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The combination of Fenton process and *Phanerochaete chrysosporium* for the removal of bisphenol A in river sediments: Mechanism related to extracellular enzyme, organic acid and iron

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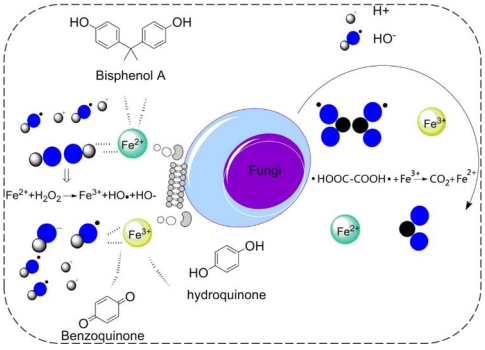
HIGHLIGHTS

- A novel technology combined Fenton process with fungal treatment was applied to remove BPA in river sediments.
- The novel combination treatment significantly increased the removal rate of BPA.
- There were synergetic effects on degradation of pollutants between this two treatment technologies.
- There was biochemical Fenton processes in the cultivation of *P. chrysosporium*.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, Fenton process combined with bioremediation technology, as a novel treatment technology, was applied for the removal of bisphenol A (BPA) from river sediments. The removal rate of BPA reached 58.23% after 24 days of combined treatment, which was higher than those in the treatment with *Phanerochaete chrysosporium* (*P. chrysosporium*) alone (21.59%) or the Fenton treatment alone (14.48%). The degradation mechanism of BPA in treatment process was investigated. According to the analyses of pH, iron, enzyme activities and organic acids, it was found that there was a synergetic effect between Fenton process and *P. chrysosporium* treatment. The organic acids generated by *P. chrysosporium* created a better acid environment for Fenton reaction, and the ferrous iron introduced by Fenton reagents might stimulate the development of *P. chrysosporium*. In addition, β-cyclodextrin (β-CD) is a good chelating agent together with excellent bioavailability, so we investigated the influence of β-CD on the combination treatment. Results showed that β-CD was able to promote the combination treatment. We also obtained that the optimal dosage of $FeSO_4$ (270 mmol/L), ratio of Fe^{2+} to H_2O_2 and dosage of β-CD (13 mmol/L) were 0.5 mL, 1:14 (mol/mol), 1 mL, respectively. The combined treatment under mild reaction conditions provides a new way for the removal of BPA from polluted river sediments.

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1. Introduction

Bisphenol A (BPA) is thought to be environmentally deleterious and is also an endocrine disrupting chemical (EDC), which has been universally used in different industry such as plastic production [1]. More and more researchers have detected BPA even in water body including lake, river and ocean sediments. Reviews about its potential biological effects have been reported [2,3]. The effects of BPA on human mainly related to reproductive system. Furthermore, the endocrine effects of BPA on other species have been confirmed [4,5], thus the elimination of BPA becomes a significant issue from the point of view of environment protection and human health [6].

Numerous technologies, including oxidation, adsorption, membrane separation, and advanced oxidation processes, have been developed for the removal of BPA. The Fenton process plays an influential role in the removal of pollutants. Fenton process with high removal rate, H_2O_2 and Fe^{2+} is oxidant and catalyst [7], respectively. H_2O_2 can act as an initiator as well as a hydroxyl radicals (HO·) scavenger, as shown in Eq. (4).

The free radical HO·, being generated in Fenton process, can remove various recalcitrant compounds because of their excellent oxidation ability. The process of classic Fenton reactions is showed [8–10]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO\cdot + OH^- \quad (k = 63 - 76\ M^{-1}s^{-1}) \tag{1}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2\cdot + H^+ \quad (k = 0.001 - 0.01\ M^{-1}s^{-1}) \tag{2}$$

In the presence of an organic substrate (R–H), the HO· abstracts a hydrogen atom from R–H and generate an organic radical (R·), which subsequently undergoes a series of chemical transformation to form various oxidation products [11,12]. However, the non-specific and enhanced reactivity of HO· towards both organic and inorganic substrates ($k > 108\ M^{-1}s^{-1}$ [13]) results in various competitive processes (Eqs. 4–6) that negatively affect the organic oxidation process [11,14,15].

$$Fe^{2+} + HO\cdot \rightarrow Fe^{3+} + OH^- \quad (k = 3.2 \times 10^8 M^{-1}s^{-1}) \tag{3}$$

$$H_2O_2 + HO\cdot \rightarrow HO_2^{\cdot} + H_2O \quad (k = 3.3 \times 10^7 M^{-1}s^{-1}) \tag{4}$$

$$HO\cdot + HO\cdot \rightarrow H_2O_2 \quad (k = 6 \times 10^9 M^{-1}s^{-1}) \tag{5}$$

Nowadays, Fenton technology has been commonly used in wastewater treatment particularly the refractory wastewater [16,17]. Its application in the remediation of polluted soil was also reported [18]. However, the setback of classic Fenton reaction such as the restricted pH (about 3.0), high reaction temperature, high costs and iron sludge are need to be solved [19]. Hence, some technologies could be used to modify Fenton process. According to the scientific literature, biological treatment was available for improving the performance Fenton process [20]. In recent years, white-rot fungi have attracted attention in the degradation of pollutants due to their superiority, such as they are involved in the degradation of various xenobiotic compounds [21], their penetration in soil via mycelia and rapid colonization of solid substrates [22]. Among these studies, mature fungal myceliums have been applied for the bioremoval of pollutants, such as heavy metals, polyaromatic hydrocarbon (PAHs) and polychlorinated biphenyl (PCBs). *P. chrysosporium*, as a kind of white-rot fungi, has an excellent adaption ability and can produce hemoperoxidases during their secondary metabolism [23], which are beneficial to the degradation of various target contaminants [24,25]. These hemoperoxidases including lignin peroxidase (LiP) and manganese peroxidase (MnP) are active and can promote the degradation of contaminants [26,27].

In previous studies, Fenton process and white-rot fungi can be used in waste treatment individually, and some researches also reported the Fenton process or white-rot fungi combined with other techniques in wastewater treatment and remediation of polluted soil [28–30]. So far the combination of these two treatment technologies for removal of contaminants in river sediments or soil has not been reported. A novel treatment technology that combining Fenton process and *P. chrysosporium* for the removal of BPA from river sediments was studied in this work. *P. chrysosporium* can secrete small molecular organic acids such as oxalic acid, malic acid and furmaric acid. These small molecular acids together with ligninase enzymes were involved in the metabolism of *P. chrysosporium* and have some effects on Fenton reaction. In this study, the biochemical Fenton process was investigated. According to the scientific literature, β-cyclodextrin (β-CD) was involved in the chemical Fenton treatment [31]. Hence, the effect of β-CD on combined treatment technology was also studied. Meanwhile, iron was important among Fenton process and the growth of white-rot fungi [32,33]. Therefore, the pH, of organic acids and ferrous iron, activity of Lip and MnP were investigated in this work.

2. Methods and materials

2.1. Preparation of contaminated sediments

The sediment samples were collected from the top 25 cm of Xiangjiang River in Hunan Province in central-south of China. Sediment samples were air dried, crushed and sieved with a 2 mm mesh to remove plant debris and gravels. The key physicochemical properties of sediments are presented in Table 1. There was no residual BPA in the collected sediment. The BPA solution ($1\ g{\cdot}L^{-1}$) was added to dried sediment (500 g) and homogenized under static incubation without light for obtaining laboratory sediment sample. The final concentration of BPA of dry weight sediments was about $250\ mg{\cdot}kg^{-1}$.

2.2. Optimization of Fenton treatment

Optimization experiment was carried out in aqueous solution. The pH was neutral, and the temperature was 25 °C. Ferrous sulfate was used to prepare the ferrous solutions. The ferrous iron (270 mM) and H_2O_2 solutions (3%, v/v), were freshly prepared every day in stock concentrations. Ultra-pure water (18.2 MΩ cm, Barnstead 133 D11911) and other solutions were deoxygenated before the reaction by introduction of nitrogen gas. The optimal dosage of $FeSO_4$, the ratio of Fe^{2+} to H_2O_2 and the dosage of β–CD were studied.

2.3. Fungal strain

The fungus strain *P. chrysosporium* BKMF-1767 (ATTC 24725) was obtained from China Center for type Culture Collection (Wuhan, China). Stock cultures was maintained on potato dextrose agar (PDA) plants at 4 °C and then transferred to PDA plates at 37 °C in constant incubator for 48 h before experiment. Spore suspension was prepared by the spore diluting in the sterile distilled water and the spore concentration was adjusted to $2.0 \times 10^6\ CFU{\cdot}mL^{-1}$.

2.4. Experiment setup

The best pH of homogeneous Fenton process was about 3. In order to avoid the damage to sediments resulted from pH change, the neutral pH was chosen in this study. BPA concentration, iron concentration, enzyme activities and organic acids concentration were analyzed. Four

Table 1
The key physicochemical properties of river sediments.

Properties	Value
pH	7.7
Temperature (°C)	20
Organic matter (%)	13.6
Organic carbon (%)	7.9
Sand (%)	65
Water content (%)	57.2

Table 2
The setting of experimental group. *P. chrysosporium*: *Phanerochaete chrysosporium*, β-CD: β-cyclodextrin.

Group	Fe^{2+} (mL)	H_2O_2 (mL)	*P. Chrysosporium* (mL)	β–CD (mL)
a	0.5	2	2	1
b	0.5	2	2	–
c	–	–	2	1
d	0.5	2	–	–

treatment groups were set up in the experiment as follows: **a**, **b**, **c** and **d**. 2 g sediment was placed in each 50 mL centrifuge tubes. In group **a**, the 2 mL of suspension of *P. chrysosporium* was added into the tubes firstly, and then the β-CD was added, thirdly the Fe^{2+} and H_2O_2 were added into the tubes immediately. Group **b** was the same as group **a**, except the β-CD was replaced by the amount water. Group **c** was the same as group **a**, except the Fe^{2+} and H_2O_2 was replaced by the amount water. Group **d** was the same as group **a**, except the *P. chrysosporium* was replaced by the amount water. The detailed information of different groups was described as Table 2. The suspension-to-sediment ratio was 4:1 in each tube. These tubes were sealed with screw caps, and placed at room temperature. All batch experiments were performed in duplicates.

2.5. BPA extraction and analysis

The extraction of BPA from sediments samples and the determination of BPA were carried out according to the procedure reported by Zeng et al. [34]. Briefly, the centrifuge tubes were centrifuged at 4000 r/min (10 min), and the residual BPA in sediments was extracted by adding 10 mL solvent mixture methanol-acetone (1:1, v/v), followed by sonication for 20 min. After setting, the organic phase was evaporated to dryness and redissolved in 2 mL acetonitrile. Then the solution filtered by a 0.22 μm syringe filter. BPA in the solution was determined by high-performance liquid chromatography (HPLC, Agilent 1100, USA) equipped with an Extend-C18 reversed-phase column (4.6 × 150 mm, Agilent Technologies). The mobile phase was acetonitrile/water (50: 50, v/v) and the flow rate was 1 mL·min^{-1}. UV-detection (1100-UV, Agilent Technologies, USA), and wave length was 226 nm. The column temperature was 40 °C. The retention time was 7 min.

2.6. Enzymes activities analysis

2.6.1. Preparation of crude enzyme liquid

The suspension in centrifuge tube was centrifuged for 10 min at 4 °C, and then supernatants were filtered through filter paper (0.22 μm). An ultraviolet spectrophotometer (UV-2700 SHIMADZU, Japan) was used for the analyses of the activities of enzyme. The ratio of fresh samples extracted with sterile distilled water was 1: 1 (w/v).

2.6.2. Lignin degradation enzymes analysis

The activity of LiP was tested by recording the oxidation of veratryl alcohol to veratryl aldehyde, which was monitored by spectrophotometrically at 310 nm (ε310 = 9300 M^{-1}·L·cm^{-1}) according to Liu et al. [35]. The mixture of reaction liquid was consisting of 1.0 mL veratryl alcohol (10 mM), 1.5 mL sodium succinate buffer solution (pH 3.0, 100 mM) and 0.4 mL crude enzyme extracts. 0.1 mL H_2O_2 (10 mM) was added to mixed solution for starting the reaction. The variation in absorbance of the solution was monitored via ultraviolet spectrophotometer at 310 nm for 3 min.

The activity of MnP was estimated by recording the oxidation of $MnSO_4$ spectrophotometrically at 240 nm (ε240 = 8100 M^{-1}·L·cm^{-1}). The mixture of reaction liquid was consisted of 0.5 mL $MnSO_4$ (15 mM), 2.0 mL sodium tartrate buffer solution (100 mM, pH 4.5) and 0.4 mL crude enzyme extracts. 0.1 mL H_2O_2 (10 mM) was added to mixed solution for starting the reaction. The variation in the solution was monitored via ultraviolet spectrophotometer at 240 nm for 3 min.

2.7. Iron analysis

Ferrous iron was analyzed using a Hach spectrophotometer (DR2800, Loveland, CO) based on the modulated Hach methods (No. 8051, 8146, and 8008).

2.8. Organic acids analysis

The supernatant after centrifuge (4000 r, 10 min) was filtered through 0.45 μm filter papers. The organic acids (oxalic acid, malic acid and fumaric acid) in the filtrate were determined by HPLC equipped with an Extend-C18 reversed-phase column (4.6 × 150 mm, Agilent Technologies). The mobile phase was phosphoric acid/water (998: 2, v:v) and the wave length was 210 nm. The column temperature was 30 °C.

3. Results and discussion

3.1. Optimal conditions of Fenton treatment

The optimal amount of $FeSO_4$, ratio of $FeSO_4$ to H_2O_2 and amount of β-CD can be recognized easily from Fig. 1. When the addition of $FeSO_4$ was less than 0.5 mL, the removal rate of BPA significantly decreased. It might be caused by the lack of HO· production [36]. And the removal rate of BPA was decreased when the dosage of $FeSO_4$ was over 0.5 mL. On one hand, this behavior was ascribed to the excess ferrous iron which can scavenge the hydroxyl radical based on Eq. (9):

$$HO_2\cdot \rightarrow O_2^{-}\cdot + H^{+} \quad (6)$$

$$O_2^{-}\cdot + H^{+} \rightarrow HO_2\cdot \quad (7)$$

$$Fe^{2+} + HO\cdot \rightarrow Fe^{3+} + OH^{-} \quad (8)$$

On the other hand, it may attributed to the complicated reaction Eqs. (1) and (2), (4), (6)–(8) among HO·, $HO\cdot_2$, Fe^{2+} and Fe^{3+} [9,13,14]. From Fig. 1b, the removal rate of BPA is closely related to the ratio of Fe^{2+} to H_2O_2. The optimal volume ratio of $FeSO_4$ solution to H_2O_2 was 1:4 (ml/ml), and, the optimal mole ratio of Fe^{2+} to H_2O_2 was 1:14 (mol/mol). β-CD has the significant effect on the reaction of Fe^{2+} and HO· in Fenton process. The reason why this Fenton process can be carried out at neutral pH condition might be the β-CD can form complexing with Fe^{2+}. Lindsey et al. [37] demonstrated the CD-iron complex formation by observing the differences in absorbance spectra of β-CD, carboxymethyl beta-cyclodextrin (CMCD), Fe^{2+} and iron-CD mixtures. And For β-CD, iron is likely coordinated by hydroxyl group on the rim of the CD [38]. CDs can form a ternary complex with iron and the hydrophobic pollutant, which allows effective direct HO· radical reaction towards contaminants [31,39].

3.2. Degradation of BPA

In order to investigate the removal rate of BPA under different treatment technology, the concentration of BPA was monitored by HPLC. The removal rate of BPA in three experiment groups was showed in Fig. 2. On day 1, the removal rate of BPA in group **a** was lower than **group d** (Fenton treated alone); from day 2 to 24, the removal rate of BPA in group **a** was higher than those in group **c** and group **d**. And it was obvious that the removal rate of BPA increased with the increase of time. On day 24, the removal rate of BPA in group **a** and **c** (*P. chrysosporium* treated alone) were 58.23% and 21.59%, respectively. Many studies have reported that *P. chrysosporium* was able to eliminate a series of contaminants [40,41], and the results in group **c** demonstrated

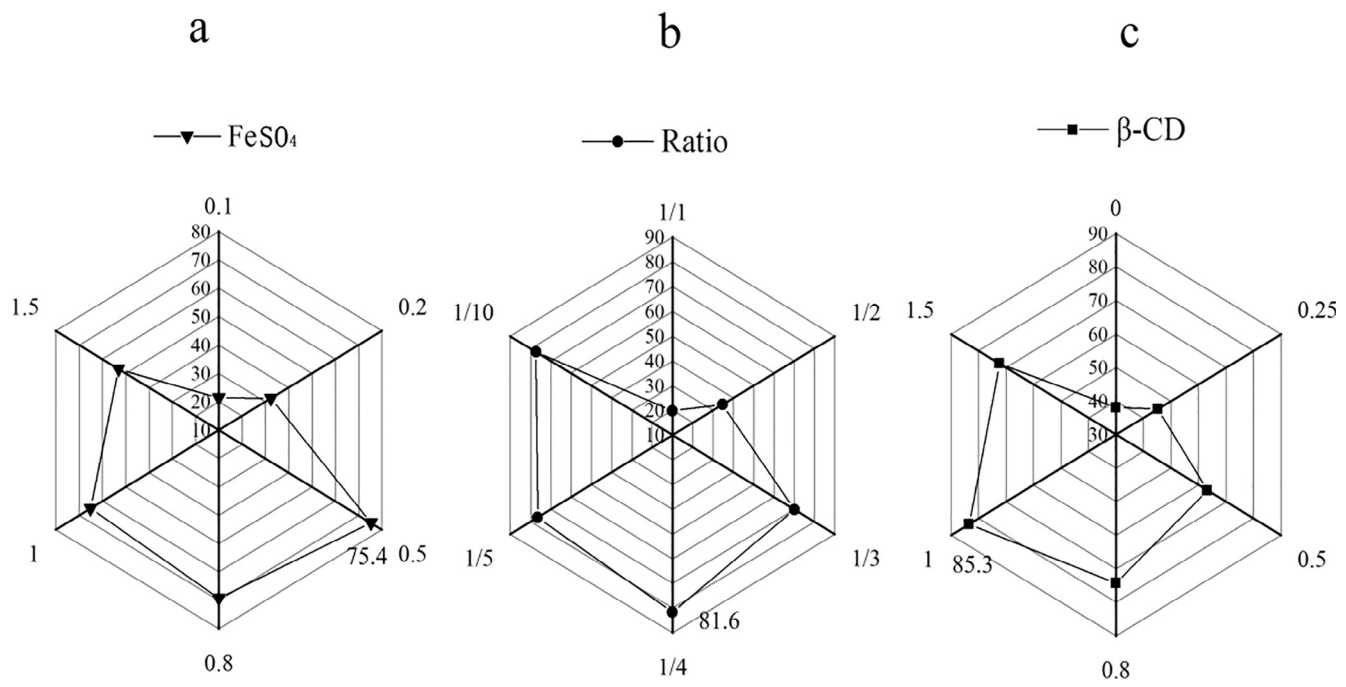

Fig. 1. Removal rate of bisphenol A in different conditions of Fenton process. The optimal amount of $FeSO_4$ (270 mmol/L), the $FeSO_4/H_2O_2$ (mL/mL), and amount of β-cyclodextrin (13 mmol/L) were 0.5 mL, ¼ (Fe^{2+}/H_2O_2, 1/14 (mol/mol)), and 1 mL, respectively.

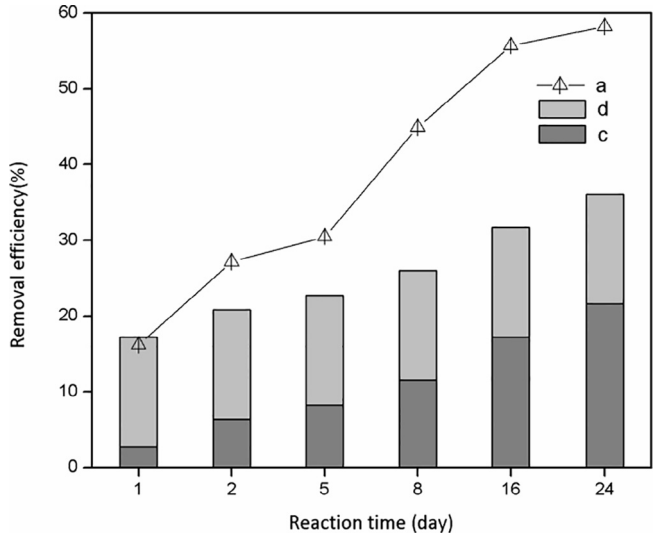

Fig. 2. Removal rate of BPA in degradation process. a: experimental group, c: *P. chrysosporium* treated alone, d: the removal rate of BPA after 1 day of Fenton reagents treatment.

that the BPA could be eliminated by *P. chrysosporium*. At the beginning days, removal rate of BPA in **group a** increased rapidly, it may ascribe to the sharp and quick chemical effect resulted from Fenton reagents. On day 5, the removal rate increased slowly, it may be attributed to the consumption of original chemicals. But after day 5, the concentration of BPA in sediments decreased faster than before, which could be correlated to the quick development of *P. chrysosporium*. On day 24, the removal rate reached 58.23%, yet the plus of **c** and **d** was only 36.07%, which indicated the combined treatment has favorable effect on elimination of BPA than individual treatment. Therefore, the combination of Fenton reagents and *P. chrysosporium* treatment was effective in remediation of BPA-polluted sediments. This combination treatment has advantage in removal effect.

3.3. Biochemical Fenton process in the treatment process

In the biodegradation process of cellulose, Paul [42] proposed Fe (II) and H_2O_2 can be generated by Fe (II) autoxidation, or by superoxide in reaction with Fe (III). Besides, the fungi could degrade intermediate products of contaminants caused by incomplete degradation of Fenton process [29,43]. In addition, oxalic acid is frequently accumulated in the extracellular of white-rot fungi [44]. Oxalic acid, a kind of secondary metabolites of fungi, plays an important role in lignocellulose degradation [45]. And the oxalic acid is able to bind and solubilize iron from iron oxide compounds [46]. Therefore, it was significant to study the concentration of oxalic acid and iron in the biochemical system. The pH and ferrous iron were related to biochemical Fenton process of *P.chrysosporium*. In order to investigate the biochemical Fenton process in the combined treatment, the pH, oxalic acid and iron content were analyzed.

3.3.1. Change of pH during biochemical Fenton process

The change of pH was showed in Fig. 3a. Before day 8, pH was continuously decreased in all groups, which was ascribed to the generation of acidic intermediate species among degradation or oxidation process. Some researchers have found that acids were secreted by microorganisms in the biochemical degradation system [47]. Oxalic acid is secreted by many white rot and brown rot fungi, which is essential for the degradation of cellulose [48]. On day 8, the pH in group **a** and **b** reached the minimum value of 5.04. At the end of process, the pH was significantly increased as shown in Fig. 3b. After day 16, original chemical Fenton reagents have been consumed according to previous report [49]. In addition, the increase of pH could be ascribed to the decline of *P. chrysosporium*'s development. Boyle et al. also found the change of pH in the development of white-rot fungi [50]. The pH in group **a** and **b** was lower than group **c** (Fig. 3a), indicating that less acids were secreted by microbes in group **a** and **b**, which can be explained by this fact: microbes were more active in group **a** and **b** than **c**. The iron in Fenton reagents can be a significant element in the metabolism of fungi [51], which promoted the development of fungi. Thus the iron in Fenton reagents in group **a** and **b** may be beneficial to the growth of *P. chrysosporium*. Some publications also reported β-CD has

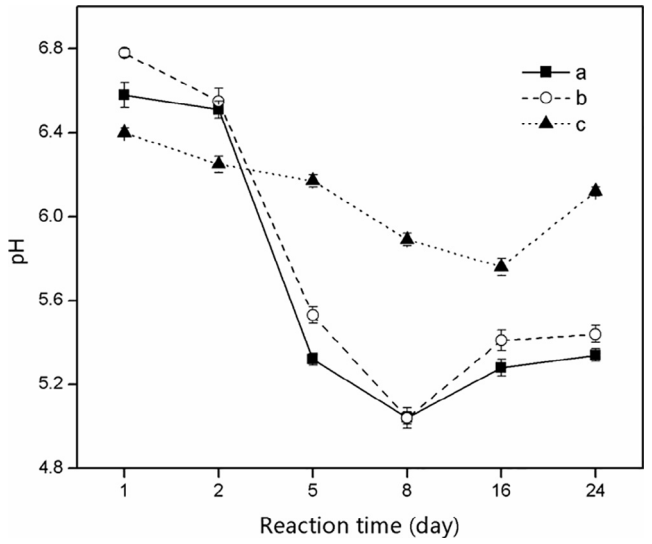

Fig. 3a. Changes of pH during degradation process, a: the experimental group, b: group a without β-cyclodextrin, c: *P. chrysosporium* treated alone which indicated that the β-CD may stimulate the cultivation of *P. chrysosporium*.

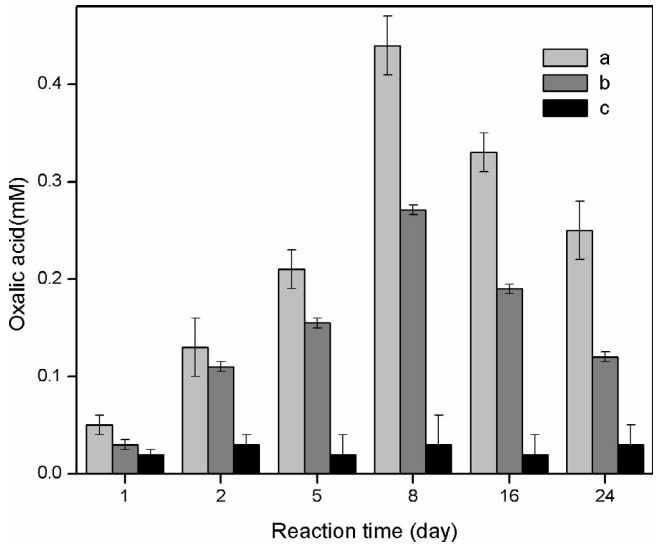

Fig. 3b. Concentration of oxalic acid during degradation process. a: the experimental group, b: group a without β-cyclodextrin, c: *P. chrysosporium* treated alone.

positive effect on fungi [52,53], pH in group **b** without β-CD was lower than **a** (Fig. 3a), which may related to this. The concentration of H_2O_2 was showed in Fig. 4. From this figure, H_2O_2 in group **a** was generally declined with time, and the H_2O_2 in group **c** reached maximum on day 8. From the figure, we know on the day1-2, the removal rate of BPA was mainly caused by chemical Fenton process. After the day 5, degradation process was mainly attributed to the fungal treatment.

3.3.2. Change of oxalic acid in the biochemical Fenton process

Oxalic acid is an important organic acid in white-rot decay system. The concentration of oxalic acid varied during degradation process (Fig. 3b). Obviously, the concentration of oxalic acid was rather low in the beginning, particularly in group **c**. On day 8, the oxalic acid in all groups reached the maximum, and then the decrease of the concentration of oxalic acid was found. Researches have reported that organics in the suspension can be degraded by *P. chrysosporium* and led to the generation of oxalic acid [23]. The high concentration of oxalic acid on day 5–8 was likely because of the fast reproduction of *P. chrysosporium*. Group **c** with a relatively lower concentration of oxalic acid to

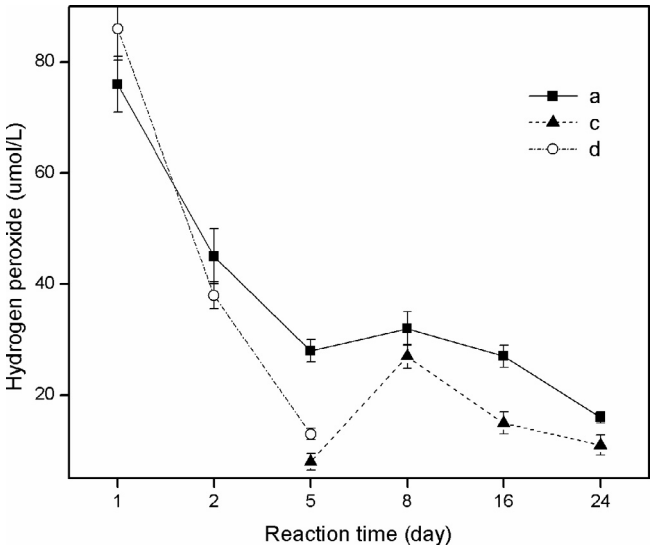

Fig. 4. Concentration of hydrogen peroxide during degradation process. a: the experimental group, c: *P. chrysosporium* treated alone, d: Fenton treatment alone.

group **a** and **b**, which indicated that the *P. chrysosporium* in group **a** and **b** have a better development or higher activities. It was suggested that the Fenton reagents prompted the growth or development of *P. chrysosporium*. As can be seen from Fig. 3a, the pH declined at first and then enhanced; from Fig. 3b, there were a highest concentration of oxalic acid on day 8 (lowest pH). It was suggested that the pH was affected by the generation of oxalic acid. Interestingly, the concentration of oxalic acid in group **b** was lower than that in group **a**, which indicated that the β-CD could stimulate the development of *P. chrysosporium*. Researchers [54] also found chelating agents (CA) can bind iron ion, facilitating the initiation of Fenton –like reactions (Eqs. (9) and (10)):

$$Fe(III) + CA \rightarrow Fe(III)-CA \quad (9)$$

$$Fe(III)-CA + H_2O_2 \rightarrow Fe(II)-CA + H^+ + HO_2^{\cdot} \quad (10)$$

This reaction could be one of the reasons for higher removal rate of BPA in group **a** and **b** than that in group **c**.

3.3.3. Change of ferrous iron in the biochemical Fenton process

Group **a** showed an obviously lower concentration of ferrous iron than group **b** from Fig. 5. It might be caused by the existence of β-CD in group **a**. β-CD can combine with iron ion and benzene ring, reinforcing the Fenton reaction without strictly initial acids environment, and then formed a steady Fenton system [55]. The concentration of ferrous iron in group **a** was lower than that in group **b**, which could be correlated with the findings that β-CD could cause positive influence on the uptake of iron by fungi [56]. In the initial stage of degradation, the concentration of ferrous iron was 0.71 $mg{\cdot}L^{-1}$ and 0.73 $mg{\cdot}L^{-1}$ in group **a** and group **b**, respectively, after the addition of Fenton reagents. However, with the degradation process, the concentration of Fe^{2+} in group **a** and group **b** were both declined. Firstly, it may be caused by the uptake of Fe^{2+} by *P. chrysosporium* and the reaction of Fe^{2+} with oxalic acid [46]. Besides, there was a possibility that the iron was absorbed on the sediment. The precipitation of Fe^{3+} also existed in the treatment process.

From above all, oxalic acid generated by fungi could decrease the pH to create a better reaction condition for chemical Fenton process. Therefore, Fenton process was promoted by the fungal treatment during the degradation of BPA. The removal rate of BPA was essentially enhanced. From Fig. 2, the combination of two treatment technologies in group **a** achieved a higher removal rate than the control with *P. chrysosporium* alone or the control with Fenton reagents alone.

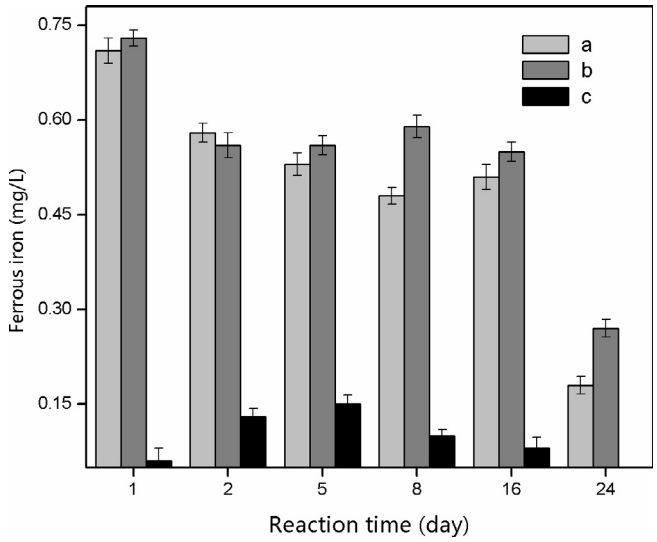

Fig. 5. Concentration of ferrous iron during degradation process. a: the experimental group, b: group a without β-cyclodextrin, c: *P. chrysosporium* treated alone.

3.4. Enzyme activities

In order to evaluate the activities of *P. chrysosporium*, the activities of LiP and MnP during degradation process were analyzed. Obviously, the activity of LiP was higher in group **a** than group **b** during the whole process (Fig. 6), which was correlated with the presence of β-CD in group **a**. β-CD is a promising bioavailability-enhancing agent [57], which can enhance the release and microbial bioaccessibility of contaminants in soils [58,59]. *P. chrysosporium* in group **a** with the addition of β-CD could prompt the degradation of BPA. In addition, at the beginning of the day in group **a**, the increase of the activity of LiP was relatively slow; with the degradation process, the activity of LiP reached maximum 20.69 $U{\cdot}L^{-1}$ on day 5. The activity of LiP in group **b** and group **c** also reached the maximum on day 5. Interestingly, before the fifth day, the activity of LiP in group **c** was much lower than group **a**. But from the day 8 to day 24, the activity of LiP in group **c** was higher than group **a**. For one thing, from day 1 to day 5, the growth of *P. chrysosporium* in group **c** might be slower than **a** and **b**. According to the conclusions above, the growth of *P. chrysosporium* was stimulated by the Fenton reagents in group **a** and **b**. For another, researchers have found that the removal of BPA was associated with the generation of LiP and MnP by white-rot fungi [60,61]. LiP in group **a** and **b** might be involved with the transformation mechanism of BPA [62]. Thirdly, LiP could generate radical signals in the presence of H_2O_2 [63], thus on day 8 to day 24, a portion of LiP in group **a** and **b** were contributions to both chemical Fenton and biochemical Fenton reaction [64,65].

The activity of MnP was showed in Fig. 6. At the beginning of the day, the activity of MnP increased rapidly in all groups. The maximum activity of MnP of group **a**, **b**, and **c** were 18.23, 16.61 and 15.3 $U{\cdot}L^{-1}$, respectively. The MnP in all groups reached maximum on day 5, indicating the development or growth of *P. chrysosporium*. The Fig. 6 also showed that the activity of MnP in groups that with Fenton reagents was higher than that without Fenton reagents. The activities of LiP and MnP in group **a** were higher than those in group **b**, which was the one of the reasons why the removal rate of BPA in group **a** was much higher than group **b**. In addition, on day 5 with the highest activities of LiP and MnP (20.69 $U{\cdot}L^{-1}$, 18.23 $U{\cdot}L^{-1}$ respectively) in group **a**, the pH reached its minimum. The decline of pH can largely be explained for the growth and the biochemical Fenton process of *P. chrysosporium*.

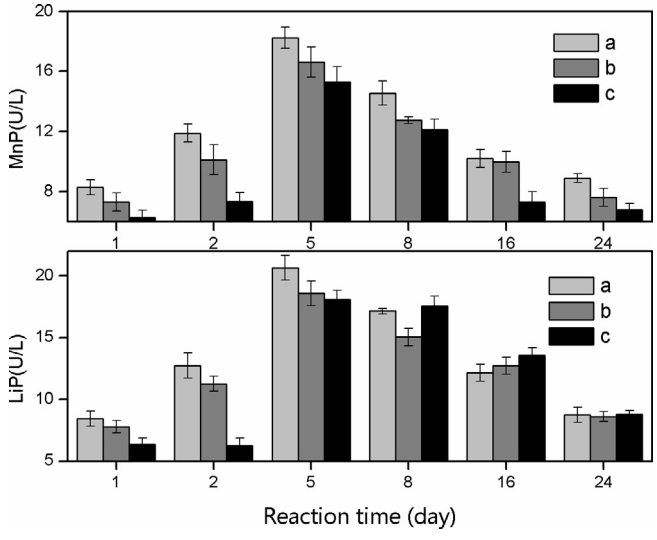

Fig. 6. Activities of LiP and MnP during degradation. **a**: experimental group, **b**: group **a** without β-cyclodextrin, **c**: *P. chrysosporium* treated alone.

3.5. Organic acids during degradation process

Oxalic acid, malic acid and fumaric acid generated by *P. chrysosporium* were measured in this study. The pH in the degradation process was also influenced by organic acids. The concentration of these representative acids also studied for the mutual effect between Fenton reaction and the addition of *P. chrysosporium*. In Fig. 7, firstly, with the addition of Fenton reagents, three organic acids were continuously increased, which was one of reasons for the decline of pH. On day 5, the maximum concentration of malic acid and fumaric acid in group **a** were 0.44 mM and 0.16 mM, respectively, indicating the active growth of *P. chrysosporium*. Yet the oxalic acid reached the maximum on day 8, there were two possible reasons: i), a portion of oxalic acid was chelated by iron, with the decrease of ferrous iron, the oxalic acid was released; ii), the maximum of oxalic acid was lagged to day 8, which was correlated with the competition between oxalic acid and other two acids. In addition, on day after 8, the concentration of these three acids was decreased similarly, which was partially resulted from the decline of chemical Fenton process at the end of degradation according to Nakagawa et al. [66].

The added Fenton reagents, together with biochemical Fenton chemicals in group **a** and **b**, were beneficial to pollutants degradation. Although using the same amount of chemicals, the combination treatment in group **a** and **b** achieved higher removal rate of BPA than in group **d**, which showed the advantage of this combination technology.

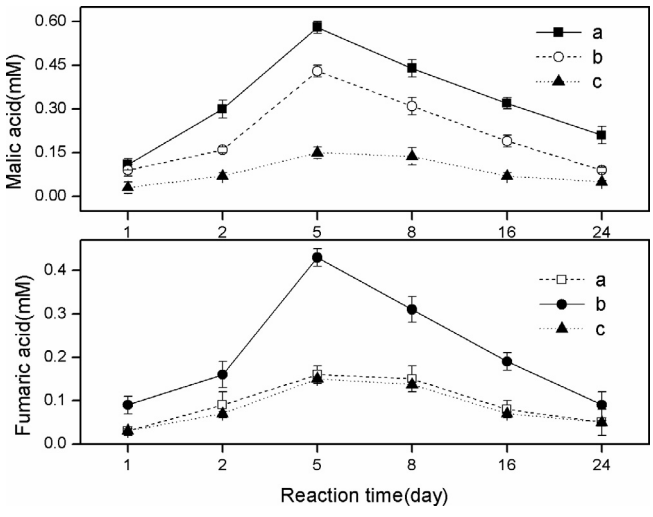

Fig. 7. The concentration of malic acid and fumaric acid during degradation process. **a**: experimental group, **b**: group **a** without β-cyclodextrin, **c**: *P. chrysosporium* treated alone.

In group **a** and **b**, The organic acids generated by *P. chrysosporium* created a better reaction condition for chemical Fenton process, which increased the removal rate of BPA. As already reported by many studies, it was suitable for Fenton reaction at the low pH (about 3). The concentration of three organic acids in group **a** were much higher than group **c** during the whole process, which can be explained for that the catalyst and oxidizer in Fenton treatment improved the activity of this white-rot fungi. Furthermore, with the enhancement of fungal's activity, the biochemical Fenton process of *P. chrysosporium* increased, thus the enzymes activities related to the degradation of BPA were promoted.

4. Conclusion

In this study, Fenton process was not pre-treatment or after-treatment but organically involved in the degradation process combined with *P. chrysosporium* treatment. After combination treatment, the removal rate of BPA in river sediments was 58.23%, which showed a higher removal rate than the only Fenton reagents treatment (14.48%) or *P. chrysosporium* treatment (21.59%). The changes of enzyme activities and concentrations of iron and organic acids indicated that there were likely synergetic effects between Fenton reagents treatment and *P. chrysosporium* treatment. On one hand, the development of *P. chrysosporium* was stimulated by Fenton reagents. On the other hand, organic acids and hydrogen peroxide generated by *P. chrysosporium* could prompt the chemical Fenton process. At the beginning of degradation, Fenton reagents played an important role in the elimination of BPA; at the end, fungal treatment functioned as a main treatment technology. In addition, the removal rate of BPA was higher in the presence of β-CD, which is probably resulted from that the CDs can form a ternary complex with iron and the hydrophobic pollutant. Therefore, the combination treatment of Fenton process and *P. chrysosporium* was effective in removing BPA from river sediments.

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