
Ulva lactuca	0.21	(Henriques et al. 2015)
Polyporus squamosus	3.54	This study

optimal conditions for maximum biosorbed Hg(II) were calculated to be 5.30, 47.39 mg/L, 20°C and 254.9 min for pH, C_o, T(°C) and time, respectively. Under the determined optimal conditions, the maximum amount of biosorbed Hg(II) and removal yield were calculated using the quadratic model to be 3.54 mg/g and 37.35%, respectively. *Polyporus squamosus*, which is abundant and readily available in nature, can be used as an effective biosorbent for the removal of heavy metal ions such as Hg(II).

Acknowledgement

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