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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

Enzymatic Bioregeneration of Activated Carbon by Laccase

Aktif Karbonun Lakkaz ile Enzimatik Biyorejenerasyonu

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Abstract

Activated carbon is widely used in combination with biological treatment systems for the treatment of organic compounds, which are refractory or toxic in conventional biological treatment systems. In these systems, compounds adsorbed on activated carbon may desorb within time due to a concentration gradient between adsorbent and the bulk liquid caused by the biodegradation of substrates in the liquid phase by microorganisms. The desorbed compounds are further biodegraded by microorganisms. This mechanism is called bioregeneration of activated carbon. Previous studies showed that bioregeneration percentages could be higher than the concentration gradient-driven desorbability. This was attributed to exoenzymatic bioregeneration occurring due to the activity of extracellular enzymes secreted by microorganisms in these systems. These extracellular enzymes can diffuse into the activated carbon pores where they can react with the previously adsorbed compounds resulting in their desorption from the carbon surface and degradation. However, the effect of extracellular enzymes on bioregeneration was not conclusively proven in any of the literature studies on bioregeneration because extracellular enzymes were not directly used for the purpose of bioregeneration. In this study, enzymatic bioregeneration of activated carbon was investigated by directly using an extracellular enzyme, laccase, which is known from the literature to catalyze the oxidation reactions of phenolic substances and is commercially available in its pure form. Therefore phenol, 2-nitrophenol, and bisphenol-A were used as the target compounds. For this purpose, batch adsorption, abiotic desorption, enzymatic degradation and enzymatic bioregeneration experiments were performed using two different activated carbon types; thermally and chemically activated ones. The results showed that there was a significant difference between the total enzymatic bioregeneration efficiencies and abiotic desorption efficiencies for each phenolic compound depending on the activated carbon type. Thereby, exoenzymatic bioregeneration has been quantitatively shown for the first time in the literature.

Key Words: activated carbon, phenol, 2-nitrophenol, bisphenol-A, bioregeneration, laccase

Öz

Aktif karbon, geleneksel biyolojik arıtma sistemlerinde refrakter veya toksik olan organik bileşiklerin arıtımında biyolojik arıtma sistemleriyle kombinasyon halinde yaygın olarak kullanılmaktadır. Bu sistemlerde, aktif karbon üzerinde adsorbe edilen bileşikler, mikroorganizmaların sıvı fazındaki substratları degrede etmesi neticesinde oluşan konsantrasyon gradyanı nedeniyle desorbe olabilir ve daha sonrasında biyolojik olarak parçalanabilir. Bu mekanizmaya aktif karbonun biyorejenerasyonu denir. Önceki çalışmalar, biyorejenerasyon yüzdelerinin konsantrasyon gradyanı kaynaklı desorplanabilirlikten daha yüksek olabileceğini gösterdi. Bu durum bu sistemlerde mikroorganizmalar tarafından salgılanan hücre dışı enzimlerin aktivitesiyle gerçekleşen ekzoenzimatik biyorejenerasyona atfedildi. Bu hücre dışı enzimler, aktif karbon gözeneklerine girerek, daha önce adsorbe edilen bileşiklerle reaksiyona girebilmekte ve bunların karbon yüzeyinden desorpsiyonuna ve degrede olmasına neden olabilmektedir. Ancak literatürde biyorejenerasyon üzerine yapılan çalışmaların hiçbirinde enzimlerin biyorejenerasyon üzerindeki etkisi kesin olarak kanıtlanamamıştır. Çünkü hücre dışı enzimler bu amaçla doğrudan kullanılmamıştır. Bu çalışmada, literatürde fenolik maddelerin oksidasyon reaksiyonlarını katalize ettiği bilinen hücre dışı bir enzim olan ve ticari olarak saf haliyle temin edilebilen lakkaz enzimi kullanılarak aktif karbonun enzimatik biyorejenerasyonu araştırılmıştır. Hedef bileşikler olarak fenol, 2-nitrofenol ve bisfenol-A kullanılmıştır. Bu amaçla termal ve kimyasal olarak active edilmiş iki farklı aktif karbon tipi kullanılarak kesikli adsorpsiyon, abiyotik desorpsiyon, enzimatik biyodegradasyon ve enzimatik biyorejenerasyon deneyleri yapılmıştır. Sonuçlar, aktif karbon tipine bağlı olarak her bir fenolik bileşik için toplam enzimatik biyorejenerasyon verimleri ile abiyotik desorpsiyon verimleri arasında önemli bir fark olduğunu göstermiştir. Böylece literatürde ilk kez ekzoenzimatik biyorejenerasyon niceliksel olarak da gösterilmiştir.

Anahtar Kelimeler: aktif karbon, fenol, 2-nitrofenol, bisfenol-A, biorejenerasyon, lakkaz

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I. INTRODUCTION

In the activated carbon process, adsorbed pollutants fill the activated carbon pores over time and the adsorption capacity of the activated carbon decreases. For this reason, activated carbon must be disposed of or regenerated at the end of the process. However, regeneration techniques using chemical and thermal methods are often expensive. Biological regeneration, or in other words bioregeneration, of activated carbon is suggested as an alternative method in the literature [1]. In activated carbon applications combined with biological processes, the lifetime of activated carbon increses significantly due to bioregeneration. Biofilm development on the surface of activated carbon in biological activated carbon filters enables bioregeneration of the adsorbent and extends its lifetime, particularly in the treatment of micropollutants [2]. Therefore, increasing the extent of bioregeneration in these applications is expected to decrease the costs related to the following regeneration techniques [3].

Bioregeneration is defined as the regeneration of the adsorption capacity of activated carbon as a result of the biodegradation of previously adsorbed organic materials [1]. Literature studies showed high bioregeneration efficiencies for activated carbon reaching up to 90 % in the biological treatment of phenolic compounds with a mixed microbial culture of acclimated activated sludge [4,5,6]. A more recent study showed about 80 % bioregeneration of phenol and paranitrophenol-loaded GACs, when phenol-acclimated biomass was used [7]. On the other hand, Immobilized pure bacterial culture of *Pseudomonas aeruginosa* could achieve 33-66 % bioregeneration of activated carbon in the treatment of phenolic compounds [8]. Another study obtained 98 % bioregeneration of activated carbon loaded with phenolics by using a pure fungus culture of *Scedosporium apiospermum.* This amount of bioregeneration was reported to be higher than conventional regeneration methods such as thermal treatment or solvent extraction [9].

The bioregeneration mechanism includes the desorption of adsorbed organic substances due to the concentration gradient between the activated carbon surface and the bulk liquid, and the following biological decomposition of these desorbed organic compounds by microorganisms [3]. The second hypothesis about bioregeneration is that extracellular enzymes produced by bacteria diffuse into the activated carbon pores and form an enzyme-substrate complex with adsorbed compounds, and as a result, the adsorption energy of the degraded or hydrolyzed organic compound decreases and empties the activated carbon pores to which it is bound to. This enzyme-substrate complex can be transformed into a degraded form by the extracellular enzymes or further degraded by the microorganisms in the bulk liquid [1]. A more recent study based on meta-omics analysis also indicated that bioregeneration was mostly caused by the enzyme reactions mechanism, i.e., the metabolism-related enzymes secreted by bacteria degraded the organic matter adsorbed by activated carbon into small molecules for further metabolism [10].

However, although the studies in the literature indicated the occurrence of exoenzymatic bioregeneration [4,5,6,11], the exoenzymatic bioregeneration could not be conclusively proven in any of these studies because the bioregeneration was provided with the microbial mixture in the activated sludge. In order to qualitatively show and quantitatively calculate the exoenzymatic bioregeneration, it was recommended to use the extracellular enzymes directly in bioregeneration experiments in the absence of microorganisms [3]. In addition, previous studies have shown that enzymatic bioregeneration may depend on the chemical properties of the activated carbon surface [1]. In literature studies, it was found that thermally activated carbons were more suitable for chemical adsorption, while chemically activated carbons led to physical adsorption. Therefore, it has been argued that occurrence of exoenzymatic bioregeneration is more probable in thermally activated carbons, where the activation energy is higher due to chemical adsorption [3].

The ability of microorganisms to adapt to micropollutants more easily when they are attached to activated carbon and to provide suitable enzymes for their degradation also increases the biodegradability of adsorbed xenobiotics, which are difficult to decompose in conventional biological treatment systems. In addition, the degradation of such compounds with purified enzymes has recently gained importance [12]. Although phenolic compounds can be removed to some extent in conventional activated sludge plants, advanced chemical oxidation methods are often needed [13] because they are quite difficult to completely biodegrade [14]. In this respect, treatment studies with various phenolic compounds in biological systems enhanced by the addition of enzymes combined with physicochemical methods such as adsorption to activated carbon gain importance.

Laccase enzyme is of increasing interest due to its ecological importance in biological remediation of soils and waters, applications in the food and textile industry, and chemical synthesis reactions [15]. Important laccase-producing microorganisms, such as white rot fungi, are used for the biological removal of different pollutants [13]. In literature, there are so many studies on the biological degradation of phenolic compounds by laccase-producing microorganisms. For example, Uhnakova et al. (2009) investigated the biodegradation of brominated phenols by *Trametes versicolor* fungus and the laccase enzyme it produces [16]. Besides, Zhang et al. (2008) studied the

degradation of 2,4-dichlorophenol (2,4-DCP), 4-chlorophenol and 2-chlorophenol with the laccase enzyme produced by *Coriolus versicolor* fungus [17]. Studies on the use of purified enzymes for the degradation of organic substances in wastewater which are difficult to biodegrade have been increasing in recent years. There are many studies in the literature on the use of laccase as an oxidoreductase enzyme for the removal of phenols, chlorophenols, endocrine disruptors, hormones, micropollutants, PAHs and textile dyes [12]. For example, it was determined that the laccase enzyme (5 U/mL) degrades phenol and bisphenol-A (BPA) at pH 5 and 35°C by 80% and 60%, respectively, during 30 minutes of mixing [13]. Another study examined the degradation of bisphenol-A with the laccase enzyme. In that study, under optimum conditions (pH: 7, 30°C, initial BPA concentration: 20 mg/L, laccase concentration >0.12 U/mL) laccase provided over 95% BPA biotransformation during the 3 hours of contact period [14].

Studies on the degradation of phenolic compounds such as bisphenol-A and similar xenobiotics with enzymes immobilized on activated carbon or nanoparticles have also started to take place in the literature [18,19]. However, bioregeneration of activated carbon was not studied in these studies and it was not determined quantitatively. Although their purpose and methods are very different, Nguyen et al. (2016) showed that micropollutants can be oxidized by enzymes immobilized on the activated carbon surface after adsorbing to the activated carbon [19]. In this study in the literature, it has been shown that electron transfer between the substrate and oxygen occurs more easily with the catalyst effect of laccase on the activated carbon surface compared to free enzymes.

This study aimed to qualitatively and quantitatively show the occurrence of exoenzymatic bioregeneration in activated carbon adsorption processes combined with biological systems. The extent of enzymatic bioregeneration was not quantified in the literature by the use of exoenzymes. For this purpose, laccase enzyme was selected as an example of exoenzyme owing to its ability to transform phenolic substances. The present study does suggest the use of purified enzymes in full-scale-bioregeneration applications due to their high costs, but tries to understand the mechanism and extent of enzymatic bioregeneration. The study mainly investigates the extent of bioregeneration that could occur due to the presence of an exoenzyme, considering that various exoenzymes are naturally present in biological wastewater treatment systems. In the literature, it has been suggested that the bioregeneration extent could be increased by increasing the activity of exoenzymes [3]. The results of the study are expected to open new perspectives in the engineered use of enzymes for the purpose of maximized bioregeneration, particularly in the presence of toxic and inhibitory organic compounds. For this reason, in this study, besides phenol, which has inhibitory effects in biological systems and is relatively easy to biodegrade in high concentrations in classical biological systems, 2-nitrophenol (2-NP) and bisphenol-A (BPA), which are difficult to degrade and also inhibitory, were chosen as the target compounds. Within the scope of the study, first of all, adsorption and desorption studies of these compounds were carried out on thermally and chemically activated carbons in abiotic environment, and their adsorption and desorption capacities were calculated. Then, suitable conditions were determined for bisphenol-A to be degraded by laccase, and finally, the biological regeneration potential of both activated carbon types was determined using laccase as an extracellular enzyme.

II. MATERIALS AND METHODS

Experimental studies include adsorption studies, desorption studies, enzymatic biodegradation and enzymatic bioregeneration studies with phenol, 2-nitrophenol (2-NP) and bisphenol-A (BPA). Two different types of activated carbon, which are thermally and chemically activated (Norit PKDA and Norit CAgran, respectively) were used in the studies. In order to determine enzymatic bioregeneration, laccase enzyme purified from *Trametes Versicolor* fungus was used. All studies were carried out in batch systems. The applied methods and the measured parameters are explained below.

2.1 Adsorption Experiments

Adsorption studies were carried out in a temperature-regulated shaker at 150 RPM at 25°C. The adsorption capacities of activated carbons for the phenolic compounds (phenol, 2-nitrophenol and bisphenol-A) were determined by contacting 200 mg/L concentration of the phenolic compound with 1 g/L dose of granular activated carbon (GAC) in 100 mL capacity flasks. In adsorption studies, the pH value of the thermally activated carbon PKDA increased to around 8-8.5 due to its basic properties, while the pH value of CAgran activated with phosphoric acid decreased to about 5.5 due to acidic functional surface groups. Adsorption studies were also performed with 250 mg/L KH_2PO_4+K_2HPO_4 buffer solution to adjust pH between 6.5-7-5. By measuring the concentrations of the target compound at the beginning and end of the adsorption, the adsorption capacities were calculated according to Equation (1).

$$q_{ads} = (C_b - C_f)/M \quad \textbf{(1)}$$

q_{ads} : adsorption capacity (mg compound/g GAC)
C_b : Initial concentration (mg/L)
C_f : Final concentration (mg/L)
M : Activated Carbon Dose (g/L)

2.2 Desorption Experiments

Activated carbons used in desorption studies were initially saturated with the target phenolic compounds as in Section 2.1. In 100mL flasks, phenolic compound at a concentration of 200 mg/L and activated carbon (1 g/L) were contacted to ensure that the activated carbons were completely saturated with the phenolic compound. Then, desorption studies were also carried out at 25°C and 150 RPM in a temperature-regulated shaker. Desorption of phenolic compounds was achieved by contacting the previously saturated activated carbon with distilled water. During the desorption studies, the pH value was measured between 6 and 7 for both types of activated carbon. Desorption studies were also performed in the presence of 250 mg/L KH_2PO_4+K_2HPO_4 buffer solution to adjust pH around 7. At the end of each desorption step, the concentration of the phenolic compound was measured and the amount of desorbed phenolic compound was calculated from the difference. The total desorption capacity cannot be determined with a single desorption step since sorption equilibrium between the activated carbon surface and bulk liquid will be quickly reached in a single abiotic desorption test. Therefore, in order to keep the concentration gradient high, the desorption tests were repeated by periodically refreshing the distilled water (every day) after measuring the concentration of the desorbed phenolic compound at the end of each desorption step. These step-wise desorption tests were continued until the phenolic compound was not desorbed from the activated carbon any more, or the desorbed amount was below measurable levels. Total desorption from activated carbon was calculated according to Equation (2) [1].

$$q_{des} = \Sigma (C_i/M) \quad \textbf{(2)}$$

q_{des} : desorption capacity (mg phenolic compound/g GAC)
C_i : Concentration in the water phase after each desorption step (mg/L)
M : Activated carbon dose (g/L)

Accordingly, abiotic regeneration efficiencies (R_{des}) of each activated carbon type were calculated according to Equation (3).

$$R_{des} = (q_{des} / q_{ads)} \text{ x } 100 \quad \textbf{(3)}$$

R_{des} : desorption efficiency (%)

2.3 Enzymatic Biodegradation Experiments

In order to determine the oxidation properties of phenolic compounds by the purified free laccase enzyme, different doses of enzymes were contacted with phenol, 2-nitrophenol or bisphenol-A at a concentration of 200 mg/L for each compound at 2 different pH (4.7 and 7.2) conditions in a temperature-regulated shaker at 25°C and 50°C for three days. In both biodegradation and bioregeneration studies, pH was adjusted to about 7.2 with phosphate buffer, or adjusted to pH value of about 4.7 with sodium acetate buffer. With enzymatic biodegradation studies, it was aimed to determine the conditions (enzyme dose, pH and temperature) where phenol, 2-nitrophenol and bisphenol-A oxidation activities of laccase enzyme are high.

The laccase enzyme used in the study is a product with activity >=0.5 U/mg purified from *Trametes versicolor* fungus, obtained from Sigma Aldrich (Product Code: 34829). The reason for using this enzyme was that it is an extracellular enzyme well known for its ability to degrade phenolic substances and is commercially available in its pure form. The activity of the enzyme was tested by the methods specified in the literature. Laccase enzyme activity was determined by color change at 420 nm as a result of contacting the enzyme with ABTS (2,2'-Azibonis-(3-ethylbenzthiazoline-6-sulphonate) under certain conditions [13]. In enzymatic degradation experiments, laccase enzyme was applied with activities of 0.2, 1 and 5 U/mL in batch tests.

2.4 Enzymatic Bioregeneration Experiments

Bioregeneration studies were carried out with enzymes under suitable conditions. As in abiotic desorption tests, the activated carbons to be used were first saturated with the target phenolic compound. Enzymatic bioregeneration studies were carried out at 25°C (and 50°C when necessary) at pH 7.2 and pH 4.7 in a temperature regulated shaker at 150 RPM. In these studies, desorption and oxidation of phenolic compounds in pre-saturated activated carbon were provided by contacting them with free enzymes dissolved in 25 mL distilled water. Thus, enzymatic bioregeneration was achieved in these flasks. Laccase enzyme was added to have an activity of 0.2 or 1 U/mL in the flasks. During the enzymatic bioregeneration tests, concentrations of the phenolic compound in the liquid phase were also measured at regular intervals, and thereby the part that desorbed but could not be oxidized by the enzymes was also followed. However, the percent enzymatic bioregeneration was determined by applying post-adsorption tests to the regenerated activated carbon at the end of each enzymatic bioregeneration test. The post-adsorption tests were carried out under the same conditions as the pre-adsorption tests and the amount of the adsorption area that was regenerated at the end of enzymatic bioregeneration was calculated according to Equation (4).

$$R_{enz} = (q_{postads} / q_{ads}) \text{ x } 100 \quad \textbf{(4)}$$

R_{enz} : Percentage of enzymatic bioregeneration (%)
q_{ads} : Adsorption capacity of unused GAC (mg compound/g GAC)
$q_{postads}$: Adsorption capacity of enzymatic bioregenerated GAC (mg compound/g GAC)

The difference ($R_{enz} - R_{des}$) between the regeneration capacities due to enzymatic bioregeneration and abiotic desorption indicates regeneration that is not dependent on the concentration gradient and is only caused by the activity of exoenzymes. Thus, it has been possible to quantitatively determine enzymatic bioregeneration for different types of activated carbon.

2.5 Analyses

The analyzes of phenolic substances were made by the direct spectrophotometric method (5530D) of 4-Aminoantipyrine (4-AAP) defined as a standard method [20]. Phenol, 2-NP and BPA concentrations were also measured by HPLC (High-performance Liquid Chromatography) method for some selected samples, and the reliability of the method and the possibility of interference by by-products were evaluated. In the case of biodegradation and bioregeneration experiments of 2-NP, the 4-AAP method measurements were interfered by the formation of a phenolic product upon oxidation of 2-NP by laccase. Therefore, only HPLC measurements were considered for determination of 2-NP concentrations in biodegradation and bioregeneration experiments.

For HPLC measurements, Dionex Ultimate 3000 HPLC device and C18 column (3µm 4.6 X 150 mm Dionex Bonded Silica Products) were used. For the measurements, BPA standard solutions and samples diluted 50 times were prepared in volumes between 0.5 and 1 mL with 10% methanol and put into HPLC sample tubes. During the measurement, 30% methanol and 70% distilled water were given to the column as the carrier phase and during the washing of the column between samples. Each injection took 25 minutes, with a flow rate of 0.5 mL/min. During the studies, the column temperature was kept constant at 30 ºC and the pressure was kept between 100 -130 bar. In the measurements, the peaks were monitored with the DAD detector at 280 nm, and the peaks were observed at 19.1, 20.4 and 19.4 minutes respectively for phenol, 2-NP and bisphenol-A.

III. RESULTS AND DISCUSSIONS

3.1 Adsorption and Desorption of Phenolic Compounds at Abiotic Conditions

Adsorption capacities were calculated according to Equation (1) as shown in Table 1 for each phenolic compound at initial concentrations of about 200 mg/L.

Table 1. Adsorption capacities of GACs for the target phenolic compounds

q_{ads} (mg/g)	phenol	2-NP	BPA
PKDA	103.6	176.7	98.9
CAgran	85.8	164.1	193.0

As shown in Table 1, phenol and 2-NP adsorption capacities of thermally activated PKDA were higher than those of CAgran. However, the difference in 2-NP adsorption capacities between the two carbon types was less compared to the case of phenol. For both types of activated carbon, 2-NP adsorption capacity was much higher than phenol adsorption capacity. In addition, it was determined that the adsorption capacity of both activated carbons decreased somewhat as a result of balancing the pH with the buffer solution (data not shown). But the difference was not noteworthy. On the other hand, for bisphenol-A, it was determined that CAgran activated carbon adsorbed almost twice as much BPA than PKDA. The reason for this difference is that, unlike the other two phenolic compounds, BPA has a larger molecule because it contains two benzene rings, and therefore it is more amenable to physical adsorption on chemically activated CAgran. Physical adsorption was determined as the dominant adsorption mechanism for adsorption of phenol and 2-NP on chemically activated carbons in previous studies [4,6].

Cumulative desorption capacities were calculated according to Equation (2) as shown in Table 2 for each phenolic compound. Desorption efficiencies were calculated according to Equation (3). The desorption efficiencies obtained by succeeding adsorption-desorption studies are given in Table 3. These cumulative desorption efficiencies were obtained in 9 steps for phenol, 30 steps for 2-NP and 74 steps, i.e. 74 days for BPA until reaching a non-measurable concentration of the phenolic compound in the bulk liquid.

As can be seen in Tables 2 and 3, the desorption capacity and efficiency of chemically activated GAC CAgran were much higher than those of thermally activated PKDA for each compound. Desorbability of phenolic compounds were highly dependent on the activation type of activated carbon. This showed that the carbon activation type eventually affects the occurrence of physical and chemical adsorption. For CAgran, high amounts of physical adsorption of 2-NP and BPA molecules led to higher desorption efficiencies, whereas chemisorption of all three compounds on the thermally activated PKDA led to much lower desorption capacities for this carbon.

Table 2. Desorption capacities of GACs for the target phenolic compounds

q_{des} (mg/g)	phenol	2-NP	BPA
PKDA	9.8	13.4	8.9
CAgran	19.2	70.4	89.8

Table 3. Desorption efficiencies (%) of each phenolic compound from the two GAC types

	phenol	2-NP	BPA
PKDA	9.5 %	7.6 %	9.0 %
CAgran	22.4 %	42.9 %	46.6 %

In each case, these desorption efficiencies obtained are not sufficient for long-term use of activated carbon, and it is suggested that desorption itself is not an efficient method for regeneration of activated carbon under abiotic conditions. These abiotic desorption efficiencies obtained will be compared with enzymatic bioregeneration efficiencies in Section 3.3.

3.2 Enzymatic Oxidation of Phenolic Compounds

Firstly, the powdered laccase enzyme was dissolved in 25 mL tubes to have activities of 1 U/mL and 5 U/mL and contacted with phenol at a concentration of approximately 200 mg/L at a fixed pH of 7.2. The change in phenol concentration was observed with respect to time and the obtained results are given in Figure 1. As can be seen, the laccase enzyme led to oxidation of phenol by 93% in less than 24 hours at both enzyme doses. However, phenol degradation rate was slightly higher at 5 U/mL enzyme dose. This is an expected result. In a literature study, it was determined that laccase enzyme (5 U/mL) degraded phenol at an efficiency of 80% during 30 minutes of mixing at pH 5 and 35°C [13]. These results are consistent with our study. In the present study, 15 mg/L phenol remained nondegradable in both tubes with 1 and 5 U/mL enzyme doses. This shows that laccase enzyme lost its activity in less than 24 hours and therefore could not degrade some of the phenol. For this reason, in the following enzymatic bioregeneration studies of phenol-saturated GAC, the duration time of bioregeneration tests were applied as 24 hours.

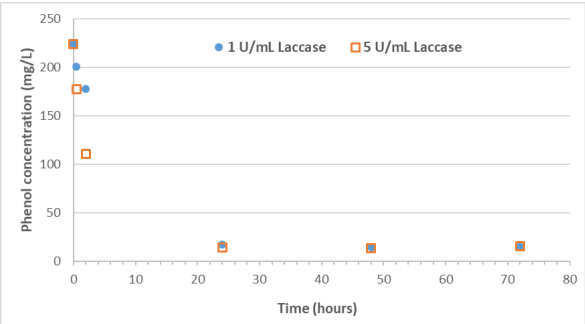

Figure 1. Phenol degradation by laccase enzyme at 1 U/mL and 5 U/mL doses at 25°C and pH:7,2.

pH can be an important parameter in enzymatic reactions [14]. In order to determine the effect of pH on enzymatic degradation with laccase, phenol degradation studies were carried out with 1 U/mL enzyme at 25 °C at 2 different pH values (7.2 and 4.7) (Figure 2). pH 7.2 was achieved with phosphate buffer and pH 4.7 with sodium acetate buffer. While the initial phenol concentration was 224 mg/L at pH 7.2, it decreased to 25 mg/L after 24 hours resulting in 89% phenol removal. On the other hand, the initial phenol concentration of 183 mg/L at pH 4.7 decreased to 8 mg/L during 24 hours resulting in 96% phenol removal. The efficiency of enzymatic degradation at pH 4.7 was slightly higher than at pH 7.2. It was also understood that at pH 4.7, the phenol concentration decreased with a greater removal rate. While the phenol concentration was halved in approximately 6 hours at pH 7.2, it was halved within 2 hours at pH 4.7 in an acidic environment. As can be seen, it is not possible to say that there was a significant difference between pH 4.7 and 7.2 in terms of degradation efficiency, but it is clear that the degradation rate for the laccase enzyme was higher at pH 4.7. This result was not surprising since the laccase enzyme used in our study is produced by fungi which usually have higher enzyme activities at lower pH values compared to bacteria.

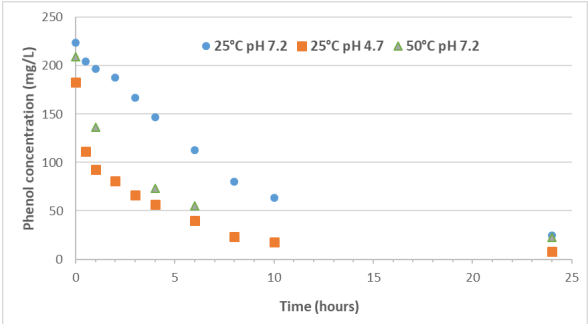

Figure 2. Phenol degradation by laccase enzyme at a dose of 1 U/mL at pH 4.7 and 7.2 at 25 °C and 50 °C.

To determine the effect of temperature on the activity of the laccase enzyme, enzymatic degradation studies were also carried out at 50°C. The time-dependent variation of the degradation of phenol and 2-NP with 1U/mL laccase enzyme at 50°C and at pH of 7.2 are given in Figure 2. Compared to the phenol removal profile for 25 °C and 7.2 pH, it is seen that the removal rate was much higher at 50 °C. While approximately 25 mg/L phenol was degraded after 1 hour at 25 °C and 115 mg/L after 6 hours, approximately 70 mg/L phenol was degraded after 1 hour at 50 °C, and 150 mg/L phenol after 6 hours. At the end of 24 hours, phenol concentration of around 20-25 mg/L remained in the environment without being oxidized by laccase at both temperatures.

In order to find out whether phenol removal also occurred due to mechanisms other than enzymatic degradation, 200 mg/L phenol was shaken under the same conditions without adding enzyme and phenol concentration was followed with respect to time for 4

days. These experiments were performed both at 25°C and 50°C at both pH values of 4.7 and 7.2. Phenol concentration did not decrease by time (data not shown) showing that phenol was not measurably removed by any mechanism other than enzymatic reaction, such as evaporation or adsorption to the glass surface, etc. Hence, enzymatic degradation was identified as the only phenol removal mechanism in these studies. Brown precipitate observed at the end of the biodegradation experiments indicated that the polymerized phenols, which were produced as a result of oxidation process catalyzed by laccase, were removed from the bulk by precipitation. Phenol, which is oxidized by the enzyme laccase, turns into phenoxy radicals. Phenoxy radicals react with each other and form polymers by covalently bonding via C bonds [1].

2-NP degradation studies were also carried out at 25 °C at pH 7.2 and pH 4.7, by adding approximately 527 and 348 mg/L 2-NP, respectively and 1 U/mL laccase enzyme to each flask. Also, 166 mg/L initial 2-NP concentration was tested with 1 U/mL enzyme at pH 7.2. In the samples of adsorption and desorption experiments in which only 2-NP and GAC were present in the medium, 2-NP concentrations had been successfully measured with the 4-AAP method. But parallel HPLC measurements showed that 4-AAP method was interfered by a by-product of 2-NP oxidation by laccase in the biodegradation experiments. Therefore, HPLC measurements were used in biodegradation and bioregeneration experiments performed with 2-NP.

To monitor the 2-NP removal in degradation experiments, 2-NP concentrations were measured by taking samples at certain time intervals within 24 hours (data not shown). It was found that 1 U/mL laccase decreased 527 mg/L 2-NP down to 329 mg/L and 166 mg/L 2-NP to 3 mg/L at pH 7.2 within 24 hours. At pH 4.7 conditions, 1U/mL laccase decreased 348 mg/L 2-NP down to 4 mg/L. Enzymatic degradation efficiencies reached up to 99 % except in the case of high initial 2-NP concentration of 527 mg/L at pH 7.2, where degradation efficiency was only 38 %. The reason for this was high initial 2-NP concentration and the fact that activity of the suspended enzyme lasted for less than 24 hours and therefore was not able to further oxidize 2-NP. These results showed that 1 U/mL enzyme dose and a 24-hour contact period were sufficient for effective oxidation of 2-NP, and they will be also sufficient for the following bioregeneration studies. To determine whether 2-NP removal occurs by evaporation, it was shaken at 25°C, pH 4.7 and without adding enzyme, and 2-NP concentration was followed for 5 days. It was also monitored at 50°C and pH 7.2 and pH 4.7 conditions. 2-NP was not removed by any mechanism other than enzymatic reaction as in the case of phenol.

In the enzymatic degradation experiments performed with bisphenol-A, laccase enzyme at a dose of 1 U/mL was contacted with bisphenol-A at concentrations of approximately 10, 50 and 100 mg/L at pH of 7.2 in 25 mL tubes. These tests lasted for 24 hours and the concentration change was observed. The results obtained are given in Figure 3 on a logarithmic scale.

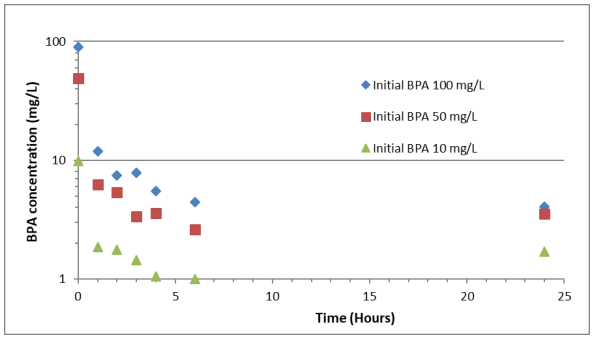

Figure 3. Degradation of BPA by laccase enzyme (1 U/mL) at pH 7.2 and 25°C conditions.

Laccase enzyme achieved most of the bisphenol-A oxidation within the first hour, and bisphenol-A concentration in the water phase stabilized within the first 5-6 hours. Laccase was able to oxidize bisphenol-A at a concentration of 100 mg/L by 90% in the first hour. The removal efficiency reached 95% within 5-6 hours. This enzymatic degradation efficiency was very similar to the 93% removal that was obtained for phenol. This showed that laccase was able to degrade BPA in a manner similar to phenol and at a relatively high rate, although BPA is known to be much more difficult to be biodegraded by microorganisms compared to phenol. In this sense, it is understood that bisphenol-A degradation with laccase and similar oxidoreductive exoenzymes will be an effective method. After the first 5-6 hours, BPA concentrations remained almost constant. This shows that the activity of the suspended laccase enzyme did not exceed a few hours. When the initial BPA concentration was 50 and 100 mg/L, the final BPA concentration decreased to 3 and 4 mg/L, respectively, at the end of the enzymatic treatment. When the initial concentration was 10 mg/L, the final concentration ranged between 1-2 mg/L (Figure 3).

In order to determine the effect of pH, degradation of BPA was also studied at an initial concentration of 50 mg/L at the pH values kept around 4.7-5 with sodium acetate buffer, again at 1 U/mL laccase enzyme activity and at a temperature of 25°C (Figure 4). As can be seen, decreasing the pH from 7.2 to 4.7 did not cause a significant difference in the degradation of BPA. However, under pH 4.7 conditions, the final BPA concentration decreased to 1 mg/L as it was around 3 mg/L under pH 7.2 conditions. Although this may not seem like a significant difference at first glance, even this small difference can be very significant, since Bisphenol-A is among the primary pollutants and even very low concentrations may cause endocrine

disrupting effects in nature. For this reason, applying low pH (around 5) in enzymatic degradation with laccase will reduce the effluent values . Also in another study, higher BPA removal was found at pH 6 compared to neutral conditions [14].

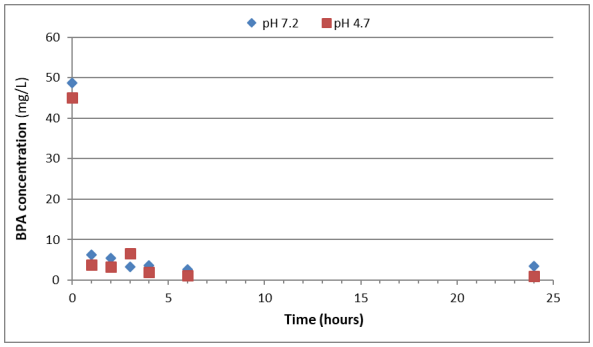

Figure 4. Degradation of BPA by 1 U/mL laccase enzyme activity at different pH conditions at 25°C.

The studies have shown that laccase enzyme provides a very effective BPA removal at an activity dose of 1 U/mL. Exceeding this dose will increase the cost of the enzyme, but will not provide any significant benefit. Therefore, it was decided to try a lower enzyme dose (0.2 U/mL). Figure 5 shows BPA removal with laccase at 2 different enzyme doses. 95% removal was observed in the case of 1 U/mL laccase dose, resulting in final BPA concentration of around 4 mg/L. The removal efficiency decreased to 70 % at 0.2 U/mL laccase dose, and the final BPA concentration remained at a very high level of around 30 mg/L. In addition, bisphenol-A degradation rate was higher at 1 U/mL enzyme dose. In the first hour, 1 U/mL laccase degraded approximately 90% of 100 mg/L BPA, while laccase at 0.2 U/mL was only able to degrade approximately 30% of the intial concentration. These studies have shown that the enzyme activity has a very important effect on BPA removal, and shoud be optimized.

In order to see whether the removal of BPA took place by evaporation, 200 mg/L BPA was shaken at 25 °C under the same conditions without adding enzyme, and BPA concentration was followed for 4 days depending on time. No decrease in BPA concentration was observed during 4 days (data not shown). Since the enzymatic bioregeneration studies will also be carried out at 50 °C, the change in the initial concentration of 120 mg/L BPA was monitored for 3 days in the absence of enzymes at two different pH conditions (pH 4.7 and 7.2) to see if BPA would be removed by evaporation at this relatively high temperature (data not shown). There was again no decrease in BPA concentration at 50 °C. These studies have shown that BPA was not removed by any mechanism other than enzymatic reaction, such as evaporation or adsorption to the glass surface, etc., at conditions of 25 and 50 °C and pH 4.7 and 7.2. For this reason, enzyme activity has been identified as the only effective BPA removal mechanism in our enzymatic degradation studies.

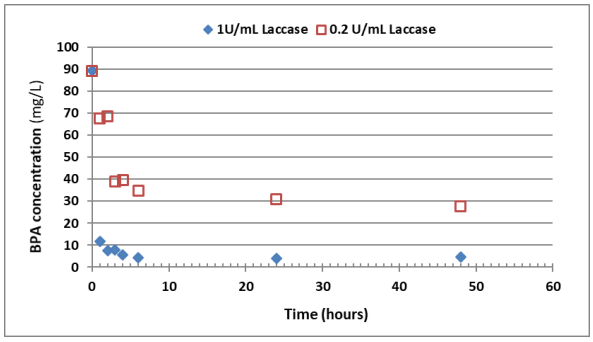

Figure 5. BPA degradation at pH 7.2 and 25°C by different laccase enzyme activites (1 U/mL and 0.2 U/mL).

To determine the effect of temperature on the activity of the laccase enzyme, enzymatic degradation studies were also carried out at 50°C. The time-dependent variation of the concentration of BPA at 50°C with 1U/mL laccase enzyme at pH 7.2 and pH 4.7 conditions are given in Figure 6. BPA oxidation took place in the first 1 hour at 50°C as well as at 25°C. However, at 25 °C, the BPA removal efficiency was around 90% and an additional 5% removal occurred in the next few hours, while at 50 °C this rate reached around 95% in the first hour and there was no further degradation in the following hours. This shows that the degradation of BPA by laccase at 50°C occurred slightly faster than at 25°C, but the enzyme activity was lost more quickly. On the other hand, no difference was observed at pH 7.2 and pH 4.7 conditions in studies performed at 50°C (Figure 6).

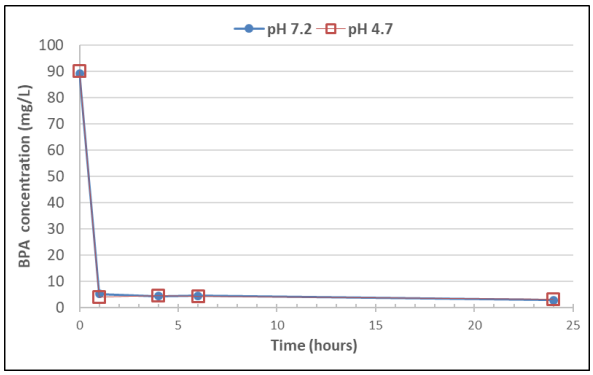

Figure 6. BPA degradation at pH 7.2 and pH 4.7 and at 50°C conditions at laccase enzyme activity of 1 U/mL.

A literature study examining the removal of BPA using crude laccase solution obtained from *Trametes versicolor* showed that the degradation yield in 24 hours reached 88.76% [21]. In another study, it was determined that laccase enzyme (5 U/mL) degraded bisphenol-A by 60% at pH 5 and 35°C during 30

minutes of contact [13]. The degradation efficiencies obtained in all degradation experiments with laccase in our study were consistent with the values obtained in the literature [13,14,19].

3.3 Bioregeneration with Laccase Enzyme

In bioregeneration experiments, 1 U/mL laccase enzyme dose was used since it was found to be sufficient in enzymatic degradation experiments. Bioregeneration studies were carried out in two different solutions such that pH was adjusted to 7.2 with a phosphate buffer or pH was fixed to 4.7 with sodium acetate buffer. The initial abiotic adsorption capacities (q_{ads}) obtained for approximately 200 mg/L of phenol and the post-adsorption capacities ($q_{postads}$) obtained after enzymatic regeneration as well as the bioregeneration efficiencies calculated from these values are shown in Table 4 for phenol as the target compound.

While the maximum total desorption efficiency was around 10% in thermally activated PKDA carbon under abiotic conditions in Section 3.1, this efficiency was increased to around 40% with laccase enzyme (Table 4). This has shown that activated carbon saturated with phenol can be enzymatically regenerated partially with laccase enzyme, the amount of which is much higher than the maximum desorption (regeneration) that could be achieved under abiotic conditions. In the case of chemically activated CAgran, the bioregeneration efficiency was much higher. The desorption capacity of CAgran, which was around 26% under abiotic conditions at pH 7, increased to 82% with the laccase enzyme. The very high bioregeneration efficiency differences between the two types of activated carbon indicate the importance of the activated carbon type. Although enzymatic bioregeneration was also valid for the thermally activated carbon, the results indicated that it will provide a more realistic solution for bioregeneration of chemically activated carbons.

Table 4. Enzymatic bioregeneration efficiencies of phenol-loaded GACs by laccase (1 U/mL).

GAC	pH	T (°C)	q_{ads} (mg/g)	$q_{postads}$ (mg/g)	Bioregeneration efficiency (%)
1 g/L PKDA	7.2	25	131.0	51.2	**39.1**
1 g/L CAgran	7.2	25	40.2	33.1	**82.3**
1 g/L PKDA	4.7	25	86.5	38.1	**44.0**
1 g/L CAgran	4.7	25	39.9	49.1	**123.2**
1 g/L PKDA	7.2	50	132.0	39.9	**30.2**
1 g/L CAgran	7.2	50	58.0	26.7	**46.0**

At pH 4.7, higher bioregeneration efficiencies were observed for both activated carbon types compared to pH 7.2. In the previous section, it was observed that degradation of phenolic compounds with laccase was higher at pH 4.7. Therefore, higher bioregeneration was an expected result. surprisingly, after the contact of laccase enzyme with CAgran at pH 4.7, the adsorption capacity exceeded that of unused activated carbon, and accordingly, the bioregeneration efficiency was found to be higher than 100%. This was probably because the enzymes attached to the activated carbon surface continued their activity for a while and resulted in further enzymatic degradation during post-adsorption tests. It has been shown in another study in the literature that the laccase enzyme immobilized on the activated carbon surface maintains its activity longer than the suspended enzyme [19]. In bioregeneration studies, after 1 day of contact with the enzyme, the phenol concentration remaining in the water phase was only between 3 and 5 mg/L in each of the 4 reactors (data not shown). This indicated that, as predicted from previous degradation studies, most of the desorbed phenol was further degraded by suspended laccase enzymes within 24 hours.

Bioregeneration experiments were also performed with phenol at 50°C in order to see the effect of high temperature at pH 7.2. Bioregeneration efficiencies were obtained as 30.2 % for PKDA and 46.0 % for CAgran (Table 4). These bioregeneration efficiencies were lower compared to the values obtained at 25 °C. At 25 °C, bioregeneration occurred at 39.1 and 82.3 % efficiencies, respectively for PKDA and CAgran. This decrease in bioregeneration efficiency was attributed to the loss of enzymatic activity at higher temperature, although enzyme activity duration increased with immobilization on GAC.

In enzymatic bioregeneration experiments with 2-NP as the target compound, bioregeneration studies were carried out in a solution containing 1 U/mL laccase enzyme and 2-NP at a concentration of approximately 140 mg/L at pH adjusted to 7.2 with phosphate buffer. In the biodegradation studies for phenol, it was observed that degradation rate was higher at 50 °C, but the final efficiencies were comparable. Based on this, the bioregeneration of 2-NP was monitored at 50°C. The adsorption capacities before and after enzymatic regeneration and the bioregeneration efficiencies calculated from these values are given in Table 5.

Table 5. Enzymatic bioregeneration efficiencies of 2-NP-loaded GACs by laccase (1 U/mL) at 50 °C.

GAC	pH	q_{ads} (mg/g)	$q_{postads}$ (mg/g)	Bioregeneration efficiency (%)
1 g/L PKDA	7.2	135.1	116.4	**86.2**
1 g/L CAgran	7.2	104.6	69.2	**66.2**

While the maximum total desorption efficiency was around 7% in thermally activated PKDA carbon with high adsorption capacity under abiotic conditions (Table 3), this efficiency was increased to around 86% with laccase enzyme (Table 5). This showed that activated carbon saturated with 2-NP was regenerated 12 times more than the case in abiotic conditions when laccase enzyme was provided. The regneration capacity for CAgran, which was around 42% under abiotic conditions, increased to 66% with laccase enzyme. When the desorption efficiencies and bioregeneration efficiencies are compared, it is obvious that bioregeneration efficiencies are considerably higher than desorption efficiencies for both GAC types. These bioregeneration efficiencies for 2-NP were higher than the 25-30 % efficiencies obtained by a biomass mixture in a literature study [22]. However, in another study, 65-90 % bioregeneration efficiencies were obtained by an activated sludge mixture, which was previously acclimated to 2-NP [6]. The laccase enzyme, when it was used in the absence of other biological agents in the present study, was able to achieve comparable bioregeneration efficiencies with an acclimated biomass that was used in the previous study [6]. This indicates that acclimation may provide the necessary exoenzymes for the degradation of xenobiotics adsorbed on activated carbon.

The bioregeneration efficiency differences between the two activated carbon types showed the importance of the activated carbon type. It appears that PKDA was more bioregenerated, although 2-NP was desorbed much more from CAgran at abiotic conditions. Considering the higher adsorption capacity and lower abiotic desorption efficiency of PKDA, it can be concluded that laccase was very effective in bioregeneration of PKDA with high 2-NP saturation. Therefore, in terms of high adsorption and high bioregeneration efficiency, the use of thermally activated carbons should be considered to be more appropriate for 2-NP removal. This finding is totally opposite to the results obtained with phenol. Since thermal activation is carried out without oxygen, it leads to a reactive surface and chemical adsorption takes place, forming strong irreversible bonds. In chemical activation, since the activated carbon has a surface with fully oxidized active sites, its interaction with oxygen does not affect the surface, and more physical adsorption takes place and more reversible weak bonds are formed [1]. In the case of phenol, it seems that laccase enzyme could not oxidize most of the chemically adsorbed phenol and could open less new adsorption sites via enzymatic bioregeneration on the thermally activated PKDA. But on the other hand, chemically adsorbed 2-NP could be oxidized to a higher amount by laccase leading to higher bioregeneration efficiencies. This is because most of the phenolics including phenol participate in oxidative polymerization on the surface of oxygen-sensitive thermal activated carbons. However, 2-NP does not participate in oxidative polymerization reactions. It is known that oxidative coupling reactions of phenolic compounds other than nitrophenols reduces the reversibility of adsorption [1].

Bioregeneration experiments were also performed for bisphenol-A (BPA) at an initial bulk concentration of 200 mg/L in the pre-adsorption process. These experiments were performed at laccase activity doses of 0.2 U/mL and 1 U/mL and at pH values of 4.7 and 7.2. The studies were first carried out under 25°C temperature conditions. The initial adsorption capacities and the adsorption capacities obtained by post-adsorption after enzymatic regeneration and the bioregeneration efficiencies calculated from these values are given in Table 6 for the enzyme activity dose of 0.2 U/mL.

While the maximum total desorption efficiency was around 45% in chemically activated CAgran carbon under abiotic conditions in Section 3.1, this efficiency was increased to around 90% with laccase enzyme (Table 6). Moreover, while only one day of contact time was sufficient for enzymatic bioregeneration, the 45% cumulative abiotic desorption took place in 74 steps, i.e. 74 days, as discussed in Section 3.1. The rate of abiotic desorption, which took place in one day, was only around 3%. In other words, this has shown that CAgran activated carbon saturated with BPA was regenerated by laccase enzyme, at least 2 times the maximum total desorption (regeneration) that can be possible only with water under abiotic conditions.

Table 6. Enzymatic bioregeneration efficiencies of 200 mg/L BPA at 25°C with laccase at 0.2 U/mL activity

GAC	pH	q_{ads} (mg/g)	$q_{postads}$ (mg/g)	Bioregeneration efficiency (%)
1 g/L PKDA	7.2	73.7	156.7	**212.5**
1 g/L CAgran	7.2	167.2	154.4	**92.3**
1 g/L PKDA	4.7	89.1	157.3	**176.5**
1 g/L CAgran	4.7	174.9	156.9	**89.7**

The bioregeneration efficiencies were much higher with thermally activated PKDA as in the case of phenol. In section 3.1, the regeneration capacity for PKDA, which was around 9% under abiotic conditions, increased to over 100% with laccase enzyme (Table 6). Efficiencies above 100% show that the activity of enzymes attached to activated carbon lasted much longer and continued to degrade BPA during post-adsorption. However, in Section 3.2, the enzyme

activity had lasted for only a few hours in the degradation experiments without activated carbon. Enzymes attached to carbon surface should have kept their activity longer compared to the enzymes, which were totally suspended in the previous biodegradation experiments. As a result of this, the calculated bioregeneration efficiencies were much higher than the total desorbabilities. It has also been observed in another study with BPA and laccase, that the activity of enzymes held on activated carbon is much longer than those of suspended enzymes [19]. It can be said that these findings obtained in our study showed that the amount of enzymatic bioregeneration could be much more than the sum of abiotic desorption and enzymatic degradation because of a synergistic effect, and therefore it was more effective than expected. In other words, it is understood that, as a treatment application, enzymatic bioregeneration may have much more efficient effects than sole enzymatic degradation of phenolic compounds.

The bioregeneration efficiency differences between the two activated carbon types also show the importance of the activated carbon type. Although CAgran activated carbon gave higher bioregeneration efficiencies in our study with phenol, the opposite was true in our experiments performed with BPA. This can be attributed to the chemical structure of BPA molecule, which has two benzene rings and much larger compared to phenol. Bioregeneration studies showed no noteworthy effect of pH on the enzymatic bioregeneration of BPA. In bioregeneration studies, after 1 day of contact with enzyme, BPA concentration remaining in the water phase was only around 2 mg/L in each of the 4 reactors. This showed that, as predicted from previous degradation studies, desorbed BPA was largely degraded by the suspended laccase enzymes within 24 hours.

Bioregeneration studies were also repeated at 1 U/mL laccase activity to see how increasing the enzyme dose would affect bioregeneration efficiency. As seen in Table 7, increasing the enzyme dose did not further increase the bioregeneration efficiency. In section 3.2, it was shown that enzymatic degradation increased with increasing enzyme dose from 0.2 to 1 U/mL. However, this increase was not observed in enzymatic bioregeneration. In other words, a dose of 0.2 U/mL enzyme activity was sufficient for enzymatic bioregeneration. This shows that the enzymes immobilized on activated carbon remain active for longer than suspended enzymes and can oxidize more BPA. Therefore, enzymatic bioregeneration can be achieved with a much lower enzyme addition compared to sole enzymatic degradation.

Table 7. Enzymatic bioregeneration efficiencies of 200 mg/L BPA at 25°C with laccase at 1 U/mL activity.

GAC	pH	q_{ads} (mg/g)	$q_{postads}$ (mg/g)	Bioregeneration efficiency (%)
1 g/L PKDA	7.2	92.2	159.1	**172.6**
1 g/L CAgran	7.2	178.2	168.1	**94.3**
1 g/L PKDA	4.7	106.7	86.6	**81.1**
1 g/L Cagran	4.7	174.3	137.1	**78.7**

To determine the effect of temperature, bioregeneration studies were also carried out at 50 °C at pH 7.2 and pH 4.7 with 1 U/mL enzyme activity doses. In these studies, unlike the previous ones, 100 mg/L BPA was used in pre- and post-adsorption. As seen in Table 8, bioregeneration efficiencies decreased at 50 °C compared to 25 °C. The immobilized enzymes may have lost their activity at 50 °C much faster than the case at 25 °C. A similar situation was also detected in the previous studies with phenol. The fact that this decrease is more pronounced especially in PKDA indicates the possibility that the activated carbon type may also affect the enzyme immobilization. It is suggested that the enzyme immobilization was higher on PKDA and therefore the decrease in bioregeneration efficiency at high temperature was higher than that of Cagran.

Table 8. Enzymatic bioregeneration efficiencies of 100 mg/L BPA at 50°C with laccase at 1 U/mL activity.

GAC	pH	q_{ads} (mg/g)	$q_{postads}$ (mg/g)	Bioregeneration efficiency (%)
1 g/L PKDA	7.2	92.4	65.9	**71.3**
1 g/L Cagran	7.2	90.0	65.2	**72.5**
1 g/L PKDA	4.7	97.9	26.0	**26.5**
1 g/L Cagran	4.7	95.8	70.2	**73.3**

In addition, as seen in Tables 6-8, PKDA bioregeneration was found to be much lower at pH 4.7 than the values obtained at pH 7.2 in all cases. This difference did not exist for CAgran. This can be attributed to the fact that PKDA, which was activated thermally by steam, has more basic surface functional groups, while CAgran, activated by phosphoric acid, has acidic surface functional groups [1].

IV. CONCLUSIONS

The results obtained in the study showed by which mechanisms and to what extent extracellular enzymes were able to bioregenerate activated carbon to fill an important gap in the scientific literature. With this study, extracellular enzymatic bioregeneration was demonstrated quantitatively for the first time in the literature by directly using purified exoenzymes. Phenol, 2-nitrophenol and bisphenol-A were degraded to a large extent with the laccase enzyme. Depending on the pH, 40-45% of thermally activated PKDA carbon and 80-100% of chemically activated CAgran carbon loaded with phenol were bioregenerated at 25 °C between pH 5-7. However, the bioregeneration efficiencies decreased at 50 °C. In the case of 2-NP, bioregeneration efficiencies as high as 86 and 66 % were achieved at 50 °C for PKDA and CAgran, respectively. At an enzyme activity of 0.2-1 U/mL, bisphenol-A was greatly degraded by the laccase enzyme and 80-100% of PKDA and 80-95% of CAgran could be bioregenerated at 25 °C between pH 5-7. The study showed that both the activation type of activated carbon and the chemical structure of the target compound affect the extent of bioregeneration. In all cases, the bioregeneration efficiencies were much higher than the abiotic total desorption efficiencies. This showed that extracellular enzymes such as laccase take an active role in the bioregeneration of activated carbon.

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