Received: 17 January 2020 | Revised: 29 May 2020 | Accepted: 30 May 2020

DOI: 10.1002/aoc.5899

Applied Organometallic Chemistry WILEY

FULL PAPER

A novel magnetically separable laccase-mediator catalyst system for the aerobic oxidation of alcohols and 2-substituted-2,3-dihydroquinazolin-4(1*H*)-ones under mild conditions

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In this study, a magnetically reusable artificial metalloenzyme has been constructed by co-immobilization of palladium nanoparticles as a strong oxidizing catalyst and laccase as an oxygen-activating enzyme into the cavities of magnetic mesocellular foams silica (Pd-Laccase@MMCF). The combination of Pd-Laccase@MMCF and hydroquinone (HQ) act as electron-transfer mediator system and make stepwise electron transfer from substrate to molecular oxygen. This catalyst system was used for the aerobic (i) oxidation of alcohols to the corresponding carbonyl compounds and (ii) dehydrogenation of 2-substituted-2,3-dihydroquinazolin-4(1*H*)-ones in phosphate buffer (0.1 M, pH 4.5, 4 mL)/THF (4%, 1 mL) as solvent under mild conditions. The co-immobilization of both laccase and Pd onto high surface area mesoporous support, high catalytic activity and magnetically separable and reusable make the present catalyst system superior to other currently available electron-transfer mediator systems.

KEYWORDS

aerobic oxidation, laccase, magnetic mesocellular foam silica, Metalloenzyme, palladium

1 | INTRODUCTION

In recent decades, the use of enzymes as safe catalysts in organic reactions has attracted considerable attention because enzyme-catalyzed reactions meet most of the criteria of green chemistry.[1] This holds particularly true for enzyme-catalyzed oxidations in the presence of O_2 as the oxidant.[2] O_2 is not only one of the cheapest oxidants available, but also regarded as an environmentally friendly oxidant. Another potential advantage of O_2 is the formation of non-toxic H_2O as the only byproduct during the course of oxidase-catalyzed oxidations with O_2. Within this context, laccase-catalyzed oxidations are receiving particular attention among synthetic chemists in recent years.[3] Laccase, a multicopper-containing oxidase usually found in plants and fungi, is able to catalyze the oxidation of a wide variety of phenolic compounds and aromatic amines in the presence of O_2 as the oxidant under mild conditions and produce H_2O as the only by-product.[4] Notwithstanding these advantages, the utilization of laccase in organic reactions is limited owing to low stability, high production costs and the difficult recovery and reuse of it.[5,6] It has been shown that the immobilization of laccase on heterogeneous system can overcome the aforementioned disadvantages by improving the stability of the enzyme, facilitating its separation from the reaction mixture, and reducing the cost factor because immobilized enzyme can be easily reused.[6–9] Morever, another major obstacle using free laccase as the catalyst in organic

reactions is the low redox potential. It is well-known that the redox potential of laccase can be broadened by natural mediators such as 4-hydroxybenzoic acid and 3-hydroxyanthranilic acid or by artificial mediators including 2,2-Azino-bis-(3-ethylbenzothiazoline)-6-sulfon0ic acid (ABTS), 1-hydroxybenzo-triazole (HBT), and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO). Although, the use of laccase-mediator systems (LMS) proceeds the oxidation of substrates with higher redox potentials [10], the mentioned mediators are quite expensive and difficult to recover from reaction mixture, which hampers the applications of LMS. In order to overcome these limitations, co-immobilization of laccase and mediators on different support materials such as cellulose beads,[4] magnetic Fe_3O_4[6] and mesoporous silica[11] have been made. Despite these developments, the co-immobilization of laccase and new more effective mediator on to high specific surface areas support is still demand.

Mesoporous silica materials have been widely explored for their unique properties such as high surface areas, large pore volumes, tunable pore sizes, and a controllable framework composition.[12] In recent years, magnetic silica materials have attracted a lot of attention as support for enzyme immobilization because they possess not only the unique features of mesoporous materials, but also magnetic properties make these materials can be simply separated by an external magnetic field and recycled.[13]

The selective oxidation of alcohols to carbonyl compounds plays a key role in organic chemistry and in industry owing to the wide application of the resulting carbonyl compounds as precursors for many drugs, agricultural chemicals and fine chemicals.[14] Although a variety of stoichiometric reagents have been traditionally used to achieve this transformation, their use causes environmental problems owing to generation of a large amount of waste.[15] To overcome this shortcoming, catalytic methodologies employing molecular oxygen as the sacrificial oxidant have been emerged.[16] Thus, much attention was given to palladium catalysts for the aerobic oxidation of alcohols.[17] However, because of the unfavored electron transfer between Pd^0 and O_2, palladium-catalyzed oxidations with direct reoxidation of Pd^0 by O_2 require high reaction temperatures, which are not suitable for thermally unstable substrates. Therefore, it seems that there is still an increasing need for the development of systems that can operate under mild conditions. In order to overcome this problem, Bäckvall and coworkers have reported several mild palladium-catalyzed aerobic oxidations that proceed through a multistep electron transfer containing a triple catalytic system.[18] These catalytic systems consist of macrocyclic transition metal complex that acts as the oxygen-activating component, benzoquinone as electron transfer mediator (ETM) and palladium as substrate-selective catalyst. They obtain a stepwise electron transfer from substrate to molecular oxygen to perform desired transformation under mild conditions (Scheme 1).

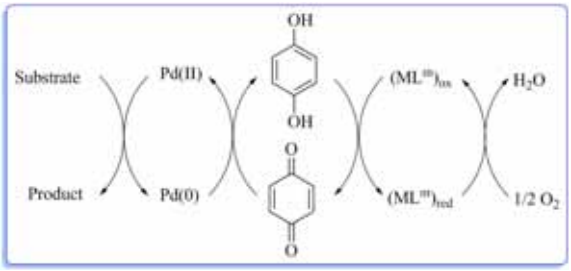
Although these systems presented an interesting breakthrough in the field of aerobic oxidation, these methods require transition metal complexes and expensive ligands which are intrinsically difficult to separate from the product and recycle. Therefore, there is an increasing demand for the development of heterogeneous cooperative catalytic systems with simple separation and recycling in the ligand free aerobic oxidation of organic compounds under mild conditions.

Based on the mentioned background and our systematic studies about the application of laccase and laccase/quinone catalytic system in organic reactions, [19] our main objectives in this study are: (1) the synthesis of a magnetically separable cooperative catalyst system *via* the immobilization of Pd nanoparticles as a strong oxidizing catalyst and laccase enzyme as an oxygen-activating and sustainable alternative to transition metal complex on MMCF (Pd-Laccase@MMCF); and (2) the investigation of the catalytic ability of this biohybrid catalyst for the aerobic oxidation of alcohols and 2-substituted-2,3-dihydroquinazolin-4(1*H*)-ones in the presence of hydroquinone instead of benzoquinone as electron transfer mediator (Scheme 2). One of the main novelty and advantage of this cooperative catalytic system is that redox catalysts are immobilized on magnetically reusable support.

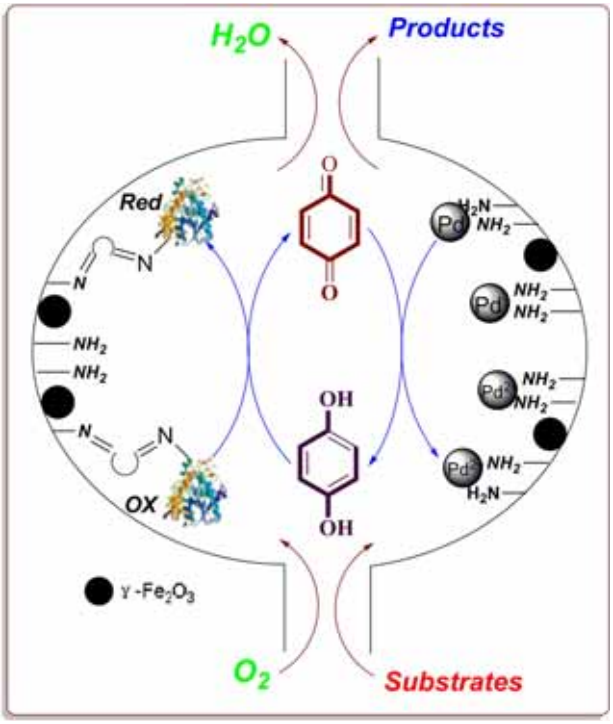
2 | EXPERIMENTAL

2.1 | Material and physical measurements

All substances were bought from the Merck and Aldrich Chemical Companies and utilized without additional purification. The 2-substituted-2,3-dihydroquinazolin-4(1*H*)-ones were prepared by previous procedures in our



SCHEME 1 Cooperative catalytic aerobic oxidation systems



SCHEME 2 Pd-Laccase@MMCF/HQ as a cooperative catalytic aerobic oxidation system

research lab.[20] The particle size was obtained by SEM using FESEM-TESCAN MIRA3. The chemical composition of the prepared nanocatalyst was determined by EDX (Energy Dispersive X-ray Spectroscopy). Pd, Cu and Fe percentages of the prepared nanocatalyst were obtained by inductively coupled plasma-optical emission spectrum (ICP-OES) on Optima 7300D spectrophotometer. Magnetic measurements were carried out using a vibration sample magnetometer (VSM, Meghnatis Daghigh Kavir Co., Iran) under magnetic fields up to 20 kOe. X-ray diffraction (XRD) measurement was done on a X'PertPro Panalytical.

2.2 | Preparation of magnetic mesocellular foam silica (MMCF)

Spherical mesocellular foam silica was prepared following a previously reported procedure.[21] The characteristics of the obtained MCFs are as follows: BET surface area = 503 m^2 g^{-1}, specific pore volume = 1.1 cm^3 g^{-1}, window size = 7 nm and Cell size = 24 nm.

Next, the magnetic particles were introduced into the MCF as follows [22]: Fe $(NO_3)_3 \cdot 9H_2O$ (2.68 g) was dissolved in methanol (5 ml), followed by addition of 1 g of foam. After evaporation of methanol at 80 °C in an oven,

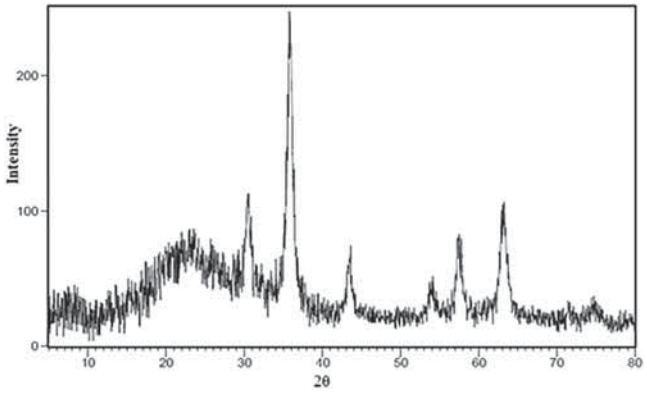


FIGURE 1 XRD pattern of magnetic mesocellular foam silica

the Fe $(NO_3)_3$-impregnated foam was reacted with propionic acid (4.6 ml) at 85 °C for 3 hr to form the iron propionate complex. Subsequently, the sample was heated to 300 °C in air (heating rate of 1 °C/min) and maintained at this temperature for 30 min. The resulting material was named as MMCF.

The synthesized MMCF was characterized by X-ray diffraction (XRD). As is shown in Figure 1, the XRD spectrum of the MMCF exhibited a broad reflection (2θ = 11-29°), approving the presence of amorphous silica.[23] This spectrum also indicated multiple peaks in the 2θ range 30°-70°, which revealed the presence of γ-Fe_2O_3 nanoparticles inside foam spheres.[22]

2.3 | Preparation of amine functionalized MMCF

The synthesized MMCF (1 g) suspended in dry toluene (20 ml) was added to 10 ml of toluene solution containing 2.7 ml of 3-aminopropyltrimetoxysilane. The contents were heated to reflux under argon atmosphere for 24 hr. Then, the carrier was magnetically collected, washed with toluene, ethanol, and dichloromethane and dried.[24] The resulting material was named as AmP-MMCF.

2.4 | Immobilization of Pd nanoparticles onto AmP-MMCF

First, 500 mg of the amine functionalized MMCF was suspended in pH-adjusted deionized water solution (pH 8) by adding LiOH (15 ml, 0.1 N) and stirring for 5 min. Thereafter, Li_2PdCl_4 was prepared by mixing $PdCl_2$ (145.41 mg, 0.82 mmol) and LiCl (69.52 mg, 1.64 mmol) in 10 ml distilled water, the suspension was

stirred at 80 °C until a homogeneous solution was achieved. The resulting solution was filtered, pH-adjusted (pH 8), and then added to the mixture of AmP-MMCF in water. After being stirred at room temperature for 24 hr, the resultant Pd (II)-precatalyst was collected by applying an external magnetic field and washed with distilled water. Then, the Pd (II)-precatalyst was re-suspended in water (15 ml), and a solution of distilled water (5 ml) containing $NaBH_4$ (310.2 mg, 8.2 mmol) was added to reduce Pd (II) to Pd(0). After 30 min, the final fabricated particles were magnetically separated, washed with water, acetone, and dried.[24] The resulting material was named as Pd-AmP-MMCF.

2.5 | Immobilization of laccase onto Pd-AmP-MMCF

500 mg of the Pd-AmP-MMCF suspended in potassium phosphate buffer (30 ml, 100 mM, pH 8.0) was mixed with glutaraldehyde solution (50% in H_2O, 230 mg, 0.99 mmol) at room ambient for 24 hr. After magnetic separation, the GA-modified particles were collected, washed with the phosphate buffer and acetone and applied to react with the amino groups of the enzyme. For this purpose, 500 mg of the Glutaraldehyde-functionalized Pd(0)-AmP-MMCF particles was re-dispersed in the enzyme solution prepared by dissolving laccase enzyme (30 U) in potassium phosphate buffer (3 ml, 100 mM, pH 7.2). The reaction mixture was then stirred at room temperature for 12 hr. Finally, the enzyme-bound magnetic particles, named as Pd-Laccase@MMCF, were collected, washed with phosphate buffer and dried under reduced pressure.

2.6 | Activity assay of immobilized laccase in Pd-laccase@MMCF

In order to assessment the amount of immobilized laccase onto support, the Bradford method[25] was used as follows: The activity of laccase in Pd-Laccase@MMCF was assayed spectrophotometrically with 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) as substrate (5 mM) in Na-acetate buffer (100 mM, pH 5) by measuring absorbance increase at 420 nm at room temperature. Suitable amount of Pd-Laccase@MMCF in Na-acetate buffer (100 ml) was added to the mixture and the initial rate was immediately measured as increase in optical density at 420 nm.[25] The molar extinction coefficient for the oxidation of ABTS at 420 nm is 3.6 × 104 M^{-1} cm^{-1}. One unit of activity is defined as the amount of enzyme required to oxidize 1 mmol of ABTS per minute. The findings of this procedure revealed that the amount of immobilized laccase was 22 U per 0.5 g of support.

2.7 | General procedure for the aerobic oxidation of alcohols

To a mixture of alcohol (1 mmol), Pd-Laccase@MMCF (0.25 g) and hydroquinone (HQ, 0.27 mmol) in an open-air round-bottom flask, NaPBS/THF (5 ml, 4/1 *v*/v) was added. Then, the resulting mixture was stirred at 40 °C for an appropriate time. The progress of reaction was monitored by TLC. After completion of the reaction, the catalyst was separated using an external magnet and the product was extracted with Et_2O. The organic phase was dried over anhydrous $MgSO_4$. Finally, the purity of the products was evaluated by GC without any chromatographic purification.

2.8 | General procedure for the aerobic dehydrogenation of 2-substuted-2,3-dihydroquinazolin-4(1H)-ones

To a magnetically stirred solution of Pd-Laccase@MMCF (0.25 g), and HQ (0.34 mmol) in NaPBS/THF (4/1 ml) under air, substrate (1 mmol) was added. Then, the reaction mixture was stirred at 40 °C for an appropriate time. After the reaction, the catalyst was easily separated using an external and the product was extracted with Et_2O (3 × 5 ml). The organic layer was dried with anhydrous sodium sulfate, the excess solvent was removed under reduced pressure. The crude product was purified by recrystallization from ethanol or chromatography on silica gel using *n*-hexane/ethyl acetate (3:1).

3 | RESULTS AND DISCUSSIONS

3.1 | Preparation of Pd-laccase@MMCF as an artificial metalloenzyme

An artificial metalloenzyme (ArM) is a metalloprotein made in the laboratory which cannot be found in the nature and can catalyze certain desired chemical reactions. Three different approaches have been developed to anchor transition metals to native enzymes to form hybrid artificial metalloenzymes.[26] A limitation with the approach of binding a metal catalyst into the active site

of the enzyme is that the natural enzyme activity is inhibited. Therefore, the search for new successful strategies in the construction of artificial metalloenzymes that maintain the activity and stereoselectivity of enzyme with the original function of the active site has become a very active area of research. In this context, several hybrid catalysts with metalloenzyme-like properties have been devolved.[27] Backvall and co-workers designed heterogenuse hybrid catalyst consisting of palladium nanoparticles and lipase enzyme that has the properties of a metalloenzyme for cooperative tandem catalysis.[28] This research prompted us to design a magnetically reusable cooperative catalyst system by the stepwise immobilization of palladium nanoparticles and laccase enzyme into the cavities of magnetic mesocellular foam (MMCF) (Scheme 3). As shown in scheme 3, initially magnetic nanoparticles were grafted onto the pore surface of the mesocellular foam silica. Subsequently, the MMCF was modified with 3-aminopropyltriethoxysilane to introduce amine groups onto their surfaces. Then, palladium nanoparticles were immobilized in the amino-functionalized magnetic material. Finally, laccase was immobilized by covalent attachment on the functionalized magnetic nanocomposite to prepare Pd-Laccase@MMCF by glutaraldehyde crosslinking. In Pd-Laccase@MMCF, each cavity might act as an artificial metalloenzyme, containing both amino acid constituents in the form of the immobilized laccase and a metal cofactor in the form of Pd nanoparticles. This hybrid catalyst was characterized using SEM, EDX, ICP, FT-IR and VSM techniques.

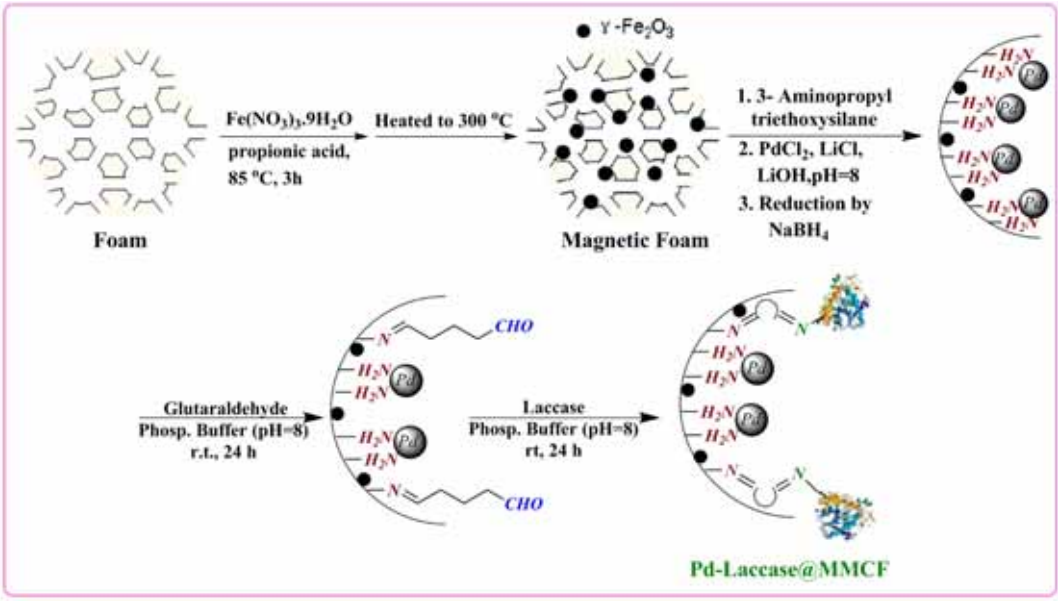
3.2 | Characterization of Pd-laccase@MMCF

Scanning electron microscopy (SEM) was performed to elucidate the surface morphology and size of the Pd-Laccase@MMCF particles. As shown in SEM image in Figure 2, the catalyst is composed of aggregated particles with a size range of 21–62 nm, confirming the nanostructure of the prepared catalyst.

In order to show the elemental composition of the catalyst, the EDX analysis of Pd-Laccase@MMCF nanocomposite was carried out. As is shown in Figure 3, EDX spectrum reveals the existence of C, N, O, Fe, Si, Pd and as well as Cu species in catalyst structure. To be noted that the attendance of Cu element in this analysis a good indication for the immobilization of laccase. In addition, the elemental mapping images also confirmed the presence of C, N, O, Fe, Si, Pd and Cu in the synthesized nanocomposite (Figure 4). On the other hand, we have determined the Pd, Cu and Fe contents of the synthesized nanocomposite by ICP-OES analysis. This analysis displayed that the Pd-Laccase@MMCF nanocomposite contained 16.5 wt % Pd, 0.025 wt % Cu and 10.4 wt % Fe.

FT-IR spectra of succeeding steps in the synthesis of the hybrid catalyst are presented in Figure 5.

The FT-IR spectrum of MMCF (Figure 5a) indicated the following peaks: Fe-O stretching vibration at 587 cm^{-1}, Si-O-Si symmetric stretching, asymmetric stretching and binding vibrations at 1096, 810 and 466 $cm^{-1}$and O-H stretching vibration at 3420 cm^{-1}.



SCHEME 3 The process of Pd-Laccase@MMCF synthesis

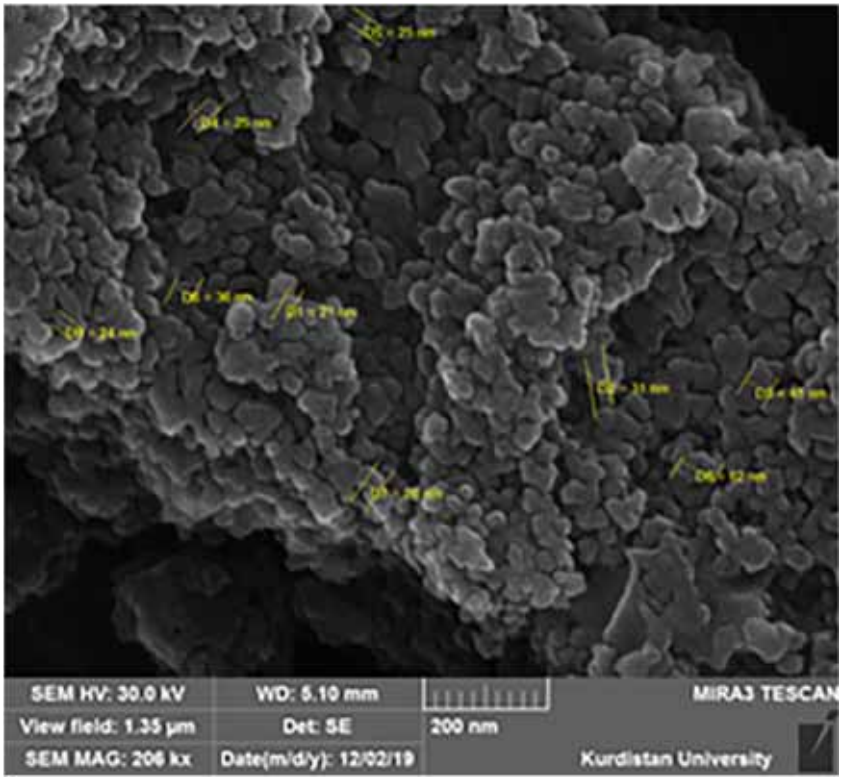


FIGURE 2 SEM image of Pd-Laccase@MMCF

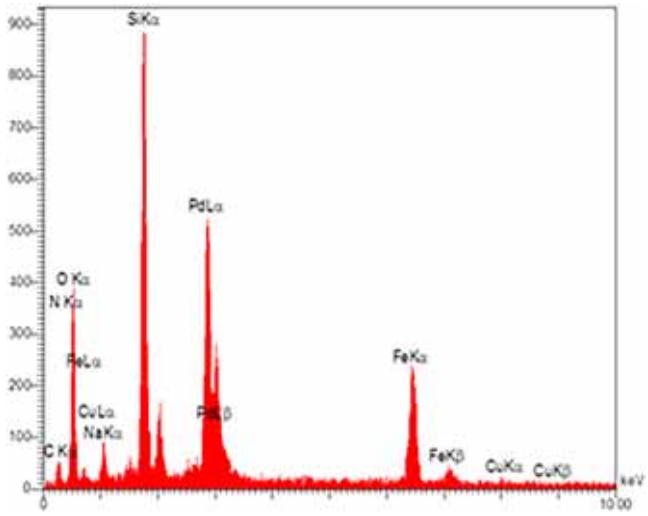


FIGURE 3 EDX spectrum of Pd-Laccase@MMCF

In the FT-IR spectrum of AmP-MMCF (Figure 5b), in addition to the above vibrations, the characteristic peaks of $-CH_2$ groups of the aminopropyl chain were detected at 2931 cm^{-1}, 2,862 cm^{-1}.[24]

In the case of FT-IR spectrum of GA-Pd-AmP-MMCF (Figure 5c), the existence of the C=N stretching vibration at 1662 cm^{-1} clearly proved that free $-NH_2$ group of Pd-AmP-MMCF reacted with –CHO group glutaraldehyde.[29]

Comparing the FT-IR spectra of Pd-Laccase@MMCF and GA-Pd-AmP-MMCF demonstrated that they are quite alike and no indicative peaks for laccase were not distinguished in the FT-IR spectrum of Pd-Laccase@MMCF (Figure 5d).

Figure 6 displays the magnetic hysteresis loops of MMCF and Pd-Laccase@MMCF nanoparticles. These curves display zero coercivity and no remanence, suggesting the synthesized samples are superparamagnetic. Meanwhile, the decrease of saturation magnetization (Ms) from 19.79 $emu.g^{-1}$ of MMCF to 9.10 $emu.g^{-1}$ of Pd-Laccase@MMCF was owing to the coating of the MMCF by non-magnetic materials.

3.3 | Catalytic activity of the Pd-laccase@MMCF nanoparticles

We examined the aerobic oxidation of alcohols to the corresponding carbonyl compounds in the presence of Pd-Laccase@MMCF under mild conditions. To determine the most appropriate conditions for the oxidation of alcohols, our investigations focused on the oxidation of 1-phenyl ethanol (1a) as a model reaction. The oxidation of 1-phenyl ethanol in the presence of Pd-Laccase@MMCF (0.25 g) in phosphate buffer (0.1 M, pH 4.5, 4 ml)/THF (4%, 1 ml) at 40 °C afforded the corresponding ketone in low yield (Table 1, entry 1). We wondered whether it is probable to increase the yield of the reaction by using a mediator. For this purpose, the model reaction was conducted in the existence of two

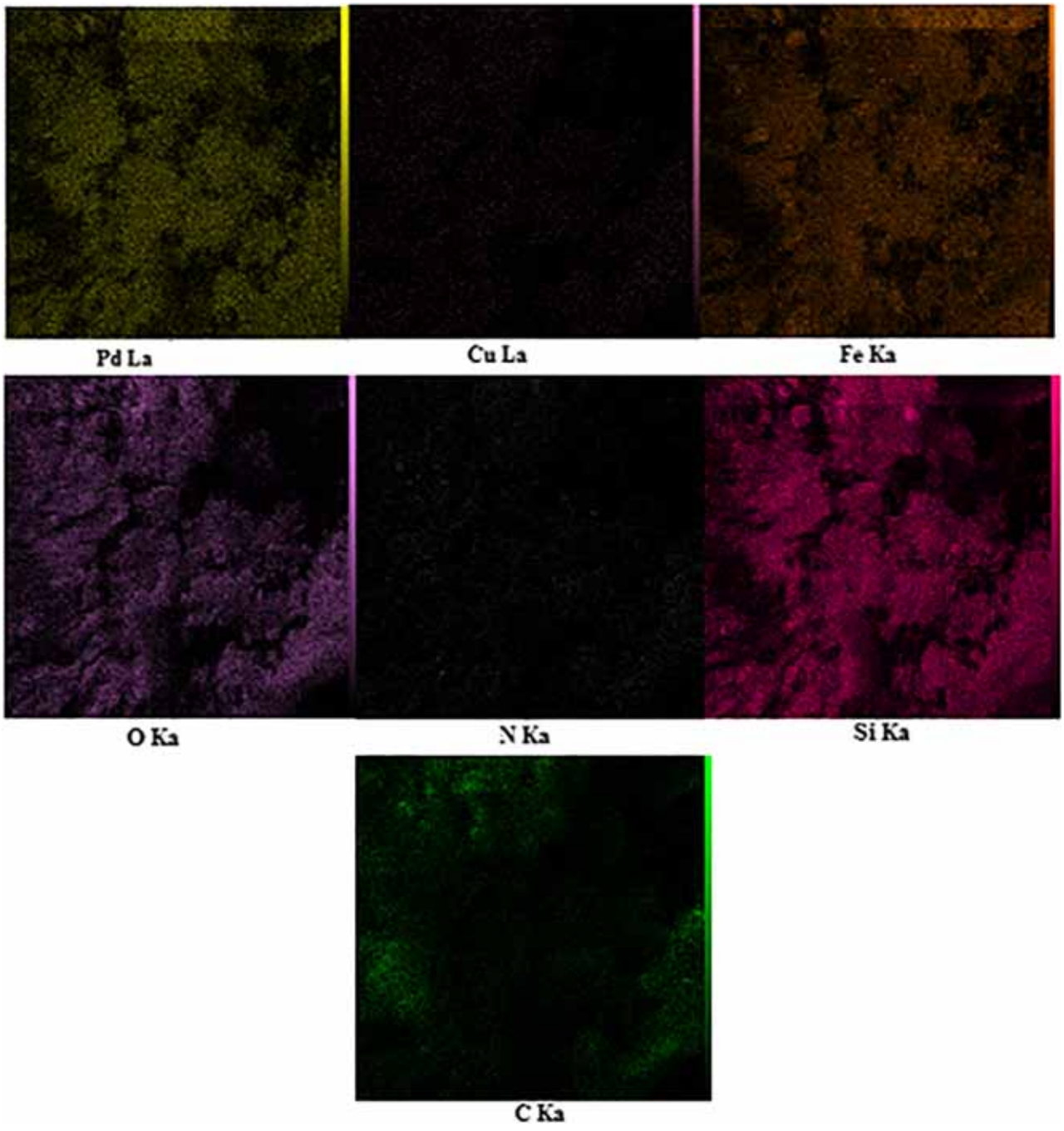


FIGURE 4 Elemental mapping of the Pd-Laccase@MMCF

different mediators including hydroquinone (HQ) and *p*-benzoquinone (BQ). Notably, the yield of 2a amounted to 99% in the existence of any of the mediators (Table 1, entries 2–3). The reaction time in the presence of BQ was shorter but HQ was selected as the mediator for the subsequent experiments owing to its low toxicity. Next, to determine the appropriate solvent, the model reaction was carried using different solvents such as THF/NaPBS, CH_3CN/NaPBS, THF and CH_3CN (Table 1, entries 2, 4–6). Even though the oxidation of 1-phenyl ethanol in either THF/NaPBS or CH_3CN/NaPBS afforded the corresponding product in high yield, the less toxicity of THF resulted in us choosing THF/NaPBS as the reaction medium. The next set of experiments was dedicated to find out the effect of the amount of the mediator and catalyst on the model reaction. The results clearly indicated that reduction in the amount of HQ or catalyst led to the decrease in the yield of product (Table 1, entries 7–8). The effect of the temperature was screened upon the model reaction. When the reaction temperature was

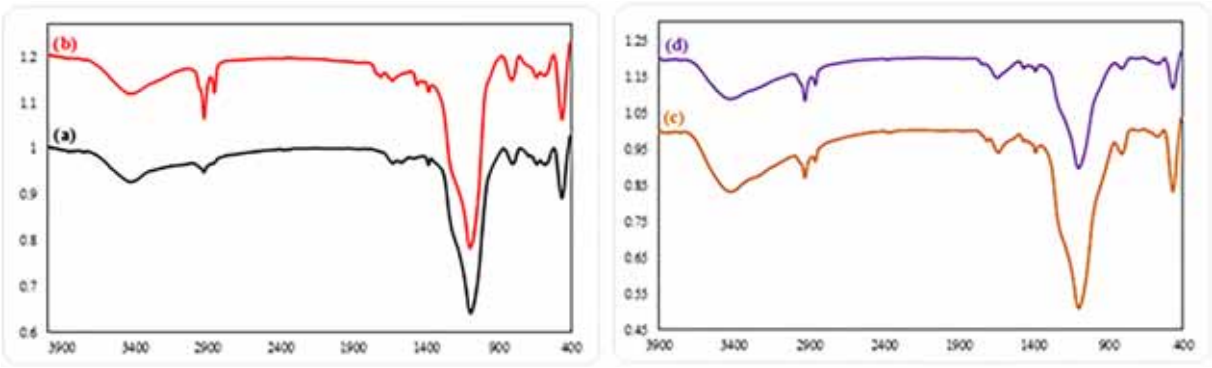


FIGURE 5 FT-IR spectra of a) MMCF, b) AmP-MMCF, c) GA-Pd-AmP-MMCF, d) Pd-Laccase@MMCF

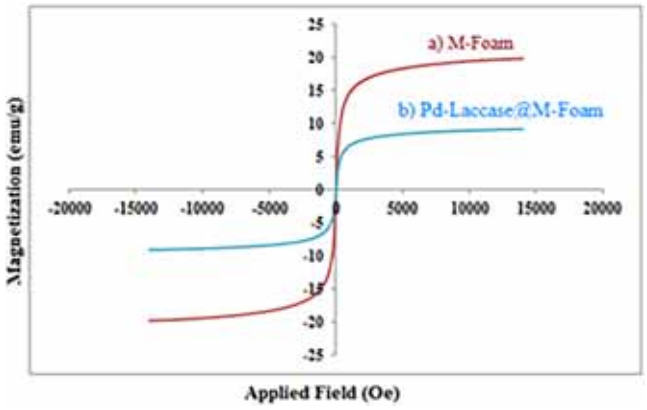


FIGURE 6 Magnetization curves of (a) MMCF (b) Pd-Laccase@MMCF

down to room temperature, the reaction didn't go to completion and only 50% yield was obtained (Table 1, entry 9).

Moreover, we surveyed other catalysts including magnetic MCF (MMCF), Pd@MMCF, and Laccase@MMCF instead of Pd-Laccase@MCF in the presence of HQ for the aerobic oxidation of 1-phenyl ethanol (Table 1, entries 10–12). MMCF failed to catalyze the desired oxidative reaction (Table 1, entry 10). Using Pd@MMCF as catalyst led to 30% conversion into the desired product (Table 1, entry 11). The model reaction was also conducted in the presence of Laccase@MMCF as catalyst. It was observed that the desired product was obtained in only 15% GC yield (Table 1, entry 12). Furthermore, we also examined the aerobic oxidation of 1-phenyl ethanol with HQ in the absence of Pd-Laccase@MMCF and found that the desired product was not formed (Table 1, entry 13). The above results clearly indicate that palladium nanoparticles and laccase immobilized into the cavities of magnetic MCF (heterogeneous catalyst) and HQ obtain a stepwise electron transfer from substrate to molecular oxygen and act cooperatively to facilitate the aerobic oxidation under mild conditions.

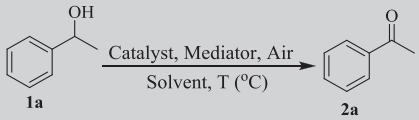
In summary, the most appropriate conditions for oxidation of 1-phenyl ethanol (1a) was achieved in the presence of Pd-Lacasse@MMCF(0.25 g), HQ (0.27 mmol) in phosphate buffer (0.1 M, pH 4.5, 4 ml)/THF (4%, 1 ml) under air at 40 °C (Table 1, entry 2).

The generality of the developed protocol has been demonstrated by oxidation of a representative range of alcohols under the optimized conditions (Table 2).

As can be seen from Table 2, Pd-Laccase@MMCF works fine in the selective oxidation of a variety of primary benzylic alcohols to the corresponding benzaldehydes in excellent yields, over-oxidation to carboxylic acids was not detected (Table 2, entries 1–5). Notably, the catalytic performance of this hybrid catalyst was influenced by the nature of the substituents in the aromatic ring and it was observed that oxidation of primary benzylic alcohols with electron-donating groups (Table 2, entry 2) occur faster than for electron-withdrawing ones (Table 2, entries 3–5). Similarly, the present catalyst system also presented high activity and selectivity for the aerobic oxidation of secondary benzylic alcohols to the expected ketones (Table 2, entries 6–7). It should be noted that heterocyclic alcohols were also smoothly converted into the respective products in excellent yield and selectivity (Table 2, entry 8). To our delight, both primary and secondary aliphatic alcohols could be successfully oxidized to the respective carbonyl compounds using the present method in good to high yields (Table 2, entries 9–11).

In sustainable organic synthesis, the recovery and reusability of the catalyst are the two most important properties of catalytic processes. Therefore, to survey this issue, we carried out the oxidation of 1-phenyl ethanol (3 mmol) using Pd-Laccase@MCF (0.75 g) and HQ (0.81 mmol) in phosphate buffer (0.1 M, pH 4.5, 12 ml)/THF (4%, 3 ml) at 40 °C. After the completion of the reaction, the magnetic heterogeneous biocatalyst was separated with external magnet and washed with Et_2O (3 × 5 ml), dried and used in the next oxidation

TABLE 1 Optimizbic oxidation of 1-phenylethanol[a]



Entry	Catalyst (g)	Solvent	Mediator (mmol)	Temperature (°C)	Time (hr)	GC Yield (%)
1	Pd-Laccase@MMCF (0.25)	THF/NaPBS	-	40	16	40
2[a]	Pd-Laccase@MMCF (0.25)	THF/NaPBS	HQ (0.27)	40	16	99
3	Pd-Laccase@MMCF (0.25)	THF/NaPBS	BQ (0.27)	40	15	99
4	Pd-Laccase@MMCF (0.25)	CH_3CN/NaPBS	HQ (0.27)	40	16	99
5	Pd-Laccase@MMCF (0.25)	THF	HQ (0.27)	40	16	80
6	Pd-Laccase@MMCF (0.25)	CH_3CN	HQ (0.27)	40	16	80
7	Pd-Laccase@MMCF (0.25)	THF/NaPBS	HQ (0.15)	40	16	80
8	Pd-Laccase@MMCF (0.2)	THF/NaPBS	HQ (0.27)	40	16	75
9	Pd-Laccase@MMCF (0.25)	THF/NaPBS	HQ (0.27)	r.t.	16	50
10	MMCF (0.25)	THF/NaPBS	HQ (0.27)	40	16	-
11	Pd@MMCF (0.25)	THF/NaPBS	HQ (0.27)	40	16	30
12	Laccase@MMCF (0.25)	THF/NaPBS	HQ (0.27)	40	16	15
13	-	THF/NaPBS	HQ (0.27)	40	16	-

[a]Reaction conditions unless stated otherwise: 1-phenylethanol (1 mmol), phosphate buffer (0.1 M, pH 4.5, 4 ml)/THF (4%, 1 ml), air, 40 °C.

reaction run. The recovered catalyst was added to fresh model reaction mixture (1-phenyl ethanol, HQ and phosphate buffer/THF) under same reaction conditions. When the reaction time was set to 16 hr, which proved to be sufficient for conversion of 1-phenyl ethanol, comparably excellent yields of acetophenone were obtained in 9 subsequent runs (Figure 7) to give a total turnover number (TON) of 90,830 for the laccase catalyst.

Due to the high catalytic efficiency of the Pd-Laccase@MMCF for the oxidation of alcohols, we became interested to test the activity of the prepared catalyst for the aerobic dehydrogenation of 2-substituted-2,3-dihydroquinazolin-4(1*H*)-ones to Quinazolin-4(3*H*)-ones. Quinazolin-4(3*H*)-ones are important nitrogen-containing heterocycles which have various biological and medicinal activities.[30,31] The cyclization of 2-aminobenzamides with aldehydes followed by subsequent oxidation is common method for the synthesis of quinazolin-4(3*H*)-ones.[32,33]

Under the conditions developed for the oxidation of alcohols, the aerobic oxidation of 2-phenyl-2,-3-dihydroquinazolin-4(1*H*)-one was conducted as a model reaction, which gave the corresponding product in 85% yield. This result managed us to acquire the optimal experimental conditions for the model reaction. The further experiments presented in Table 3 revealed that larger amount of HQ resulted in complete substrate conversion to the corresponding product.

With the reaction conditions explained above (Table 3, entry 3), dehydrogenation of a number of 2-substituted-2,3-dihydroquinazolin-4(1*H*)-ones was evaluated. The results summarized in Table 4 assert that 2-substituted −2,3-dihydroquinazolin-4(1*H*)-ones with electron-donating groups are more appropriate substrates than for electron-withdrawing ones. The oxidation of 2-substituted-2,3-dihydroquinazolin-4(1*H*)-ones carrying the electron-withdrawing groups also afforded the respective products in high yields but in these conditions longer reaction times were required (Table 4, entries 8–10).

3.4 | Selected spectral data of 2-substituted-2,3-dihydroquinazolin-4(1H)-ones

2-phenylquinazolin-4(3*H*)-one (Table 4**, entry 1)**:[34] ^{1}H NMR (DMSO-d_6, 250 MHz): δ (ppm) = 12.50 (s, 1H), 8.16–8.14 (m, 3H), 7.80–7.69 (m, 2H), 7.54–7.49 (m, 4H).

TABLE 2 Aerobic oxidation of various alcohols[a]

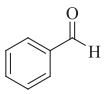
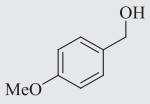
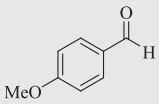
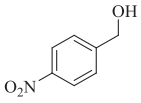
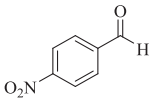
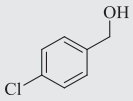
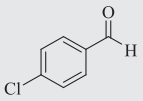
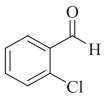
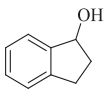
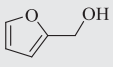
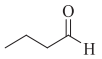
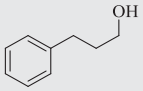
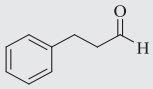
Entry	substrate	product	Time (hr)	GC Yield (%)[b]
1			18	99
2			17	99
3			20	97
4			19	97
5			20	97
6			16	99
7			19	99
8			19	99
9			24	97
10			20	97

TABLE 2 (Continued)

Entry	substrate	product	Time (hr)	GC Yield (%)[b]
11			20	96
				

[a]General procedure: Substrate (1 mmol), Pd-Laccase@MMCF (0.25 g), HQ (0.27 mmol), phosphate buffer (0.1 M, pH 4.5, 4 ml)/THF (1 ml), air, 40 °C.
[b]GC yields were determined by internal standard method.

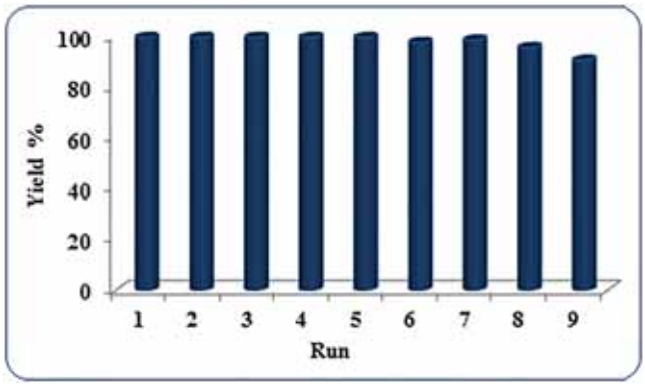


FIGURE 7 The recyclability of Pd-Laccase@MMCF as a catalyst in the aerobic oxidation of 1-phenyl ethanol

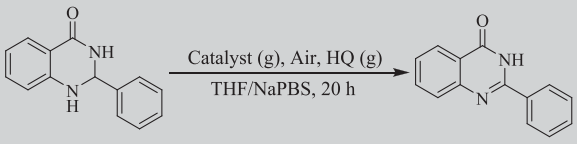
2-(4-Methylphenyl)quinazolin-4(3*H*)-one (Table 4, **entry 2)**: [34] ^{1}H NMR (DMSO-d$_6$, 250 MHz): δ (ppm) = 12.61 (s, 1H), 8.19–8.09 (m, 3H), 7.79 (t, *J* = 7.25 Hz, 1H), 7.67 (d, *J* = 8 Hz, 1H), 7.45 (t, *J* = 7.25 Hz, 1H), 7.05 (d, *J* = 8.5 Hz, 2H), 2.47 (s, 3H).

2-(4-Methoxyphenyl)quinazolin-4(3*H*)-one (Table 4, **entry 3)**:[34] ^{1}H NMR (DMSO-d$_6$, 250 MHz): δ (ppm) = 12.38 (s, 1H), 7.04–8.18 (m, 8H), 3.81 (s, 3H).

2-(3-Fluorophenyl)quinazolin-4(3H)-one (Table 4, **entry 8)**: ^{1}H NMR (DMSO-d$_6$, 250 MHz): δ (ppm) = 12.59 (s, 1H), 8.15 (d, *J* = 7.75 Hz, 1H), 8.057–7.970 (m, 2H), 7.86–7.27 (d, *J* = 7.5 Hz, 2H), 7.63–7.40 (m,3H).

2-(3-Bromophenyl)quinazolin-4(3*H*)-one (Table 4, **entry 9)**:[35] ^{1}H NMR (DMSO-d$_6$, 250 MHz): δ

TABLE 3 Optimization of reaction conditions for oxidative dehydrogenation of 2-phenyl-2,3-dihydroquinazolin-4(1*H*)-one[a]



Entry	Catalyst amount (g)	HQ (mmol)	Temperature (°C)	GC Yield (%)
1	Pd-Laccase@MMCF (0.25)	0.27	40	85
2	Pd-Laccase@MMCF (0.3)	0.27	40	85
3	Pd-Laccase@MMCF (0.25)	0.34	40	99

[a]Reaction conditions: Pd-Laccase@MMCF (0.25 g), HQ (0.34 mmol), air, phosphate buffer (0.1 M,pH 4.5, 4 ml)/THF (4%, 1 ml), 20 hr, 40 °C.

TABLE 4 Aerobic dehydrogenation of 2-substituted −2,3-dihydroquinazolin-4(1*H*)-ones[a]

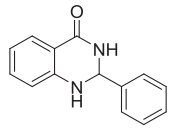
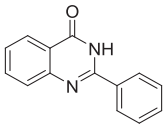
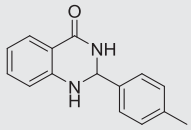
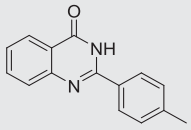
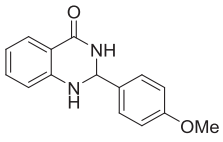
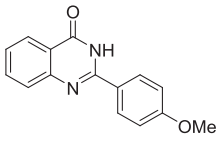
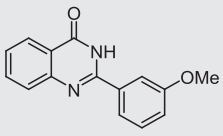
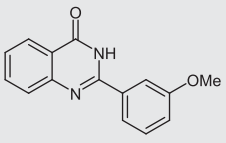
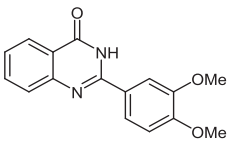
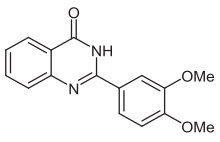
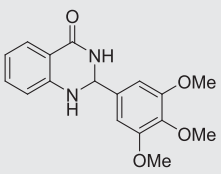
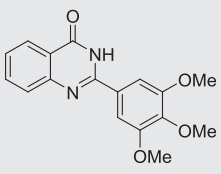
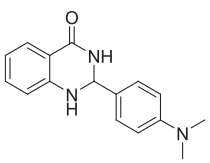
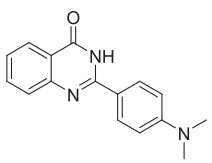
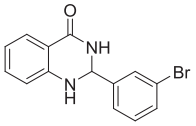
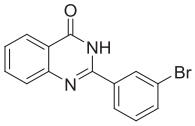
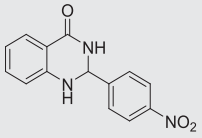
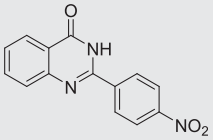
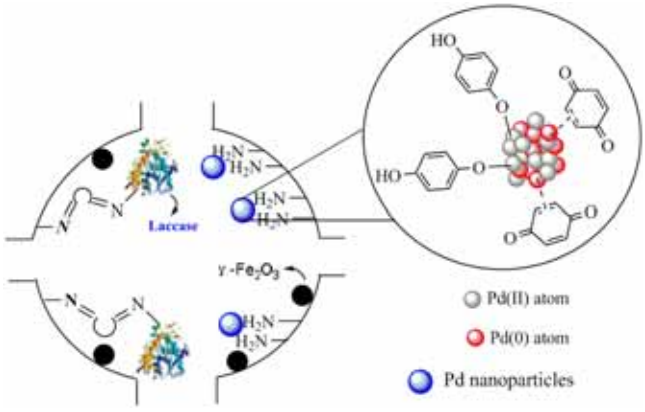
Entry	Substrate	Product	Time (hr)	Isolated Yield (%)	M.P. (°C) [Ref.]
1			20	92	236–238 [34]
2			18	95	238–240 [34]
3			16	94	244–247 [34]
4			16	95	181–182 [34]
5			16	92	242–243 [35]
6			16	95	257–260 [34]
7			18	91	235–238 [36]
8			30	90	288–289

TABLE 4 (Continued)

Entry	Substrate	Product	Time (hr)	Isolated Yield (%)	M.P. (°C) [Ref.]
9			30	87	295–296 [35]
10			32	87	363–364 [37]

[a]Reaction conditions: Substrate (1 mmol), Pd-Laccase@MMCF (0.25 g), HQ (0.34 mmol), air, phosphate buffer (0.1 M, pH 4.5, 4 ml)/THF (1 ml), 40 °C.



SCHEME 4 Pd (II) atoms are generated after treatment of BQ with Pd(0) atoms on Pd-Laccase@MMCF

(ppm) = 12.62 (s, br, 1H), 8.37 (s, 1H), 8.20 (d, J = 7.5 Hz, 1H), 8.17 (d, J = 7.5 Hz, 1H), 7.88–7.76 (m, 3H), 7.57–7.50 (m, 2H).

Although, the actual role of present cooperative catalyst system is not exactly clear now, however on the basis of previously reported mechanisms for the application of Pd@MCF/BQ catalyst system in the oxidation reactions[24,38] and aerobic oxidation of HQ to BQ in the presence of laccase,[19] it is hypothesized that Pd (II) atoms are actual oxidizing agents. After treatment of Laccase with HQ, the active Pd (II) atoms required for the oxidation reaction are generated through adsorption of BQ to the Pd(0) nanoparticles in Pd-Laccase@MMCF[38] (Scheme 4). Laccase can be reoxidized by molecular oxygen, thus completing the catalytic cycle (Scheme 2).

4 | CONCLUSIONS

To summarize, we have prepared a magnetically recoverable cooperative catalyst oxidation system by immobilizing palladium nanoparticles and laccase on MMCF, which were then applied an excellent catalyst for aerobic oxidative dehydrogenation of C–O and C–N bonds using an ideal oxidant under mild conditions. The advantages of these protocols are the use of air as the cheapest and greenest oxidant available, the mild reaction conditions and good to excellent yield of the products. It is also noteworthy that the immobilization of both of laccase and Pd onto magnetic foam provides a heterogeneous catalyst system that can be easily separated using an external magnetic field and reused several times without a significant loss of activity.

ACKNOWLEDGEMENTS

We gratefully acknowledge financial support of this research by University of Kurdistan.

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How to cite this article: Shokri Z, Azimi N, Moradi S, Rostami A. A novel magnetically separable laccase-mediator catalyst system for the aerobic oxidation of alcohols and 2-substituted-2,3-dihydroquinazolin-4(1*H*)-ones under mild conditions. *Appl Organomet Chem*. 2020;e5899. https://doi.org/10.1002/aoc.5899