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ORIGINAL ARTICLE

Biotransformation of phenol in synthetic wastewater using the functionalized magnetic nano-biocatalyst particles carrying tyrosinase

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Abstract

Low conversion efficiency and long-processing time are some of the major problems associated with the use of biocatalysts in industrial processes. In this study, modified magnetic iron oxide nanoparticles bearing tyrosinase (tyrosinase-MNPs) were employed as a magnetic nano-biocatalyst to treat phenol-containing wastewater. Different factors affecting the phenol removal efficiency of the fabricated nano-biocatalyst such as catalyst dosage, pH, temperature, initial phenol concentration, and reusability were investigated. The results proved that the precise dosage of nano-biocatalyst was able to degrade phenol at the wide range of pHs and temperatures. The immobilized tyrosinase showed proper phenol degradation more than 70%, where the substrate with a high concentration of 2500 mg/L was subjected to phenol removal. The nano-biocatalyst was highly efficient and reusable, since it displayed phenol degradation yields of 100% after the third reuse cycle and about 58% after the seventh cycle. Moreover, the immobilized tyrosinase was able to degrade phenol dissolved in real water samples up to 78% after incubation for 60 min. It was also reusable at least seven cycles in the real water sample. The results proved the effectiveness and applicability of the fabricated nano-biocatalyst to treat phenol-containing wastewaters in a shorter time and higher efficiency even at high phenol concentration. The developed nano-biocatalyst can be promising for the micropollutants removal and an alternative for the catalysts used in traditional treatment processes.

Keywords Nano-biocatalyst · Phenol · Wastewater treatment · Tyrosinase · Magnetic nanoparticles

Introduction

From an international view, the world community confronts huge challenges in the field of water supply (Chandrasekara and Pashley 2017). Nowadays, the aquatic environment is globally contaminated by various industrials or human activities. Therefore, it is essential to make appropriate use of current technologies for wastewater treatment, as well as developing new methods and guidelines for reusing wastewater streams. The candidate methods should meet some requirements such as being robust and available at low cost, and offering a short processing time and minimal impact on the environment (Dey et al. 2017; Oprčkal et al. 2017; Nelson et al. 2017; Al-Obaidi et al. 2017; Hassanzadeh et al. 2017).

Phenolic compounds are pollutants presenting acute toxicity, low biodegradability, with high accumulation and persistence in the environment. In this regard, the U.S. Environmental Protection Agency has categorized such chemicals as priority toxic pollutants (Yu et al. 2017; Víctor-Ortega et al. 2016; Rayati and Nejabat 2017; Soni et al. 2017). Several methods including adsorption, membrane separation, and advanced oxidation processes have been reported for removing phenolic pollutants (Yu et al. 2017; Chagas et al. 2015; Savic et al. 2014). These methods suffer from drawbacks such as high cost, low efficiency, sensitivity to concentration, and formation of hazardous by-products (Chiong et al. 2016; Lloret et al. 2012; Singh et al. 2017; Alneyadi and Ashraf 2016; Elsayed et al. 2017). Therefore, enzyme technology has been introduced as an alternative to many conventional processes because of its intrinsic features like mild reaction conditions and energy saving (Shukla et al. 2017). Furthermore, enzymes display extraordinary

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characteristics including high catalytic efficiency and substrate specificity with less toxic biodegradation by-products, bringing about the superiority of the enzyme-catalyzed reactions over chemical ones. Thus, enzyme-based systems have been keenly researched for the degradation of a wide range of organic contaminants, especially those which cannot be effectively treated by the traditional methods (Bilal et al. 2017; Niladevi and Prema 2008; Aber et al. 2016; Na and Lee 2017; Dammak et al. 2016). Tyrosinase (E.C. 1.14.18.1) is a copper-containing enzyme which is extensively found in nature and frequently obtained from the common button mushroom (Soltani-Firooz et al. 2017). This enzyme is considered as an attractive catalyst for phenol oxidation due to its ability to work at low substrate concentrations in the presence of atmospheric oxygen as the electron acceptor. The ortho-hydroxylation of monophenols to *o*-diphenols and the oxidation of *o*-diphenols to *o*-quinones are the two types of reactions which are catalyzed by this enzyme (Muñoz-Muñoz et al. 2013; Martínková and Chmátal 2016; Vicentini et al. 2016; Martínková et al. 2016). The generated *o*-quinones are extremely reactive and undergo a non-enzymatic polymerization to form water-insoluble oligomers. These aggregates can be removed by simple processes like sedimentation or filtration (Liu et al. 2016). However, the recovery and reusability of free enzymes are difficult and the use of soluble enzymes is limited due to their poor stability, relatively short catalytic lifetime, and high cost. These drawbacks could be overcome by the use of immobilized enzyme systems. Compared with free ones, immobilized enzymes should maintain the same functionality and offer more advantages such as enhanced stability against different denaturing conditions, higher catalytic activity, easier enzyme recovery and reusability, and reduced susceptibility to organic contaminants (Shukla et al. 2017; Bilal et al. 2017; Ji et al. 2017; Chang et al. 2015). Therefore, immobilized enzymes are currently drawn attention for various industrial applications like wastewater treatment (Tavares et al. 2017). Support materials play a significant role in the immobilization of enzymes; accordingly, they should be low cost and provide a large surface area (Chagas et al. 2015). For instance, magnetic nanoparticles (MNPs) have been extensively used as enzyme carriers in different processes including biosensing and wastewater treatment. Beside high specific surface area, MNPs display fascinating properties like superparamagnetic behaviour and ease of surface modification (Chang et al. 2015; Yazdani et al. 2016). Due to these properties, MNPs exhibit aggregation behaviour under the magnetic field, which can be resuspended in the mixture immediately by removing the external magnet. In addition, owing to their electron hopping nature and practical polymorphism, these magnetic nanoparticles have been classified as potential candidates in various applications and processes. Recently, the surface of the iron oxide nanoparticles was modified by the creation of active layers supported by polymers and bioactive molecules to enhance the use of the particles in advanced technologies and applications (Vallabani and Singh 2018). Furthermore, iron oxide magnetic nanoparticles demonstrating enzyme-like has gained phenomenal interest in biosensing, catalysis, and degradation of organic pollutants in water too (Vallabani et al. 2017). Several studies indicated that the loading capacity of MNPs, as well as the catalytic activity of the corresponding biocatalysts, could be substantially improved by the surface functionalization of the particles using appropriate agents (Shukla et al. 2017; Abdollahi et al. 2017).

However, the development of biocatalysts with high conversion efficiency and short processing time being able to operate at various conditions and high pollutant concentration to treat wastewater is still challenging. In the current work, the application of a nano-biocatalyst developed by immobilization of tyrosinase onto cyanuric chloride-functionalized magnetic nanoparticles for phenol removal was investigated to address such problems. Based on our knowledge, this is the first report concerning the use of such a magnetic nano-biocatalyst to treat phenol-containing samples under different conditions. Such a nano-biocatalyst can be easily separated from the wastewater using an external magnet and re-dispersed rapidly after removing the magnetic field. As a result, the costs associated with the separation of the nano-biocatalyst reduce.

Materials and methods

Materials

The edible mushroom was purchased from a local supermarket and used on the day of purchase for enzyme extraction. Iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), cyanuric chloride, ammonium sulfate, ethanol (99%), Iron(II) chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), tetraethyl orthosilicate (TEOS), tetrahydrofuran and 3-aminopropyltriethoxysilane (APTES) were acquired from Merck. All other chemicals used in this work were analytical grade.

Synthesis and surface functionalization of iron oxide nanoparticles

Bare magnetic nanoparticles were synthesized and modified in accordance with the previous works (Ranjbakhsh et al. 2012; Abdollahi et al. 2017). Briefly, the chemical co-precipitation method was used to synthesize the nanoparticles. For this purpose, 0.1 M solutions of Fe^{3+} and Fe^{2+} were separately prepared and added to 150 mL of deionized water which was deoxygenated by nitrogen gas. To adjust the pH of the mixture, 15 mL of 1 M NaOH solution was added

slowly and dropwise. Then, the solution was stirring for 5 min in a water bath at 60 °C under a nitrogen atmosphere. Finally, the bare magnetic nanoparticles were separated using an external magnetic field and washed three times with deionized water. Coating of the as-prepared magnetic nanoparticles was carried out using the sol–gel method and hydrolysis of TEOS. In this regard, a suspension containing 220 mg of the particles and 60 mL of ethanol was prepared and ultrasonicated under nitrogen atmosphere for 10 min. Subsequently, 4.5 mL of NH_3 solution, 0.6 mL of TEOS and 10 mL of deionized water were successively added to this mixture and it was stirred for 5 h at room temperature. The silica-coated iron oxide nanoparticles were magnetically separated from the reaction mixture and washed with ethanol and deionized water. For surface functionalization of the magnetic nanoparticles, the particles (220 mg) were resuspended in 3 mL of ethanol, followed by addition of 1.5 mL of APTES and the resulting mixture was stirred for 3 h at room temperature. Then, the reaction temperature was increased to 50 °C and the solution was stirred for 2 h. A strong magnet was used to recover the amine-functionalized magnetic nanoparticles from the suspension. After washing with deionized water and ethanol, the particles were vacuum-dried for 2 h at 60 °C.

Enzyme immobilization on the modified iron oxide nanoparticles

Before immobilization of tyrosinase onto the modified MNPs, the particles were further functionalized with 2,4,6-trichlorotriazine as an activating agent to obtain cyanuric chloride-functionalized magnetic nanoparticle (Cy-MNPs). For this purpose, the dried amine-functionalized nanoparticles (220 mg) were added to a mixture of dry tetrahydrofuran (20 mL) and cyanuric chloride (100 mg). The suspension was mechanically stirred in a water bath at 0 °C for 2 h. The cyanuric chloride MNPs were removed from the mixture, washed with tetrahydrofuran and vacuum-dried at 40 °C.

For covalent attachment of tyrosinase onto the modified iron oxide nanoparticles, a simple and convenient method was used to extract the tyrosinase from edible mushrooms (*Agaricus bisporus*). In this regard, the sliced mushrooms were added to cold acetone (− 22 °C) and stirred for 30 min. Then, the mixture was centrifuged at 7000 rpm and 0 °C and the pulp was separated and resuspended in phosphate buffer (pH 7.0). After stirring for 20 min at 0 °C, the supernatant was collected by centrifugation at 10,000 rpm. Ammonium sulfate was added to the supernatant to provide a 30% saturated solution to salt out the protein impurities. The precipitate was discarded after mixing (20 min) and centrifugation (10,000 rpm) the mixture. Finally, the saturation of the supernatant was adjusted to 60% by adding ammonium sulfate. The precipitate was obtained by mixing and centrifuging the mixture at 0 °C. The tyrosinase-containing precipitate was dissolved in the phosphate buffer and stored at − 22 °C to be used as an enzyme stock. The enzyme solution was freshly prepared by adjusting the activity of the stock to 4800 U/mL using phosphate buffer (pH 7.0) to be used for immobilization. Accordingly, 1500 µL of phosphate buffer (0.05 M and pH 7.0) was added to 5 mg of the Cy-MNPs and the suspension was sonicated for 30 s to thoroughly disperse the nanoparticles. Subsequently, the tyrosinase solution (500 µL) with an activity of 4800 U/mL was added to this mixture and it was shaken at room temperature for 18 h. Then, the tyrosinase-immobilized magnetic nanoparticles (tyrosinase-MNPs) were collected with the help of a strong magnet and washed three times with phosphate buffer to remove the unreacted enzymes. Finally, the particles were added to 2 mL of phosphate buffer and stored at 4 °C for further experiments. The tyrosinase was attached to the modified nanoparticles with an immobilization yield of approximately 69% (194 mg/g MNPs). To measure the activity of the immobilized enzyme, an adequate amount of the fabricated nano-biocatalyst was added to 550 µL of the substrate solution and the suspension was shaken at room temperature. At the same time intervals (2 min), nanoparticles were separated from the reaction media by an external magnet and the absorbance of the supernatant was recorded by spectrophotometer at 475 nm. Then, the particles were resuspended in the substrate solution and shaken again for the next sampling. The activity of the magnetic nano-biocatalyst was between 177 and 191 U/g. In addition, the comprehensive characterization of the MNPs and immobilized tyrosinase have been conducted and reported elsewhere. TEM images indicated that the most of the tyrosinase-MNPs had a semi-spherical shape with an average size of 17 nm. The kinetic parameters of the nano-biocatalyst and free tyrosinase were calculated using Michaelis–Menten model. The υ_{max} and K_m values were about 62,122 U/mL and 1.14 mM for the free enzyme, and 39,880 U/mg and 2.52 mM for the nano-biocatalyst, respectively. The obtained results indicated that immobilization causes conformational changes in the structure of enzyme to improve its catalytic performance (Abdollahi et al. 2016, 2017).

The colorimetric determination of phenolic compounds in aqueous solutions

The colorimetric method was used to determine the concentration of phenolic compounds in water samples using 4-aminoantipyrine solution (20 g/L in the $NaHCO_3$ buffer) and ferricyanide solution (83.4 mM in the $NaHCO_3$ buffer). In this reaction, the primary amine of 4-aminoantipyrine exerts an electrophilic attack on the para position of the phenolic compound which results in the formation of an

intermediate compound. Subsequently, potassium ferricyanide oxidizes the intermediate compound to develop a red quinone-type dye showing absorbance characteristic at 510 nm upon completion of the reaction (Kinsley and Nicell 2000). Initially, a standard curve was prepared using different concentrations of phenol. For this purpose, 100 µL of an aqueous phenol sample with a known concentration was added to 700 µL of 0.25 M sodium bicarbonate buffer and the mixture was thoroughly mixed. Then, 100 µL of 20 g/L 4-aminoantipyrine and 100 µL of 83.4 mM potassium ferricyanide were added to the mixture and it was shaken for 1 min. After 10 min, considering the complete development of the color of the reaction mixture, the absorbance of the solutions was measured by the UV–Vis spectrophotometer at a wavelength of 510 nm to plot the standard curve (Gómez et al. 2012). The solutions were prepared freshly and used on the day of preparation. The concentration of phenol in different solutions was calculated with the help of this standard curve. Phenol removal ratio was calculated by the following expression:

$$\text{Phenol removal ratio}\,(\%) = \left[\frac{P_{\mathrm{i}} - P_{\mathrm{r}}}{P_{\mathrm{i}}}\right] \times 100,$$

where P_{i} and P_{r} are the initial and the remaining phenol concentrations in the solutions, respectively. This colorimetric assay has been considered as a reliable, cheap and fast method for measuring the concentration of phenolic compounds in aqueous media (Zhang et al. 2009; Vicentini et al. 2013; Li et al. 2016; Alver and Metin 2017).

Effect of the dose of tyrosinase-MNPs

The effect of catalyst dosage on phenol degradation considering the reaction time was investigated. For this purpose, the different amounts of the fabricated nano-biocatalyst (5, 10, 15 and 30 mg) were individually added to a phenol solution with the concentration of 100 mg/L in phosphate buffer (0.05 M, pH 7.0). The mixtures were shaken at room temperature and samples were collected every 20 min by removing the nano-biocatalysts with a magnet, and the residual phenol concentration in the supernatant was measured spectrophotometrically. Then, the reaction was continued for another 20 min. The phenol solutions used in this work were prepared freshly and on a daily basis.

Dephenolization by the nano-biocatalyst at different pHs

The ability of the tyrosinase-MNPs to remove phenol from aqueous solutions at different pHs was explored. Thus, the solutions containing 250 mg/L phenol in the pH range of 5.5–8.0 were prepared in 0.05 M phosphate buffer or citrate phosphate buffer. Then, 15 mg of the fabricated nano-biocatalysts was added to 20 mL of the phenol solutions at different pHs and the mixtures were shaken in the same conditions for 3 h at room temperature. The samples were taken after 1 and 3 h from the start of the reaction, and the phenol degradation was determined in these two-time intervals for each solution.

Impact of different temperatures on phenol degradation

To investigate the performance of the tyrosinase-MNPs in phenol removal from water samples at different temperatures, a set of experiments was performed. In this regard, 20 mL of a phenol solution with the concentration of 250 mg/L was added to 15 mg of the tyrosinase-MNPs. The suspensions (pH 7.0) were incubated in a shaker incubator at a temperature range of 15–45 °C under gentle agitation for 3 h. Samples were collected after 1 and 3 h.

Effect of initial phenol concentration on the dephenolization

Conversion of phenol with different initial concentrations by the fabricated nano-biocatalyst was examined. The phenol concentrations in industrial wastewater are typically in the range of 0.5–16 mM (Alver and Metin 2017). Therefore, various phenol solutions in phosphate buffer (0.05 M, pH 7.0) with specified concentrations (250–2500 mg/L) were prepared to evaluate the ability of the fabricated nano-biocatalyst in phenol removal at the concentrations being similar to that of industrial effluents. The immobilized tyrosinase (15 mg) was suspended in 20 mL of the phenol solutions and the mixture was agitated at room temperature. Samples were taken after known periods, and the corresponding phenol concentrations were measured. Then, the reaction was continued for a further 12 h and the final samples were collected in the same way described before.

Reusability of the tyrosinase-MNPs

Reusability of the nano-biocatalyst for phenol degradation was tested at room temperature. Hence, 20 mL of a phenol solution (250 mg/L) was poured on 30 mg of the immobilized tyrosinase and the mixture was shaken for 2 h, considered as one reaction cycle. At the end of each treatment cycle, the immobilized enzymes were removed from the reaction medium and the supernatant was collected to assay phenol content. Afterward, the nanoparticles were resuspended in 30 mL of fresh phenol solution to perform the next reaction cycle. The concentration of phenol in the supernatant was measured by the colorimetric method.

Phenol removal from a real water samples

The practical application of the tyrosinase-MNPs for degrading phenolic compounds in real water samples was investigated. For this purpose, a phenol solution (250 mg/L) was prepared using well water as the solvent and its pH was adjusted to 7.0. Then, 15 mg of the immobilized tyrosinase was added to this solution and the suspension was gently agitated for 3 h at room temperature. The samples were taken after the specified period, and their residual phenol concentrations were determined. In addition, the reusability of the immobilized tyrosinase to remove phenol from a real water sample was examined. In this regard, a mixture of tyrosinase-MNPs (30 mg) and phenol solution (20 mL) was prepared using the well water. The suspension was shaken at room temperature and each reaction cycle lasted 2 h. Finally, the supernatant was collected at the end of each treatment cycle and the residual phenol concentrations were determined. New cycles were started by adding 20 mL of fresh phenol solution to the nanoparticles separated from the previous cycle.

Results and discussion

Catalyst dosage

Different amounts of the immobilized enzyme affect the phenol removal efficiency and reaction time. In this work, different doses of the tyrosinase-MNPs were used to treat artificial wastewater containing phenol. As illustrated in Fig. 1, phenol was eliminated completely by 15 and 30 mg of the tyrosinase-MNPs after 60 min. In another study, only less than 80% of phenol was degraded after 1 h by tyrosinase cross-linked enzyme aggregates (Xu and Yang 2013). Additionally, the other doses of the nano-biocatalyst displayed complete phenol removal efficiency after incubation for 2 h. According to the obtained results, 15 mg of the tyrosinase-MNPs was chosen for the further experiments to select an appropriate amount of the nano-biocatalyst for the balance between the phenol degradation, the enzyme cost and treatment time. In addition, the phenol removal process by the fabricated nano-biocatalyst is represented in Fig. 2.

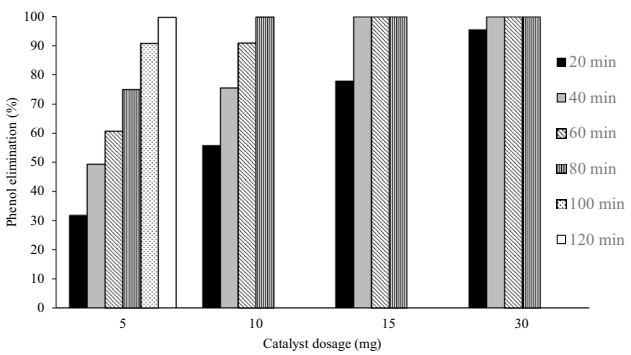

Fig. 1 Phenol removal by different doses of the nano-biocatalyst

Dephenolization by the tyrosinase-MNPs at different pHs

The pH of the phenol solution affects the activity of tyrosinase and it needs to be studied. Therefore, the degradation of phenol in aqueous solutions by the fabricated nano-biocatalyst was investigated at different pHs ranged from 5.5 to 8.5 with an initial phenol concentration of 250 mg/L (Fig. 3). The phenol conversion reached about 91%, where the phenol solution was incubated for 1 h at pH 7.0. The other researchers reported the phenol degradation less than 80% in an aqueous solution containing 2.5 mM phenol by the tyrosinase cross-linked enzyme aggregates after incubation for 1 h (Xu and Yang 2013). By extending the incubation time to 3 h, the tyrosinase-MNPs were able to remove 100% of phenol from water samples at different pHs. The results proved that the enzymatic treatment using tyrosinase-MNPs is effective over a broad range of pHs. These improvements can be attributed to the precise covalent immobilization of the tyrosinase onto the modified iron oxide nanoparticles, which result in stabilization of this enzyme. As reported in the previous studies, enzyme immobilization on proper supports using appropriate procedures can increase the conformational rigidity of three-dimensional enzymes structure and improve the enzyme stability (Khan et al. 2017; Atacan et al. 2016).

Phenol removal at different temperatures

The performance of the immobilized tyrosinase in degradation of phenol and its enzymatic activity depend on the operating temperature. As shown in Fig. 4, the phenol removal efficiency of approximately 95% was achieved after 1 h incubation at 35 °C. The complete phenol removal was observed after incubation for 3 h at all temperatures, except at 45 °C which displayed the phenol degradation efficiency of 71%.

The activity of tyrosinase decreased at a temperature above 35 °C due to enzyme deactivation. This is a reason why the tyrosinase-MNPs were not able to completely remove phenol from a water sample at 45 °C. However, a considerable amount of phenol was degraded even at this temperature by the nano-biocatalyst. In all experiments, the magnetic nano-biocatalyst was separated from the medium in a short time (less than 1 min).

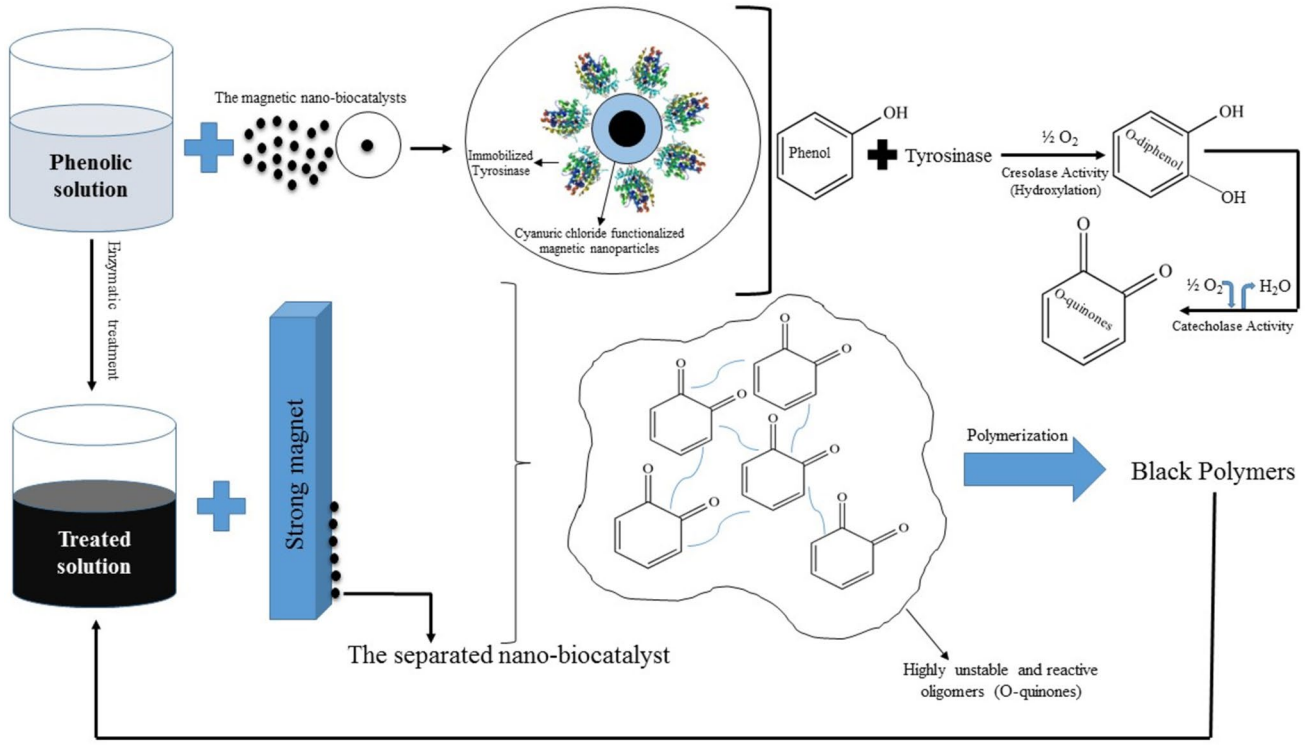

Fig. 2 Schematic illustration of the phenol removal by the magnetic nano-biocatalyst

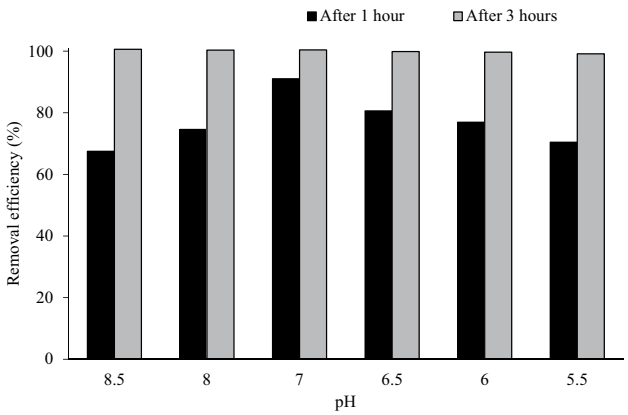

Fig. 3 Effect of different pHs on the phenol removal ability of the tyrosinase-MNPs

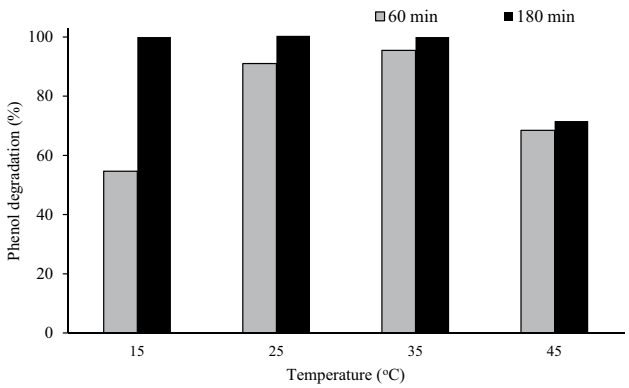

Fig. 4 Dephenolization by the fabricated nano-biocatalyst at various temperatures

Effect of initial phenol concentration

Tyrosinase-MNPs were used to degrade phenol in aqueous solutions with different initial phenol concentrations. As shown in Fig. 5, the degradation efficiencies of phenol by the nano-biocatalyst decreased as the initial concentration increased from 250 to 2500 mg/L. It is reported that the enzymatic treatment of wastewaters containing phenol with high concentrations led to the production of a large volume of reactive *o*-quinone product. Furthermore, tyrosinase has been introduced as an enzyme susceptible to the substrate and product inhibition. The *o*-quinone with a high concentration acts as an inhibitor in the reaction medium. As a result, the phenol removal efficiency could be decreased due to the production of a large volume of the *o*-quinones, and subsequently deactivation of the enzyme (Xu and Yang 2013; Haghbeen et al. 2004). However, the immobilization of tyrosinase onto the cyanuric chloride-functionalized magnetic nanoparticles resulted in proper phenol degradation more than 70% with a high concentration of 2500 mg/L after incubation for 4 h. In comparison, the lower degradation

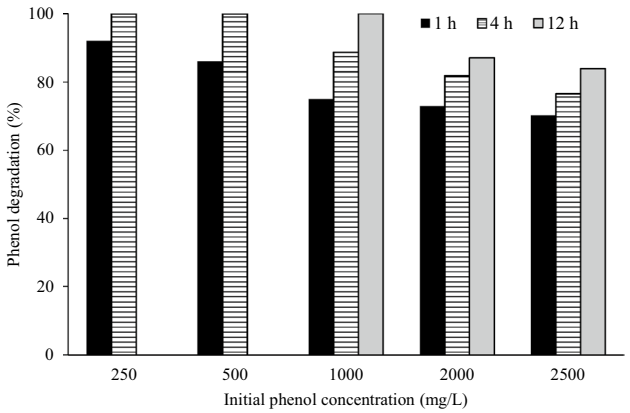

Fig. 5 Enzymatic treatment of the phenolic wastewater with different initial concentrations

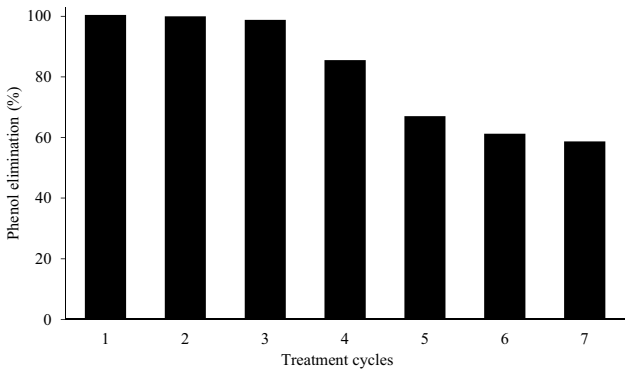

Fig. 6 Reusability test of tyrosinase-MNPs for phenol degradation in different reaction cycles

efficiency (less than 25%) was reported using the same initial phenol concentration (about 25 mM) in the previous literature (Xu and Yang 2013). The higher phenol removal efficiency might be achieved by removing the *o*-quinones from the reaction mixture.

Reusability experiments

Immobilized enzymes have several advantages such as a decrease in the cost of the enzyme in practical applications, since they can be separated from the reaction solution and reused throughout multiple cycles (Zhang et al. 2010). In this work, tyrosinase-MNPs were employed in several treatment cycles to degrade phenol (Fig. 6). In the first three cycles, phenol was removed completely by the nano-biocatalyst and after that phenol removal efficiency was decreased. This reduction is attributed to enzyme deactivation after reusing in several cycles. However, the phenol degradation of about 58% was achieved after the seventh cycle. In another study, a phenol degradation less than 50% was reported by tyrosinase using a 2.5 mM phenol solution after the fifth cycle (Xu and Yang 2013), while the tyrosinase-MNPs degraded about 70% of phenol after five treatment cycles. These results confirm that a considerable volume of phenolic wastewater can be treated efficiently by the tyrosinase-MNPs in a relatively short time causing this nano-biocatalyst applicable in various industrial wastewater treatment processes.

Phenol removal from real water samples

To investigate the practical application of the fabricated nano-biocatalyst to eliminate phenolic compounds, water samples from a local well [in Chemistry and Chemical Engineering Research Center of Iran (CCERCI)] were used to prepare phenol solutions (250 mg/L) (Analysis is shown in Table 1).

After 30 min reaction, the remaining phenol concentration in the real water sample reached 193 mg/L of the total 250 mg/L (22.7% removal). In the same experiment using phosphate buffer (0.05 M, pH 7.0), the tyrosinase-MNPs degraded 30% of the phenol. By extending the incubation time to 60 min, the degradation efficiency using the real water sample and phosphate buffer were 78% and 90.8%, respectively. The lower phenol removal efficiency using real water could be due to the presence of some impurities in this water sample. Furthermore, the reusability of the tyrosinase-MNPs for phenol removal from a real water sample was examined. As illustrated in Fig. 7, the immobilized enzyme degraded phenol completely in the first cycle but the removal efficiency decreased in the next cycles. However, the fabricated nano-biocatalyst showed a great performance in removing phenol from real water samples and only 48% of phenol remained in the mixture in the sixth treatment cycle. These results confirm that the tyrosinase-MNPs could be potentially employed as an efficient catalyst to treat real wastewater.

Treatment periods

As discussed before, one of the most important advantages of the fabricated magnetic nano-biocatalyst, which could be used for phenolic wastewater treatment, was its ability to degrade phenol with high efficiency and short time. In the previous works, the biocatalysts fabricated by immobilizing copper-containing enzymes (tyrosinase and laccase) on the different carriers were able to degrade phenol (0.5–5 mM) over a period of 4–12 h. (Liu et al. 2012, 2016; Wu et al. 2017; Seetharam and Saville 2003; Wang et al. 2012). In this study, the higher degradation efficiency was obtained using a shorter period between 1 and 4 h, even where the phenol concentration was much higher than that of the other studies. These findings prove the considerable performance of this developed nano-biocatalyst for wastewater treatment.

Table 1 Analysis of the well water

Row	Properties/description of test	Test results	Units	Normal range
1	Nickel (Ni)	Less than 0.02	mg/L	Maximum 0.07
2	Boron (B)	Less than 0.1	mg/L	Maximum 0.5
3	Manganese (Mn)	Less than 0.1	mg/L	Maximum 0.4
4	Chlorine (Cl)	47	mg/L	Maximum 400
5	Ammonia (NH_3)	Less than 0.02	mg/L	–
6	Nitrate (NO_3)	10.8	mg/L	Maximum 50
7	Total dissolved solids (TDS)	425	mg/L	Maximum 1500
8	Total hardness (TH)	196	mg/L	Maximum 500
9	Sulfate (SO_4)	98	–	Maximum 400
10	Turbidity	0.29	NTU	Maximum 5
11	Color	0	–	Maximum 15
12	Chromium (Cr)	Less than 0.02	mg/L	Maximum 0.05
13	Arsenic (As)	Less than 0.001	mg/L	Maximum 0.01
14	Sodium (Na)	74.6	mg/L	Maximum 200
15	pH	6.8	–	6.5-9
16	Magnesium (Mg)	25	mg/L	–
17	Fluorine (F)	0.76	mg/L	0.5–1.5
18	Calcium (Ca)	38	mg/L	–
19	*Escherichia coli*	Negative	In 100 mL	Negative

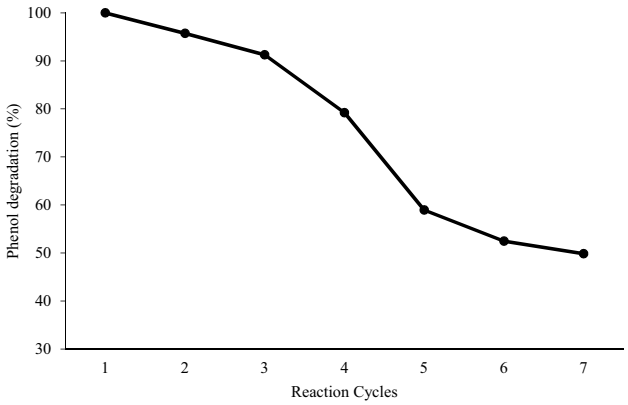

Fig. 7 Phenol removal efficiency in different treatment cycles using well water as real water samples

Conclusion

In this study, a tyrosinase-carrying magnetic nano-biocatalyst was fabricated and employed to treat phenol-containing wastewater. Biodegradation of phenolic compounds with tyrosinase immobilized onto the Cy-MNPs introduces an attractive option to augment the typical wastewater treatment methods for the micropollutants removal such as phenol. The fabricated nano-biocatalyst degraded phenol with different concentrations efficiently and in a shorter time in comparison with the previous studies. For high phenol concentration of 2500 mg/L, the immobilized tyrosinase displayed a degradation yield of approximately 70% which was much higher and faster than that of the previous studies at similar concentration. The tyrosinase-MNPs were successfully reused in different treatment cycles and more than 55% of phenol removal was observed after the seventh cycle. In addition, the nano-biocatalyst had a remarkable ability to remove phenol from real water samples. The results indicate the effectiveness and applicability of the fabricated nano-biocatalyst for the treatment of phenol-containing wastewater in a shorter time and higher efficiency even at high phenol concentration. The fabricated nano-biocatalyst can be promising for the micropollutants removal and an alternative for the catalysts used in the conventional water and wastewater treatment processes.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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