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Composting of 4-nonylphenol-contaminated river sediment with inocula of *Phanerochaete chrysosporium*

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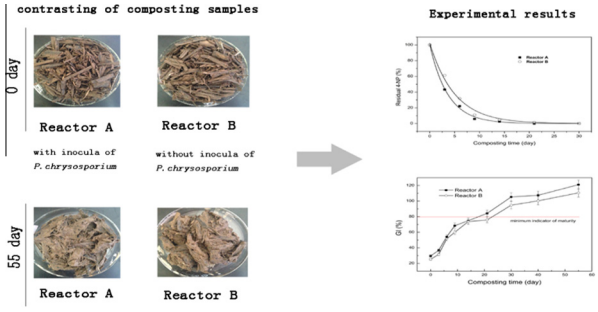
HIGHLIGHTS

- Composting of 4-nonylphenol-contaminated river sediment was studied.
- Inocula of *Phanerochaete chrysosporium* (*Pc*) could accelerate 4-NP's degradation.
- 4-NP contents in sediment were negatively correlated with *Pc*'s laccase actives.
- *Pc* increased the activities of catalase and polyphenol oxidase in the sediment.
- Composting time was reduced by inocula of *Pc* into 4-NP contaminated sediment.

GRAPHICAL ABSTRACT



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ABSTRACT

A composting study was performed to investigate the degradation of 4-nonylphenol (4-NP) in river sediment by inoculating *Phanerochaete chrysosporium* (*Pc*). *Pc* was inoculated into composting Reactor A, C and D, while Reactor B without inocula was used as control. The results showed that composting with *Pc* accelerated the degradation of 4-NP, increased the catalase and polyphenol oxidase enzyme activities in contaminated sediment. The dissipation half-life ($t_{1/2}$) of 4-NP in Reactor A, C and D with inocula of *Pc* were 2.079, 2.558, 2.424 days, while in Reactor B without inocula of *Pc* it was 3.239 days, respectively. Correlation analysis showed that the contents of 4-NP in sediment in Reactor A and D were negatively correlated with the actives of laccase, whereas no obvious correlation was observed in Reactor B and C. All these findings also indicated that *Pc* enhanced the maturity of compost, and the best composting C/N ratio was 25.46:1.

1. Introduction

4-Nonylphenol (4-NP), one kind of typically representative of phenolic endocrine disruptor compounds, which is an ultimate degradation product of nonylphenol ethoxylate (NPE) (Soares et al., 2008). NPE are widely used for different kinds of industrial, commercial and household applications, 4-NP could be found in various environments (Babaei et al., 2013).

Due to the high octanol-water partition coefficient (average log Kow 4.48), 4-NP could be easily adsorbed onto surface water particles and sediments (Soares et al., 2008; Writer et al., 2011). 4-NP was found at the highest concentration of 119,100 μg kg^{-1}

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dw in Donghu Lake of Wuhan, China (Yang et al., 2005). NP was even found with a concentration more than 3520 mg kg^{-1} in sediments of natural waters (Babaei et al., 2013). In the sludge of wastewater treatment plants, the concentration of NP frequently reached several thousand mg kg^{-1} (Shan et al., 2011). Moreover, NP can desorb from the sediment and re-enter to the water phase. Finally, the hydrophobic property led to the predominantly occurrence of NP in sediments which act as a source for the long-term release to the environment, and finally it may biomagnify in the ecosystem, posing a serious threaten to humans (De Weert et al., 2010; Yang et al., 2005; Zeng et al., 2013a). The study found that half-lives ($t_{1/2}$) for NP aerobic degradation in sediments ranged from 13.0 to 99.0 days (Yuan et al., 2004). It is necessary to control the NP level in the environment due to their high toxicity. Therefore, microbial reactions with the potential to reduce NP have received increasing attention.

To date, bacterial degradation of NP under aerobic conditions has been well-documented (Toyama et al., 2011). A few scholars isolated different kinds of aerobic NP-degrading bacteria from various environments, such as *Pseudomonas* (Yuan et al., 2004), *Metarhizium* (Rozalska et al., 2015), *Rhizobium* (Wang et al., 2014a) and *Sphingobium* (Toyama et al., 2011; Wang et al., 2014a). Besides bacteria, archaea (Wang et al., 2014b) and fungus *Umbelopsis isabellina* (Janicki et al., 2016) were also found to degrade NP. Under laboratory conditions, a high level of NP was significantly dissipated in a few days by the microorganisms. *Phanerochaete chrysosporium* (*Pc*), which could produce various extracellular ligninolytic enzymes, including lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac), has a high capacity to degrade the contents of organic pollutants. However, detailed studies about *Pc* degradation of NP in dredged river sediment are lacking.

Dredged sediments are considered as highly contaminated and must be treated before storage (Zeng et al., 2013b). As a promising *ex-situ* bioremediation technology, composting can decrease a variety of organic contaminants (Namkoong et al., 2002). Composting is also considered to be one of the most attractive technologies applied on municipal solid waste or sewage sludge on account of low environmental impact and cost (Lu et al., 2008). Das and Xia (2008) found that 80% of the total 4-NP in biosolids mixed with wood shaving was removed by composting within two weeks. Biodegradation of 4-NP in the dredged sediment can reduce the toxicological risk of the contaminants.

The purpose of this study is to investigate the effect of inoculating *Pc* to degrade 4-NP in river sediment by aerobic composting. *Pc* was inoculated into Reactor A, C and D, while Reactor B without inocula was used as control. Here, we focus on the difference of degradation of 4-NP in the four reactors, and research the relationship between the degradation of 4-NP and the physicochemical parameters, discuss the interaction between the enzymes activities and the degradation of 4-NP.

2. Methods

2.1. Fungal strain

Pc strain (ATTC 24725) was obtained from China Center for Type Culture Collection (Wuhan, China). Stock cultures were preserved on potato dextrose agar (PDA) plants stored at 4 °C. Before composting experiment, PAD plant was transferred to a 37 °C constant incubator for 48 h. The spores were diluted in sterile distilled water and controlled a concentration of 2.0×10^6 CFU mL^{-1}. The prepared spore suspension would be added into the mixture of compost.

2.2. Materials preparation

Rice straw, vegetable residues and bran were obtained from a market of Changsha, China. River sediment was collected from Xiangjiang River, which site is in Changsha Hunan province, Central-south China. The river sediment was slightly acidic (pH 6.6), with organic matter (OM) of 8.0 g kg^{-1}. At the time of sediment collection, no residual NP was detected. 4-NP (98%, Aladdin Reagent Co., Ltd.) was used for the composting degradation tests. For aerobic incubation, contaminated sediment was performed by adding 100 mL 4-NP solution (500 mg L^{-1} concentration) at 7-day intervals at 28 °C without light for 1 month under static incubation. The sediment was polluted with 35 ± 1.5 g kg^{-1} of final concentration dry weight sediment of branched 4-NP isomers. The contaminated sediment was air-dried and ground to pass through a 2-mm nylon screen. Rice straw and vegetable residues were air-dried and cut into pieces about 20 mm in size.

2.3. Composting set-up and sampling

The experiment was set up four treatments which were performed as follows: (A, C and D) sediment + 35 g kg^{-1} 4-NP + *Pc*, (B) sediment + 35 g kg^{-1} 4-NP. The detailed information was presented in Table 1. Sediment, rice straw, vegetable residues and bran were mixed in three different carbon-to-nitrogen (C/N) ratios. The C/N ratio in Reactor A and B was 25.46:1, while it was 18.77:1 and 9.53:1 in Reactor C and D, respectively. Four boxes were regarded as composting reactors with a 70% filling level of composting mixture and the size was 50 × 36 × 30 cm. Bran was used to adjust the initial carbon-to-nitrogen (C/N) ratio of composting. At the beginning, the average organic matter (OM) content of the mixture reached 43.7% in reactor A and B, while it was 31.79% and 24.45% in Reactor C and D, respectively. The water content was controlled about 60% after adding spore suspension and adjusting by sterile distilled water. The composting materials were installed loosely in plastic cases under indoor conditions and the composting masses were turned once every three days. All composting lasted for 55 days. Triplicate samples were collected from each reactor at day 1, 3, 6, 9, 14, 21, 30, 40 and 55. Samples for testing 4-NP were immediately transferred to a −21 °C freezer to stop the degradation.

2.4. Physicochemical parameters analysis

The temperatures were monitored every day. The pH values were determined by water suspensions, which were obtained from fresh samples mixed with sterile distilled water at a ratio of 1:10 (w/v) and mechanically shaked at 200 rpm for 40 min. Moisture contents were measured by the changes of weights after drying the samples at 105 °C for 24 h. The dried samples were analyzed for total organic carbon (TOC) by dry combustion overnight at 550 °C before re-weighting. OM values were commonly calculated by a conversion factor of 1.724 to convert total organic carbon: OM (%) = TOC (%) × 1.724. Total organic nitrogen (TN) was measured by the Kjeldahl method (K435, Buchi, Switzerland). C/N ratios were determined by the quotient values of TOC and TN.

2.5. 4-NP extracted and analysis

The residual 4-NP in sediment was extracted and determined according to the piece of literature (Wang et al., 2014b). Briefly, the residual 4-NP in sediment (5 g) was extracted triple using 20 mL solvent mixture acetone–hexane (1:1, v/v) with an ultrasonic processor. The mixture were dried under a stream of nitrogen and redissolved in 2 mL acetonitrile, then the solution filtered using a 0.22-μm syringe filter, and the filtrate was subject to

Table 1
Materials addition and the common characteristics in the composting samples.

Reactor	Sediment (kg)	Rice straw (kg)	Vegetable residues (kg)	Bran (kg)	Absolute weight (kg)	C/N	With or without inocula of *Pc*
A	1.0	1.0	0.07	0.2	2.27	25.46:1	With
B	1.0	1.0	0.07	0.2	2.27	25.46:1	Without
C	2	0.22	0.06	0.06	2.34	18.77:1	With
D	2	0.1	0.04	0.04	2.18	9.53:1	With

high-performance liquid chromatography (HPLC) analysis. The quantitative analysis of NP was carried out HPLC system equipped with UV-detection (1100-UV, Agilent Technologies, USA) and an Extend-C_{18} reversed-phase column (4.6 × 150 mm, Agilent Technologies, USA). Detection was carried out at 277 nm. The mobile phase was acetonitrile / water (85:15, v/v) and the flow rate was 1 mL min^{-1}. The column temperature was 25 °C. The elution of 4-NP was carried out by 8 min.

2.6. Enzymes activities analysis

2.6.1. Preparation of crude enzyme liquid

Crude extracts of the enzyme were obtained from fresh samples extracted with sterile distilled water at a ratio of 1:8 (w/v) at 200 rpm for 1 h. The suspensions were centrifuged at 6000*g* for 10 min at 4 °C and then filtered through Filter papers. The supernatant was used for the enzyme activity analyses with an ultraviolet spectrophotometer (UV-2700, SHIMADZU Corporation, Japan).

2.6.2. Lignin degradation enzymes analysis

The activities of lignin peroxidase (LiP) were tested in supernatants by monitoring the oxidation of veratryl alcohol to veratryl aldehyde spectrophotometrically at 310 nm (ε_{310} = 9300 M^{-1}.L.cm^{-1}) (Feijoo et al., 1995). The reaction mixture contained 1.5 mL sodium succinate buffer solution (100 mM, pH 3.0), 1.0 mL veratryl alcohol (10 mM) and 0.4 mL crude enzyme extracts. After intensive mixing, the reaction was started by addition of 0.1 mL H_2O_2 (10 mM), and the variation in absorbance of the mixture was recorded via ultraviolet spectrophotometer at 310 nm for 3 min. Enzyme activity was expressed as units (U), where 1 U = 1 μM product formed min^{-1}.

Manganese peroxidase (MnP) activity was estimated by monitoring the oxidation of $MnSO_4$ spectrophotometrically at 240 nm (ε_{240} = 8100 M^{-1}.L.cm^{-1}) (Rogalski et al., 2006). The reaction mixture contained 2.0 mL sodium tartrate buffer solution (100 mM, pH 4.5), 0.5 mL $MnSO_4$ (15 mM) and 0.4 mL crude enzyme extracts. After intensive mixing, the reaction was started by addition of 0.1 mL H_2O_2 (10 Mm), and the variation in absorbance of the mixture was recorded via ultraviolet spectrophotometer at 240 nm for 3 min. Enzyme activity was expressed as units, which was defined as the amount of enzyme required to oxidize 1 μM of Mn^{2+} to be Mn^{3+} per minute.

Laccase activity (Lac) was determined by measuring the oxidation of 1 mM ABTS buffered with 0.1 M sodium citrate buffer (pH 5.0). The change in absorbance was monitored at 420 nm (ε_{420} = 36,000 M^{-1}.L.cm^{-1}) for 3 min (Zhang et al., 2007). One unit of enzyme activity was defined as the amount of enzyme required to oxidize 1 M of substrate oxidized per min.

2.6.3. Oxidation-reduction enzymes analysis

Catalase (CAT) activity was assayed according to Xu et al. (2015) by monitoring through H_2O_2 (100 mM) consumption at 240 nm by UV–Vis spectrophotometer. 0.1 mL extract was added to 2.4 mL phosphate buffer solution (50 mM, pH 7.4), the reaction was started by addition of 0.5 mL H_2O_2 (0.1 M). One unit of CAT activity was defined as the amount of enzyme required to decompose 0.1 M of H_2O_2 in 1 min.

Polyphenol oxidase (PPO) activity was measured by the following method: 2.34 mL of phosphate buffer 0.05 M (pH 5.5) and 0.6 mL of catechol (0.1 M) were used as substrate. The mixture was maintained at 37 °C for 10 min. Then 0.3 mL of enzymatic extract was added and the change in absorbance was read in 3 min at 420 nm (Aquino-Bolaños and Mercado-Silva, 2004). One U of PPO enzyme activity was defined as the change in 0.1 unit of absorbance per min.

Peroxidase (POD) enzyme activity was assayed following the method described by Aquino-Bolaños and Mercado-Silva (2004). 60 μL of 2,2-azino-bis-(3-ethyl-benzthiazoline-6-sulfonic acid) (ABTS) (50 mM), 2.68 mL of phosphate buffer (0.1 M pH 6) and 100 μL enzymatic extract were used as substrate. The reaction started with the addition of 160 μL of 0.92 mM H_2O_2 and the reaction was carried out at 30 °C. The changes in absorbance were read at 414 nm for 3 min. One unit of POD enzyme activity was corresponded to the change in 0.1 unit of absorbance unit for 1 min.

2.7. Toxicity analyses

The germination index (GI) was assayed following the method described by Zeng et al. (2007). A filter paper was put into a sterilized Petri dish, and then pipetted 10.0 mL of each extract. Ten radish seeds were evenly placed on the filter paper and cultured at 25 °C in the dark for 48 h. Triplicates were analyzed for each pile sample. The germination index was depended on seed germination and root length.

2.8. Data analysis

Correlation analyses were performed to obtain more integrated information about the occurrence of composting process. The software package SPSS v19.0 (IBM, USA) was used and assigned a significance threshold of $P < 0.05$.

3. Results and discussion

3.1. Physicochemical change during composting

The temperature pattern shows the occurrence of the composting process. A typical composting temperature trend was showed in Fig. 1(a), including the mesophilic phase (the first 2 days), the thermophilic phase (>50 °C days 3–9), the cooling phase (days 10–35) and the maturation phase (days 35–55). The highest temperature (55–58 °C) was recorded at around 24–48 h after the beginning of the process. The temperatures were exceeded 55 °C for more than 3 days in both of the two reactors. It is the minimum requirement for a proper disinfection of pathogenic bacteria (Zeng et al., 2010). It was not much difference among the four reactors during the composting process whether inoculated *Pc* or not. But four of them became thoroughly decomposed at the end of experiments, and the composting process did not effected by *Pc*. It might be connected with the situation that *Pc* was inoculated at the beginning of the composted, which was similar with the phenomenon describe by Zeng et al. (2010).

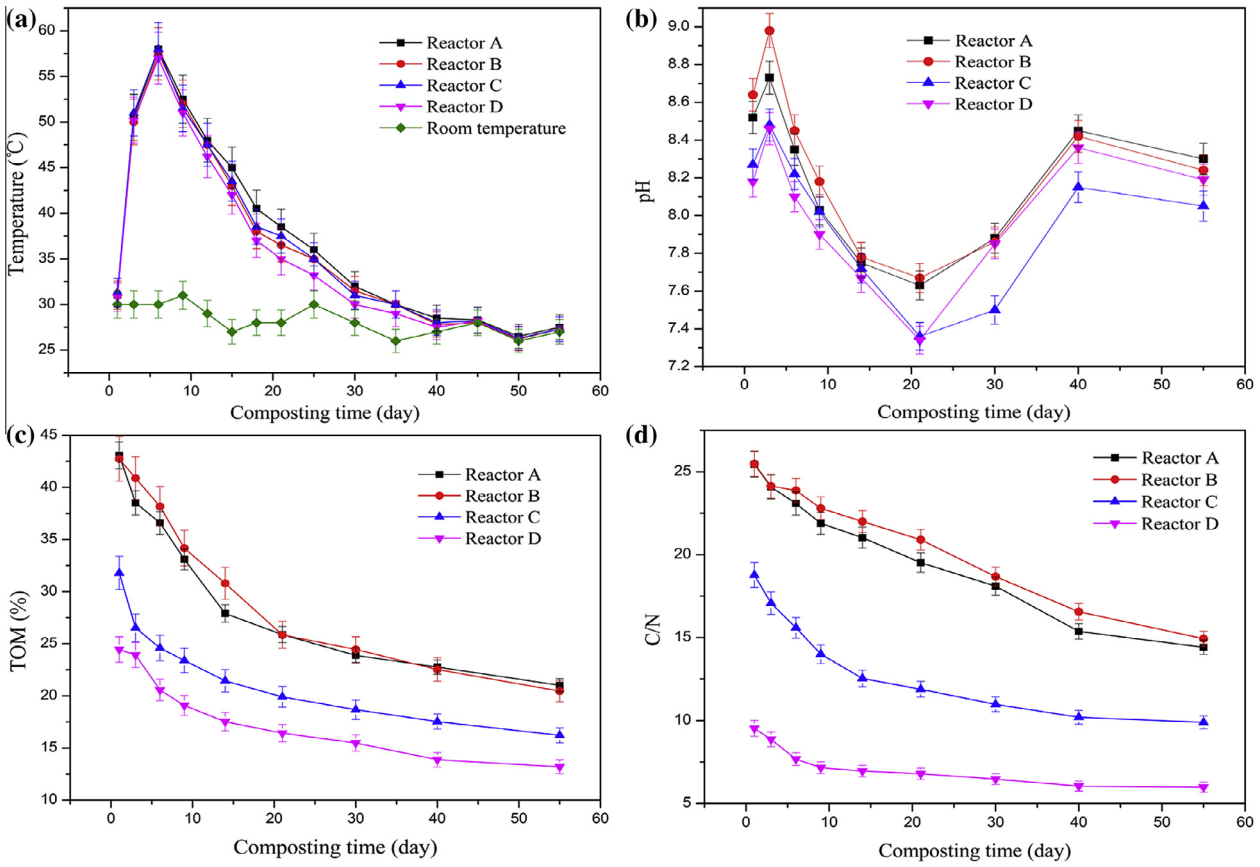

Fig. 1. Changes of physicochemical during composting process with inocula of *Phanerochaete chrysosporium* (Reactor A, C and D), compared with those in the control without inocula (Reactor B), a: Temperature; b: pH; c: organic matter content; d: C/N ratio. The error bars represent the standard deviation of the means ($n = 3$).

In this study, pH ranged between 7.6 and 9.0. A good microbial activity was supported by a pH ranging from 6.7 to 9.0 during composting (Bernal et al., 2009). Fig. 1(b) displayed the pH trends during the composting processes. It showed that pH of samples from the four reactors increased quickly in the early stage of composting and reached peak value on the day 3. Generally speaking, compost microorganism can degrade the materials which can be easily degraded in the thermophilic stage, resulting in the production of water soluble ammonia with a higher pH value. After day 3, the pH values of the four samples decreased significantly until day 21 and then gradually increased, later tended to stabilize. The decline of pH may be related to the degradation of 4-NP. On the day 55, pH of the Reactor A, B, C and D turned to be 8.3, 8.24, 8.05 and 8.19, respectively, which were within the normal and the optimum range of 8–9 for mature compost. The findings showed that it could be successful without adjusting pH during compost process, while composting 4-NP-contaminated sediment in the four reactors.

In Fig. 1(c), TOM decreased rapidly from day 1 to day 21, and which in the same C/N ratio composting reactor, Reactor A was slightly quicker than B. This difference may be affected by inoculated with *Pc*, because it is considered that the microorganisms require 30 parts of C per unit of N. After day 21 TOM in the two reactors was gradually decreased. Maximum TOM degradation values ranged between 20% and 40%. At the same time, the loss of TOM increased quickly before day 21, and then became slightly in the afterward. Similar phenomena occurred in the reactor C and D. The changing trends of TOM loss were related to the microbial activity, especially in thermophilic stage (Huang et al., 2008). Arora et al. (2002) found that the loss of TOM was primarily associated with the fungal consumption of carbohydrates.

C/N ratio is an important parameter to define the nutritional balance and evaluate the degree of compost maturity. The C/N ratio trend is presented in Fig. 1(d) showed an obviously declining trend from initial values of around 25 to final values of 14.42 and 14.93 for Reactor A and B, respectively. The final values for the C/N ratios in the two reactors were under 15, which were considered as the established limit for mature compost (Huang et al., 2010). It can be also observed that inoculation with *Pc* did not observably promote the decrease of the C/N ratio during the first fermentation phase like temperature. Compared with lowly initial C/N ratio in composting Reactor C and D, the falling of C/N ratio in Reactor A was faster. Connecting the following analysis, it was found that the best composting condition was at the C/N ratio of 25.46:1 and with inocula of *Pc*.

3.2. Degradation of 4-NP during the composting

Fig. 2 showed the changes in 4-NP remaining percentage in the two composting reactors. The NP biodegradation data in this study was modeled using first order kinetics, $C = C_0 \exp(-kt)$, where C_0 is the initial concentration (mg kg^{-1}), C is the residual concentration (mg kg^{-1}), t is the sampling time (day), and k is the biodegradation rate constant (day^{-1}). In this model, it was deduced the dissipation half-life ($t_{1/2}$) of 4-NP as follows: $t_{1/2} = -\ln 2/k$. The 4-NP degradation rate constants in Reactor A, C and D with inocula of *Pc* and in Reactor B without inocula of *Pc* were 0.335, 0.271, 0.286 and 0.214 day^{-1}, respectively. And the dissipation half-life ($t_{1/2}$) of 4-NP in Reactor A, C and D with inocula of *Pc* were 2.079, 2.558, 2.424 days, while in Reactor B without inocula of *Pc* it was 3.239 days, respectively. For the degradation rate constant and half-life for 4-NP, the results in Reactors A, C and D were quicker

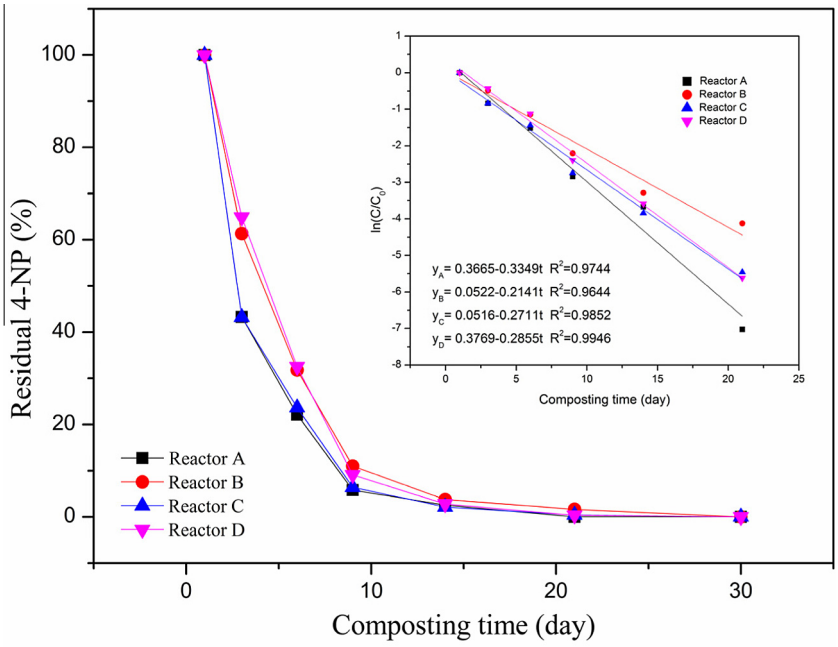

Fig. 2. Degradation of 4-NP during composting process with inocula of *Phanerochaete chrysosporium* (Reactor A, C and D), compared with those in the control without inocula (Reactor B). Each point is the average concentration of triplicate samples ($n = 3$).

than those in Reactors B. Moreover, the trends in Reactor C and D were slower than those in Reactors A. It was concluded that the best composting condition of 4-NP degradation was the C/N ratio of 25.46:1 with inocula of *Pc*. The results provided strong evidence in support of the argument that under the same composted conditions, *Pc* accelerated the degradation of 4-NP to a certain extent. Although the initial rates of biodegradation varied considerably, the trend of the biodegradation curves of the four composting reactors during the composting period were similar. For the Reactor A and B, 4-NP removal was fast during the first days of incubation, with approximately 80% of the initial amount of 4-NP being removed during the initial 6 days and 7.5 days of incubation, respectively. Moreover, NP concentration in Reaction A was below the detection limit on day 21. It could be concluded that it was degraded completely. But in Reactor B, there were still a small amount of NP residual. It may have a relationship with inoculating *Pc* in Reactor A and its extracellular ligninolytic enzymes catalysed the oxidation, but did not in Reactor B.

3.3. Changes of enzyme activities

3.3.1. Changes of lignin degradation enzymes activities

The changes of LiP, MnP and Lac activities during composting with Reactors A, B, C and D were presented in Fig. 3. Apparently, the results in Fig. 3(a) showed that LiP remained higher in the Reactor A during the whole composting process, while it was quite low in Reactor B. At the beginning of composting, it increased rapidly in Reactor A, C and D, which indicated the rapid propagation of *Pc*. The maximum LiP enzyme activity values in Reactor A, C and D were 120.98 U g^{-1}, 80.67 U g^{-1} and 68.39 U g^{-1} in day 14, while in Reactors B it was 44.52 U g^{-1}, respectively. Similar phenomena occurred according to the changes of MnP and Lac activities.

It can be clearly observed in Fig. 3(b) that the MnP level in Reactor A, C and D increased more sharply than that in Reactor B before day 21. After it, the level tended to diminish and later increased slightly. The maximum MnP activities of Reactor A, C and D were 79.45 U g^{-1}, 57.23 U g^{-1}, 61.34 U g^{-1}, while in Reactors B it was 41.67 U g^{-1}, respectively. The results further indicated that the activities of MnP were high in samples inoculated with *Pc*.

In Fig. 3(c), the significant peaks in Lac activity were found both in Reactor A and B on days 14 and 30. But the Lac activity in Reactors A, C and D fluctuated markedly than that in Reactor B during composting. The maximum Lac activities of 8 U g^{-1}, 6.55 U g^{-1} and 5.23 U g^{-1} were found in Reactor A, C and D on day 14, while in Reactors B it was 4.22 U g^{-1}, respectively.

The results indicated that the enzyme activities were higher in samples with inocula of *Pc* than that without inocula of *Pc*. At the same time, all the maximus enzyme activities were in Reactor A with inoculation and the C/N ratio of 25.46:1. It is worth noting that the values of LiP in the samples with the same C/N ratio collected from the inoculated Reactor A were three times higher than the none-inoculated Reactor B on day 21. Since *Pc* could produce the LiP, MnP and Lac extracellular enzymes, it is to be considered that inoculated *Pc* may stimulate the enzyme activities, the enzyme activities were positively affected by inoculation (Zeng et al., 2010). Moreover, studies demonstrated that LiP, MnP and Lac were shown to eliminate or oxidate of a variety of recalcitrant aromatic compounds, such as phenolic compounds and polycyclic aromatic (Cheng et al., 2015; Huang et al., 2015; Zeng et al., 2015). Soares et al. (2005) found that NP removal was associated with the production of Lac and MnP by white-rot fungi. The results led the authors to explain the bisphenol A (BPA) and NP transformation mechanisms as due to the polymerization and partial degradation of the chemicals brought about by enzymatic oxidation (Tsutsumi et al., 2001). The present study conclude that the degradation of 4-NP was connected with the extracellular enzymes of *Pc*.

3.3.2. Changes of oxidation-reduction enzymes

CAT in composting reactor is related to microbial activity and respiration which reflects the intensity of composting processes. CAT enhances oxidization of compounds by H_2O_2, and it exists in all microorganisms. Fig. 4(a) showed that CAT level increased slightly in four reactors from day 1 to day 3. There was no significant difference of it in two reactors within three days, but afterward, the difference was obvious. CAT increased rapidly from day

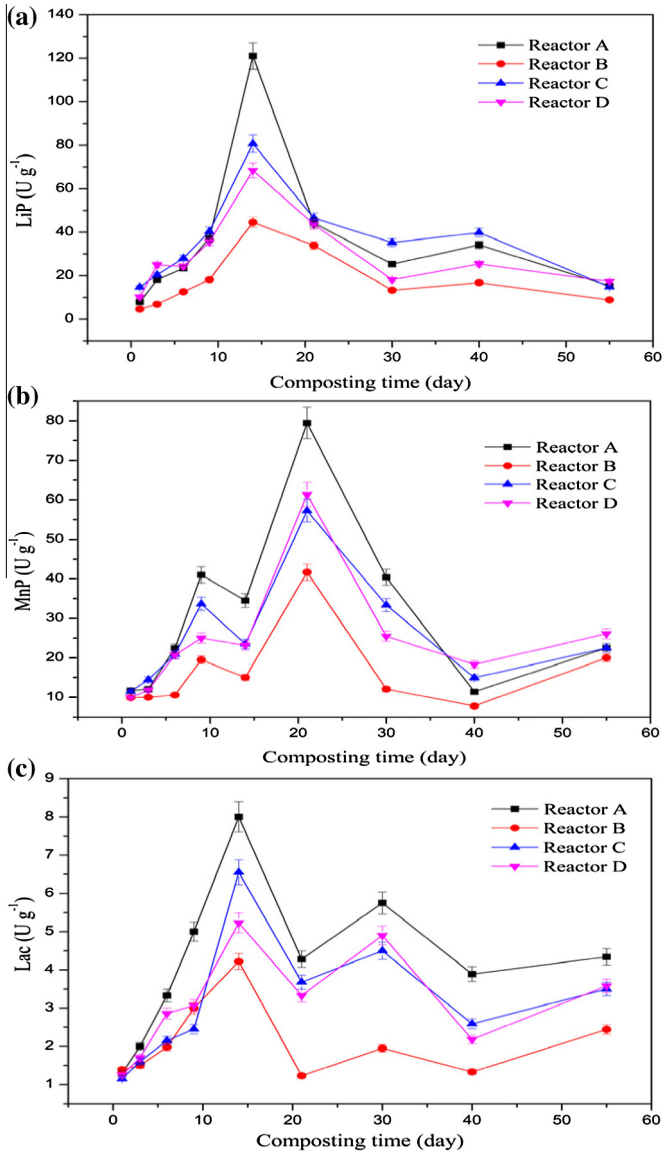

Fig. 3. Changes of lignin degradation enzymes activities during composting process with inocula of *Phanerochaete chrysosporium* (Reactor A, C and D), compared with those in the control without inocula (Reactor B), a: LiP activity; b: MnP activity; c: Lac activity. The error bars represent the standard deviation of the means ($n = 3$).

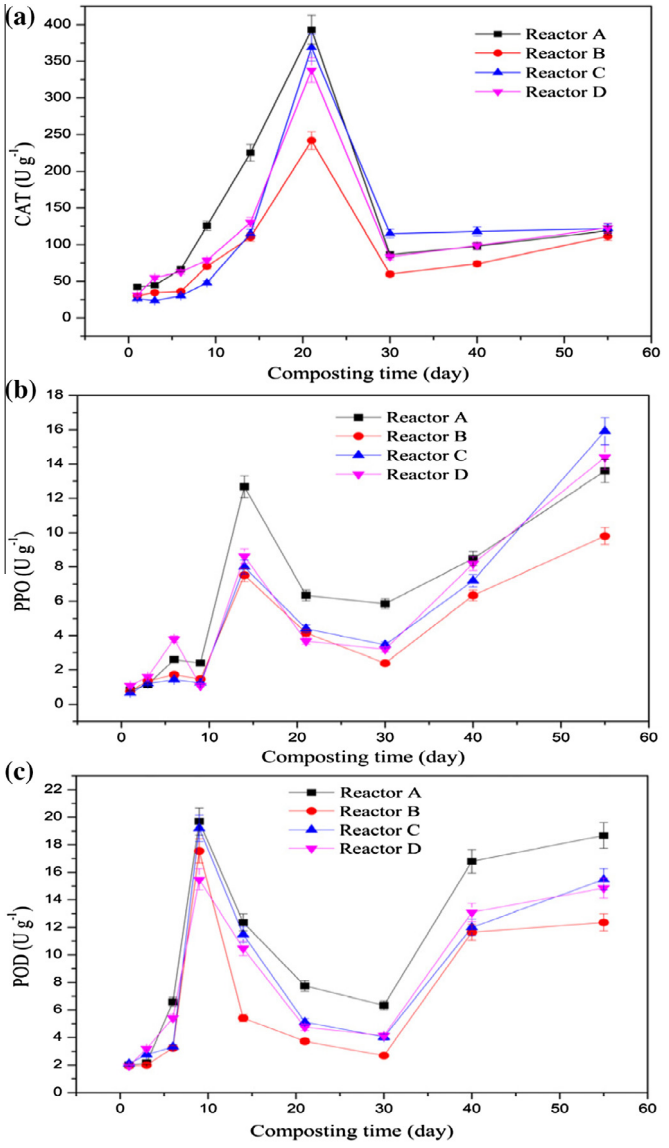

Fig. 4. Changes of oxidation-reduction enzymes activities during composting process with inocula of *Phanerochaete chrysosporium* (Reactor A, C and D), compared with those in the control without inocula (Reactor B), a: CAT enzymes; b: PPO enzymes; c: POD enzymes. T The error bars represent the standard deviation of the means ($n = 3$).

3 to day 21 in Reactor A (from 44.7 U g^{-1} to 393.06 U g^{-1}), and then decreased to 86.4 U g^{-1} at day 30, slightly increasing afterward, while in Reactor B, it increased from day 6 to day 21 (from 34.56 U g^{-1} to 241.52 U g^{-1}), then decreased to 59.67 U g^{-1} at day 30. The final CAT turned to be 119.12 U g^{-1} and 111.51 U g^{-1}- for Reactor A and B, respectively. Similar trend occurred in Reactor C and D, and the maximum CAT values reached 368.75 U g^{-1} and 338.00 U g^{-1}, respectively. The phenomenon was similar to that of a previous report on the degradation of Tetrabromobisphenol A (TBBPA) in soil (Shu and Su, 2011). Fig. 4(a) which means CAT activity increased with an increase of mesophiles number during composting in the present study. Since CAT in composting mass was reacted at normal temperature, CAT activity was supposed to only reflect the intensity of metabolic activity for mesophiles.

PPO is an important oxidoreductase and that catalyze oxidation of aromatic compounds into more easily degraded intermediate products. In Fig. 4(b), it was clearly observed two peaks from the changes of PPO activities during composting process in the four reactors, the first peak on day 6 and the second peak on day 14. At the beginning of composting, PPO activity rose sharply to a peak value on day 14, then decreased and again showed a tardily increase from day 30 onwards. The increase trend in the maturation stage was attributed to fungal autolysis (Arora et al., 2002), and implied that humification of composting mass was improved. The maximum PPO activity reached to 13.60 U g^{-1}, 15.92 U g^{-1} and 14.40 U g^{-1} in Reactor A, C and D on day 55, while it was 9.80 U g^{-1} in Reactor B, respectively.

POD can catalyze oxidation substrates, including aromatic amines, phenols, and various other compounds, when H_2O_2 present. POD enzyme activity was showed in Fig. 4(c). POD in the four reactors increased until day 9 followed by a sharp decline. However, it tardily increased from day 30 onwards, which is similar to the changes of PPO activity. The maximum POD activity reached to 19.69 U g^{-1} 19.20 U g^{-1} and 15.48 U g^{-1} in Reactor A, C and D on day 9, while it was 17.55 U g^{-1} in Reactor B, respectively. Serra-Wittling et al. (1995) thought that the changes of POD during the

Table 2
Pearson correlation between physicochemical and the contents of 4-nonylphenol (4-NP) in sediment.

	Reactor	pH	TOC	OM	C/N	LiP	MnP	Lac	CAT	POD	PPO	GI
4-NP	A	0.581	0.848**	0.848**	0.688*	−0.407	−0.471	−0.734*	−0.457	−0.624	−0.617	−0.795*
	B	0.720*	0.856**	0.856**	0.747*	−0.537	−0.383	−0.355	−0.536	−0.550	−0.537	−0.869**
	C	0.566	0.718*	0.718*	0.879**	−0.508	−0.520	−0.637	−0.479	−0.505	−0.543	−0.807**
	D	0.468	0.900**	0.900**	0.960**	−0.449	−0.558	−0.729*	−0.509	−0.535	−0.622	−0.817**

TN, total nitrogen; OM, organic matter; C/N, ratio of carbon to nitrogen; LiP, lignin peroxidase enzyme; MnP, manganese peroxidase enzyme; Lac, laccase enzyme; CAT, catalase enzyme; POD, Peroxidase enzyme; PPO, Polyphenol oxidase enzyme; GI, seed germination index; ** and * indicate $P < 0.01$ and $P < 0.05$, respectively.

whole process were related to development of lignolytic microorganisms and degradation of easily metabolizable constituents, such as lignin.

3.4. Change of toxicity during composting

The GI is a comprehensive biological indicator, which is used to evaluate the degree of maturity and the toxicity of compost (Zeng et al., 2007). During the biodegradation, 4-nonylphenol metabolites were not detected, which were further degraded to unknown compounds. Its toxicity was reflected by GI of radish seeds. As shown in Fig. 5, at the beginning of the composting, GI in all reactors were quite low due to phytotoxicity of 4-NP and increased slowly, but all of them achieved more than 110% finally. The figure showed that GI was 121.06%, 110.53%, 119.79% and 126.32% for Reactors A, B, C and D on day 55, respectively. The results indicated that the four composting reactors were phytotoxin-free. The correlation analysis between phytotoxicity of compost and 4- NP degradation see below.

Moreover, it is generally acknowledged that the compost maturity was sufficient when GI reaches 80% (Zeng et al., 2007). In Fig. 5, it was also apparent that GI in Reactor A, C and D mounted up to above 80% after 21 days of composting, while 30 days were needed for Reactors B. The results showed that all compost piles were mature and composting time was reduced by inoculating *Pc.*

3.5. The correlation between physicochemical parameters and 4-NP degradation

Table 2 showed the relationship between physicochemical parameters and 4-NP concentration variations. 4-NP degradation in all the composting reactors were positively correlated with its TOC content, OM content and C/N ratio, but negatively correlated with the germination index. These analysis results were similar with other reports. Gong et al. (2011) found that, there was a positive correlation between NP and TOC in Pearl River of China, which indicated that sedimentary organic carbon was a key factor in eliminating the endocrine disruptors.

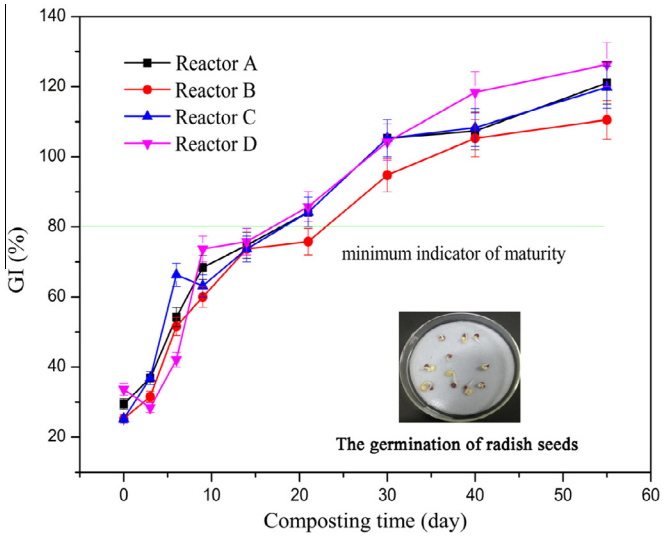

Fig. 5. Changes of germination index during composting process with inocula of *Phanerochaete chrysosporium* (Reactor A, C and D), compared with those in the control without inocula (Reactor B). The error bars represent the standard deviation of the means ($n = 3$).

At the same time, it was found that the concentrations of 4-NP was negatively correlated with Lac activity in Reactor A and D, but not related with Lac activity in Reactor B and C. It was indicated that *Pc* promoted the degradation of 4-NP. Other researchers reported that one kind of mechanisms of t-NP degradation by fungi was that t-NP was attacked by extracellular laccase at the beginning reaction stage (Babaei et al., 2013; Martin et al., 2009). It was the reason why 4-NP concentration was negatively correlated with Lac activity in Reactor A and D. Likewise, the 4-NP concentrations were negatively correlated with GI, which indicated that the composting sample had no phytotoxicity for radish seeds at the end of the experiment.

Other inter-variable correlations were presented in the same table. However, the results showed that the degradation of 4-NP had no significantly correlation with neither LiP nor MnP in this study, but also with CAT, PPO and POD. Notwithstanding, it didn't imply that those parameters were not important the composting process. It could be only concluded that those parameters hadn't significant correlation with organic compounds degradation in the composting process in this research. For example, it was reported that NP and BPA were removed significantly by MnP in a short time (Tsutsumi et al., 2001).

4. Conclusions

The results showed that it was feasible to remediate 4-NP contaminated river sediment by composting with *Pc* and the best C/N ratio was 25.46:1. Inocula of *Pc* increased the catalase and polyphenol oxidase enzyme activities of sediment and 4-NP degradation rate. Moreover, the concentrations of 4-NP were negatively correlated with Lac. Results also showed that the phytotoxicity of 4-NP in inoculated reactors were lower than that non-inoculated reactor. All these findings also indicated that composting of 4-NP-contaminated sediment with inoculating *Pc* could promote compost maturity, which provided a promising prospect for the application in remediation of contaminated river sediment.

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