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Response Surface Methodology Based Nickel Bioremoval by *Penicillium citrinum* Grown in Dilute Acid Pretreated Lignocellulosic Material

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Abstract

The present study demonstrates the effectiveness of Ni (II) bioremoval by *Penicillium citrinum* fungus cultivated in carrot pomace medium. Experimental model for Ni (II) removal was developed using central composite design (CCD) based on response surface methodology (RSM). According to the model, the effects of some key parameters such as pH, initial Ni (II) loading, and initial carrot pomace loading on Ni (II) bioremoval was found as significant ($p < 0.05$). The highest bioremoval was oberved as 82.01% in the presence of pH 5, 50 mg/L initial Ni (II), and 100 g/L initial biomass loadings, respectively. Results revelaed that the usage of *Penicillium citrinum* were proven to be effective in removing of Ni (II).

Keywords: *Penicillium citrinum;* Bioremoval; Response surface methodology.

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Seyreltik Asit Ön-İşlemi ile Muamele Edilen Lignoselülozik Materyalde Geliştirilen *Penicillium citrinum*'un Yüzey Tepki Metodolojisi Temelli Nikel Biyogiderimi

Öz

Bu çalışma, havuç posası içeren besiyerinde geliştirilen *Penicillium citrinum* fungusunun Ni (II) biyogiderimini etkin bir şekilde gerçekleştirdiğini göstermektedir. Çalışmada Ni (II) giderimi adına yanıt yüzey metodolojisine (RSM) dayalı merkezi bileşik tasarım (CCD) kullanılarak deneysel bir model geliştirilmiştir. Modele göre, başlangıç Ni (II) ve havuç posası konsantrasyonu ile pH gibi bazı önemli parametrelerin Ni (II) biyogiderimi üzerindeki etkileri anlamlı bulunmuştur ($p < 0.05$). En yüksek giderim 50 mg/L Ni (II), 100 g/L biyokütle ve pH 5'te %82.01 olarak gözlenmiştir. Sonuçlar, *Penicillium citrinum* kullanımının Ni (II) 'nin gideriminde etkili olduğunu ortaya koymaktadır.

Anahtar Kelimeler: *Penicillium citrinum*; Biyogiderim; Tepki yüzey metodolojisi.

1. Introduction

As a result of industrialization, hazardous substances such as heavy metals cause serious problems for the environment, animals, and plants. These molecules can be harmful when the proper treatment methods are not performed [1]. The toxic effects of heavy metals are well known in the literature. They affect the brain, kidney, skin, or lung negatively, and they also have carcinogenic effects on certain organisms [2]. Therefore, different removal techniques such as physical, chemical, and biological are carried out for bioremoval of heavy metals from the wastewaters.

Although physicochemical methods can provide effective heavy metal removal, high capital costs and expensive regeneration processes limit their usage [3]. On the other hand, bioremoval is a cheap and effective alternative to the other techniques. Microorganisms produce high biomass yields; they have a fast growth rate and suitable for genetic manipulations [4]. For these reasons, different microorganisms such as fungi are used for textile dye or heavy metal removal from aquatic environments [5]. *Penicillium citrinum* is a widespread mesophilic fungus naturally found on different plants such as wheat or citrus species. Biotechnological applications of *P. citrinum* such as uranium (IV) biosorption [6], metabolite and enzyme production [7, 8], or textile dye removal [9] were shown in the literature previously.

Lignocellulosic biomass is one of the most abundant and underutilized raw materials on Earth. Cellulose and hemicellulose present in the lignocellulose contain fermentable sugars, which are very suitable for microbial growth. However, to obtain fermentable sugars from lignocellulose, efficient pretreatment techniques are required [10]. Carrot pomace is an essential by-product of the juice and food industries. Millions of tons of carrot pomace are generated each year. After carrot processing, 50% of the raw material remains as pomace and this pomace contains neutral sugars, carotenoids, macroelements, and minerals (N, P, K, Mg, Na, Ca, Cu, Mn, Fe and Zn) which are essential for microbial growth and metabolic activity. Furthermore, carrot pomace has high sugar and nutritional content, and it can also accumulate the free sugars in its vacuoles [11, 12]. Therefore, numerous studies about the carrot pomace are carried out in the literature [13, 14].

Most of the biological optimization studies focus on the evaluation of one variable at a time. However, the combined effect of different variables is significant for optimization. Response Surface Methodology (RSM) allows identifying the interaction between controllable factors. RSM also defines the effects of different factors on the response alone or combined [15]. For these reasons, RSM studies have been used in many other areas such as biofuel production [16], dye biosorption [17], or heavy metal bioremoval [18].

The aim of the current study was to investigate the experimental model for Ni (II) bioremoval by *P. citrinum*. Within this context, effects of initial Ni (II) concentration, pH and initial biomass loading were optimized by response surface methodology. This is the first report about the Ni(II) bioremoval of *P. citrinum* cultivated in carrot pomace by RSM optimization.

2. Materials and Methods

2.1. Raw materials and pretreatment

Ground carrot pomace (approximately smaller than 0.2 mm particle size) was obtained from BELSO Co./Ankara and was dried in an oven at 70 °C overnight. Dried carrot pomace was kept in screw cap bottles until pretreatment. Pretreatment was carried out in autoclave with 1% H_2SO_4 for 15 min at 121 °C. The slurry was filtered through Whatman No:1 paper and was used in bioremoval experiments. The sugar concentration of carrot pomace was also determined before and after the experiments.

2.2. Microorganisms

P. citrinum was obtained from Ankara University Culture Collection. The fungus was kept in Potato Dextrose Agar (PDA) at +4 °C until experiments.

2.3. Culture conditions

250 mL Erlen-Meyer flasks working volume with 100 mL were used for the experiments. Agitation speed and temperature were set as 125 rpm. and 30 °C, respectively. Uninoculated flasks with different pH, initial biomass nickel loadings were used as control. All experiments were performed triplicate.

2.4. Bioremoval with RSM studies

Design Expert Software® 12 was used for RSM experiments. Central composite design (CCD) with three levels was used to evaluate the effects of three variables on Ni(II) bioremoval of *P. citrinum*. Total 17 runs were created for RSM. pH (3-7), initial nickel loading (50-150 mg/L), and initial biomass loading (50-150 g/L) were selected as independent factors. The data obtained from experiments, was statistically evaluated using analysis of variance (ANOVA) at significance level of $p < 0.05$ by using Design Expert Software® 12.

2.5. Analytical methods

Ni(II) removal was measured spectrophotometrically (Shimadzu) at 340 nm. wavelength, according to Snell and Snell [19]. Total reducing sugar concentrations were measured according to the DNS method [20].

3. Results

3.1. Composition of carrot pomace

In the current study, carrot pomace was used for Ni (II) bioremoval of *P. citrinum*. Carrot pomace was pretreated with dilute acid (1% H_2SO_4 v/v). Reducing sugar concentrations of different carrot pomace loadings were examined. Results were shown in Fig. 1. It was observed that increasing biomass loadings resulted in increased sugar contents. The highest sugar was obtained from 150 g/L carrot pomace as 52.72 g/L. Sugar concentrations reduced to 24.69 g/L when 50 g/L carrot pomace was used. Moreover, in 100 g/L carrot pomace loading, 38.63 g/L sugar was detected.

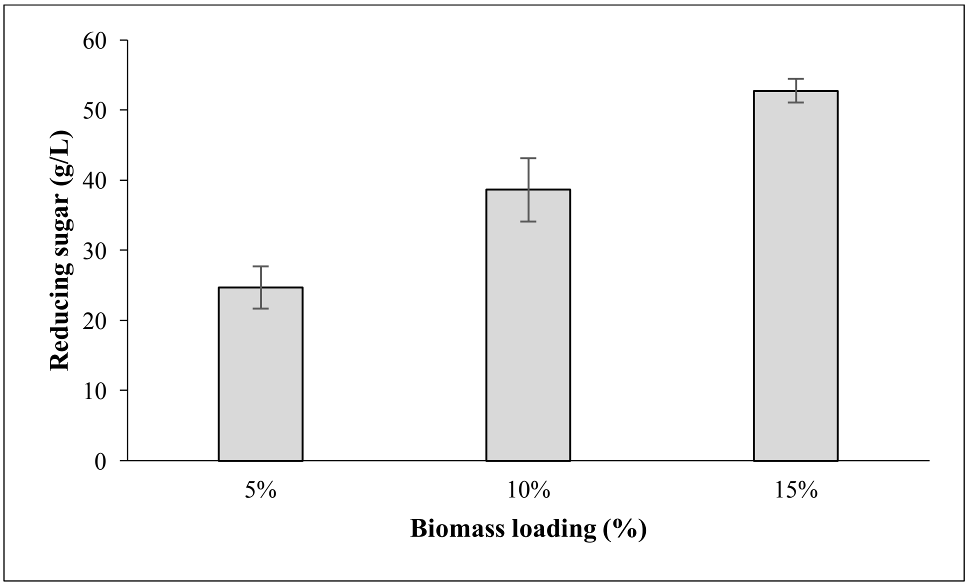

Figure 1: Reducing sugar concentrations of different carrot pomace loadings (Pretreatment: 1% H_2SO_4 for 15 min at 121 ºC)

3.2 Response Surface Methodology for Ni(II) Bioremoval

In RSM experiments, central composite design was employed to investigate the effect of pH, initial nickel, and biomass loadings. The descriptive table of the independent variables and bioremoval were shown as coded values in Table 1.

Table 1: Descriptive table of coded level for RSM analysis

Factor	Name	Minimum	Maximum	Coded Low	Coded High
A	**pH**	3	7	3	7
B	**Initial nickel concentration**	50	150	50	150
C	**Biomass loading**	5	15	5	15

The equation for the bioremoval yield was given in Eq (1). The final equation in terms of coded factors:

Eq (1): $\text{Bioremoval} = -231.79 + 102.33*A + 0.26*B + 2.48*C - 0.028*AB + 1.095*AC - 0.025*BC - 10.39*A^2 + 0.000030*B^2 - 0.152*C^2$

where A, B, C are the coded values of pH, initial nickel loading (mg/L), and biomass loading (%), respectively.

The effects of different variables on bioremoval were shown in Table 2. Response surface graphs were also shown in Fig. 2 A, B, and C. The highest removal was found at the end of the 3rd day of incubation. Therefore, RSM studies were carried out for the results of the 3rd day of incubation.

Table 2: Effect of different variables on Ni(II) bioremoval of *P. citrinum*

Std	**Run**	**Factor 1 A: pH**	**Factor 2 B: Initial nickel concentrations (mg/L)**	**Factor 3 C: Biomass loading (%)**	**Response 1 Bioremoval (%)**
4	**1**	7	150	5	20.71
12	**2**	5	150	10	59.06
10	**3**	7	100	10	40.43
2	**4**	7	50	5	15.68
6	**5**	7	50	15	81.72
15	**6**	5	100	10	73.54
9	**7**	3	100	10	17.31
11	**8**	5	50	10	82.01
8	**9**	7	150	15	46.00
3	**10**	3	150	5	14.08
16	**11**	5	100	10	77.64
5	**12**	3	50	15	19.82
17	**13**	5	100	10	75.09
13	**14**	5	100	5	53.87
7	**15**	3	150	15	11.18
1	**16**	3	50	5	13.22
14	**17**	5	100	15	79.45

According to model *p* values of the pH, initial nickel and biomass loading were found as 0.0003, 0.0155, and 0.0004, respectively, and pH was found as the most significant parameter and was followed by initial biomass loading. The highest Ni (II) removal was observed as 82.01% when pH, initial nickel, and biomass loadings were adjusted as 5, 50 mg/L, and 100 g/L. Moreover, it was found that increasing Ni (II) concentrations caused lower removal yields. The lowest removal was 11.18% in pH 3, 150 mg/L initial Ni (II) loading, and 150 g/L biomass loading, respectively. According to the data in Fig. 2 A, B, and C, higher removal rates were observed in pH 5 and lower Ni (II) loadings. Furthermore, higher Ni (II) removal was observed from increased biomass loadings.

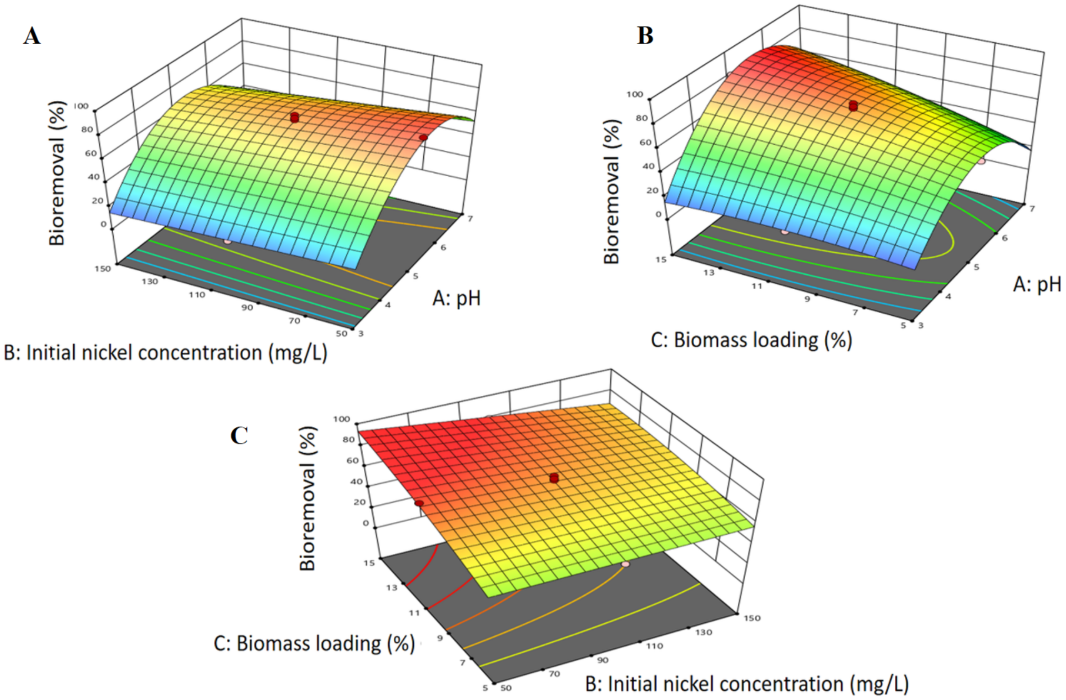

Figure 2: Response surface graphs of Ni bioremoval by *P. citrinum* in the presence of different conditions. **A:** Effect of pH and initial nickel concentration on bioremoval, **B:** Effect of pH and biomass loading on bioremoval, **C:** Effect of biomass loading and initial nickel concentration on bioremoval (Pretreatment: 1% H_2SO_4 for 15 min at 121 ºC, incubation time 72 hours, agitation speed: 125 rpm, T: 30 ºC)

Bioremoval of Ni (II) in the presence of pH 5, 50 mg/L initial Ni (II), and 50 g/L initial carrot pomace loadings were also given in Fig. 3. According to the figure, it was observed that *P. citrinum* consumed reducing sugars, and almost complete depletion occurred at the end of the 96 hours incubation period. Moreover, the highest removal was detected at the end of the 72 hours of incubation. After 72 hours, a slight decrease was found in the removal rates. Ni (II) removal was obtained as 22.84%, 72.69%, 82.01%, and 71.76% at the end of the 24, 48, 72, and 96 hours of incubation.

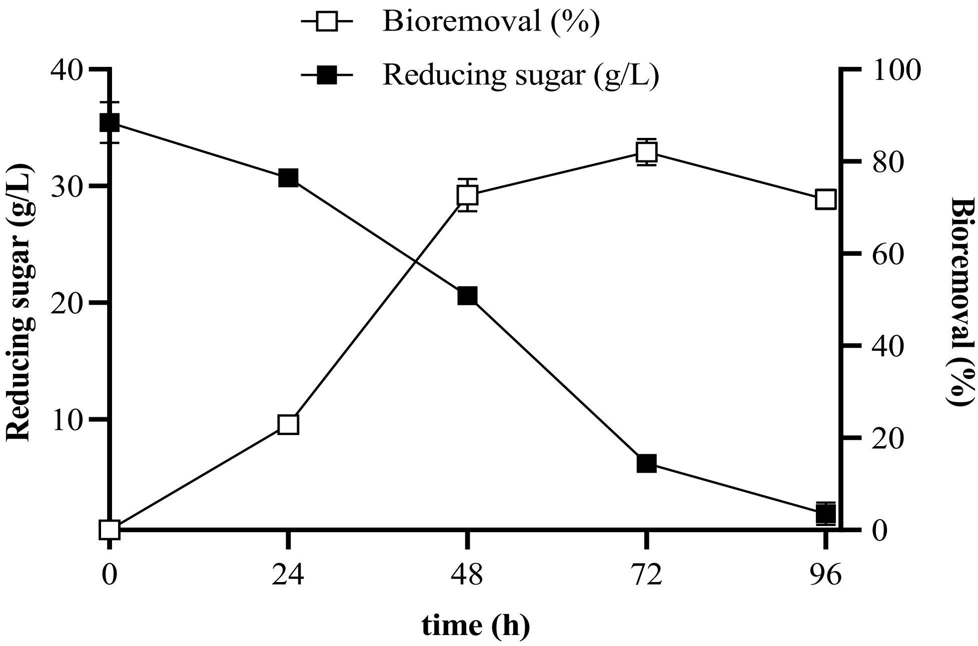

Figure 3: Time course of Ni (II) removal and reducing sugars during incubation of *P. citrinum* (Pretreatment: 1% H_2SO_4 for 15 min at 121 ºC, pH:5, initial Ni (II) concentration: 50 mg/L, initial carrot pomace loading: 100 g/L, agitation speed: 125 rpm, T: 30 ºC)

4. Discussion

During acidic pretreatment, some inhibitory compounds derived from biomass such as phenolics, weak acids, or furan derivatives can be released into the medium. These molecules have a negative impact on microbial growth and metabolism [21]. Therefore, the concentration of reducing sugars from acid pretreated carrot pomace was determined. It was observed that increasing biomass loadings resulted in higher sugar. In the literature, similar reports showed that increased biomass loading caused higher sugar amounts. In a study about switchgrass, approximately 15 g/L reducing sugar was observed in the presence of 50 g/L initial biomass loading. This value increased around 45 g/L when biomass loading was increased to 200 g/L [22].

Despite the higher biomass loadings, such as 150 g/L, any lagging or inhibition was not observed in the growth of *P. citrinum*. This tolerance can be attributed to the inhibitor-resistant nature of *P. citrinum* cells. Similarly, Karpe et al. [23] reported that *P. citrinum* could virtually degrade hydrothermally pretreated grape wastes, which contained inhibitors such as 5-HMF. Furthermore, Tejas et al. [24] showed that *P. citrinum* could remove 70% phenolic substances from paper mill wastewaters with fungal fermentation. These results indicate that *P. citrinum* is a suitable microorganism for bioremoval studies.

During RSM studies, it was indicated that *P. citrinum* effectively removed Ni(II) from carrot pomace medium, and pH 5 was favorable for *P. citrinum*. Similarly, in one study, the highest Cu, Pb, and Zn removal rates of *P. citrinum* were observed in pH 6 [25]. Medium pH is one of the most critical elements of metal uptake. In lower pH values, repulsive forces between the fungal cell surface and metal ions causedecreased metal binding to the surface. On the other hand, when pH increased, precipitation can occur, which may lead to reduction in bioremoval yields [26].

Moreover, lower bioremoval rates were observed when initial Ni(II) loading was increased (Figure 1a). This decline can be explained by the inhibitory effect of Ni(II). In the literature, it was previously shown that increased Ni(II) loadings led to lower bioremoval by *Aspergillus versicolor* [27]. Moreover, in a study about Cu(II) and Ni(II) bioremoval of *Aspergillus sp*., the highest removal in the presence of 50 mg/L initial Ni(II) was found as 86%. On the other hand, when the initial Ni(II) loading increased to 100 mg/L, bioremoval decreased to 78% [28].

Initial biomass loading is an essential parameter for microbial growth and bioremoval. Lower biomass loading can cause lower microbial growth. On the other hand, in higher biomass loading, inhibitory compounds can be released into the removal environment. Therefore, the effect of initial biomass loading on bioremoval was also investigated in the present study. According to Figure 1c, increased biomass loadings resulted in higher Ni(II) removal. In the literature, it was reported that higher sugar concentrations caused higher bioremoval rates. For instance, Kapdan and Kargi [29] found the decolorization of dyestuff by *Coriolus versicolor* as 54% in the presence of 5 mg/L glucose. Decolorization increased to 77% when the initial glucose loading was 10 mg/L.

5. Conclusions

In the present study, response surface methodology was applied for Ni(II) removal by *P. citrinum.* Carrot pomace, which is a cheap and abundant by-product of food and juice industries, was used as a growth environment. Some critical parameters such as pH, initial nickel loading, and initial biomass loading were optimized. *P. citrinum* effectively removed Ni(II) from carrot pomace medium. The highest bioremoval was observed as 82.01% in the presence of 50 mg/L Ni(II), pH 5, and 100 g/L initial carrot pomace loading. It was also detected that increased biomass loading resulted in higher removal rates, and removal rates decreased in the presence of increasing Ni(II) concentrations. This study reveals that the carrot pomace is a suitable raw material for the growth of microorganisms such as fungi, and *P. citrinum* is a promising bioagent for heavy metal removal.

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