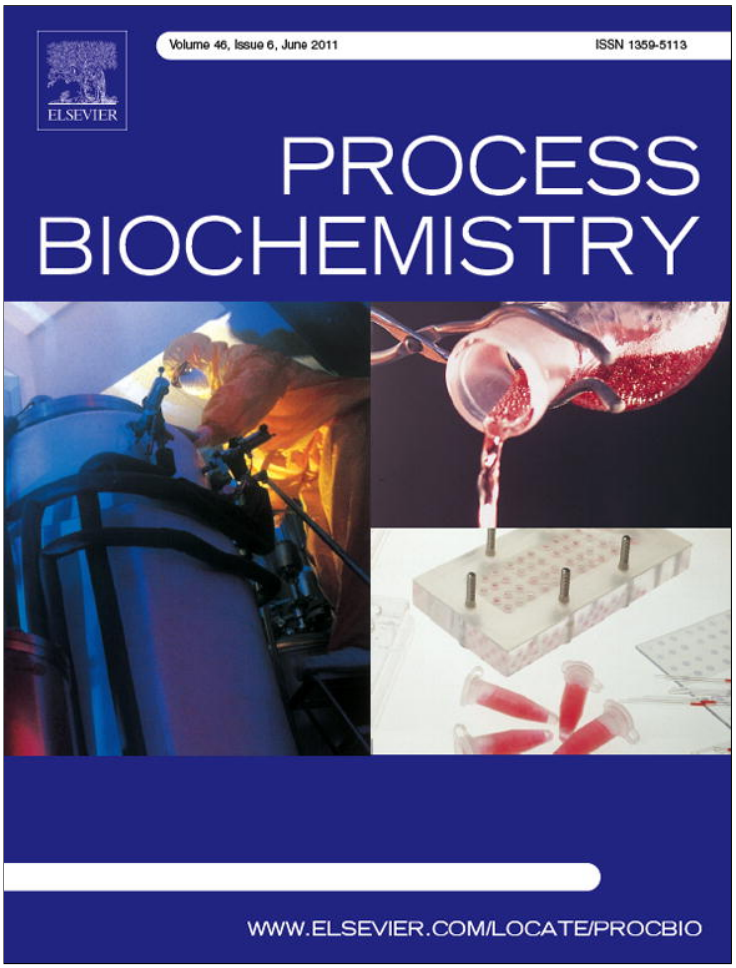

Process Biochemistry 46 (2011) 1285–1291

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Effects of inoculation with *Phanerochaete chrysosporium* on remediation of pentachlorophenol-contaminated soil waste by composting

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ARTICLE INFO

Article history:
Received 8 November 2010
Received in revised form 20 January 2011
Accepted 18 February 2011

Keywords:
Phanerochaete chrysosporium
Pentachlorophenol
Inoculation
Composting
Extracellular ligninolytic enzymes
Bacterial community composition

ABSTRACT

The effect of inoculation with *Phanerochaete chrysosporium* (*P. chrysosporium*) during different fermentation phases on remediation of pentachlorophenol (PCP)-contaminated soil waste (PSW) was investigated over 60 days. This was accomplished by evaluating physico-chemical and biochemical properties of composts, as well as bacterial community composition using denaturing gradient gel electrophoresis (DGGE). Results showed that the inoculations could significantly enhance composting efficiency and PCP removal. The best degree of maturity and highest PCP removal occurred in Run C (inoculation during the second fermentation phase) were compared with Runs A (control treatment) and B (inoculation during the first fermentation phase). A positive effect on production of manganese peroxidase (MnP) and lignin peroxidase (LiP) was found in inoculated runs, especially in Run C, while the production of laccase (Lac) was limited by *P. chrysosporium* inoculants. As a result of DGGE analysis, the compost bacterial community composition was altered by different inoculations, as indicated by the differences between the final composts. This study highlights the different effects of the inoculations on remediation performance of PSW. The inoculation during the second fermentation phase is more effective than that during the first fermentation phase.

1. Introduction

Pentachlorophenol (PCP) that is rated as a priority pollutant by the EPA, has been extensively used throughout the world as a wood preservative, pesticide and general biocide in agriculture and industry [1,2]. Large areas of soils and sediments in lakes or rivers have been polluted by PCP. After then, PCP can enter the food chain, which is thought to be teratogenetic and carcinogenic to humans [3,4]. Therefore, it still represents an environmental hazard.

As a promising bioremediation technology, composting has been successfully applied to the remediation of PCP-contaminated soil waste (PSW) [5,6]. In comparison with other technologies, composting has many advantages, which include relatively low capital and operating costs, simplicity of operation and design, and relatively high treatment efficiency [7]. Bioremediation can be enhanced by the manipulation of environmental factors to create an optimum environment for microbial degradation [8,9].

Phanerochaete chrysosporium (*P. chrysosporium*), as a kind of white rot fungus, has demonstrated a high capacity to degrade a variety of structurally diverse organopollutants including PCP [10–12] due to the production of extracellular ligninolytic enzymes, manganese peroxidase (MnP), lignin peroxidase (LiP) and to a lesser extent laccase (Lac) [10,11]. Therefore, it had been the subject of extensive investigation. There were some studies about the biodegradation of PCP in soil, water and some bioreactors by *P. chrysosporium* [12–14]. Recently, the application of inoculation with *P. chrysosporium* to treat hazardous wastes has been shown to be effective in PCP degradation and remediation of PSW at laboratory-scales [6]. However, little information about the effect of inoculation was reported due to lack of further research.

The success of remediation of PSW by composting are determined by microbial activity, as microorganisms play the most critical role in the composting process [15]. Meanwhile, the aim of optimizing composting factors is also to ensure that microorganisms are metabolically active and able to degrade contaminants [8,16]. Although the microbial communities naturally in soil waste usually carry out the composting process, the inoculation of microorganisms could potentially improve this process [17,18]. Therefore, the effect of inoculation on microbial community composition should not be neglected. Usually, microbial communities within contaminated ecosystems tend to be dom-

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doi:10.1016/j.procbio.2011.02.018

Table 1
The common physical and chemical properties of composting materials.

	Moisture (%)	OM (g kg^{-1})	TOC (g kg^{-1})	TKN (g kg^{-1})	TOC/TKN	pH
Soil	26.13 ± 0.58	95.0 ± 5.3	55.1 ± 2.1	2.4 ± 0.1	22.7 ± 0.98	4.73 ± 0.17
Rice straw	11.73 ± 0.29	738.0 ± 39.5	428.0 ± 16.1	8.8 ± 0.5	48.8 ± 2.24	–[a]
Vegetables	79.06 ± 1.66	167.6 ± 7.7	97.2 ± 3.3	5.0 ± 0.2	19.6 ± 0.76	–[a]
Bran	14.06 ± 0.35	817.4 ± 31.4	474.1 ± 20.1	41.2 ± 1.9	11.5 ± 0.53	–[a]

[a] Sample not quantified.

inated by those organisms capable of utilizing and/or surviving toxic contamination [19]. Considering the dominance of bacterial communities in hydrocarbon-contaminated condition [19], the profiling of bacterial communities is enabled by contemporary advances in molecular techniques using the method of polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE) [20,21]. Interpretation of the data from DGGE profiles allows assessment of how any treatment impacts on bacterial community composition.

The objective of present study was to assess the effect of different inoculation time on remediation of PSW by composting. Normally, physico-chemical, and biological analyses were used in the evaluation of compost maturity and PCP removal. Meanwhile, the extracellular ligninolytic enzymes' activities correlating with PCP removal were clarified. PCR-DGGE was used to investigate the response of bacterial community composition to the inoculations.

2. Materials and methods

2.1. Fungus and inoculant preparation

P. chrysosporium strain (BKM-F-1767) was purchased from the China Center for Type Culture Collection (Wuhan, China). Stock cultures were maintained on potato-dextrose agar (PDA) slant stored at 4 °C until use. The fungus was transferred to 250 ml Erlenmeyer flasks each containing 100 ml of potato-dextrose medium. A sterile glass bead (8 mm) was added to each flask. Flasks were incubated at 37 °C on a rotary shaker at 135 rpm for a week. The culture was collected by filtration, and then washed thrice with 100 ml of 0.12 M NaH_2PO_4–Na_2HPO_4 buffer. Afterwards, those mycelia preparations (<3 mm) were used as inoculants.

2.2. Composting establishment and sample collection

PCP was purchased from American ADL Co. with a purity >98%. The raw soil was obtained from Yuelu Mountain (Changsha, China). The soil was air-dried and ground to pass through a 2-mm mesh, and then stored at 4 °C. A stock solution of PCP (10 g L^{-1}) prepared in acetone was added to sieved soil to achieve the initial PCP concentration of 133 mg (kg dry wt)$^{-1}$. The PCP-spiked soil was stored in three 2-L specimen containers, and then left uncovered in a fume hood to allow the acetone to evaporate. Rice straw and vegetables, which were dried and then chopped into 10–20 mm pieces, were prepared as other composting materials. Bran was used to adjust the initial C/N ratio. The common physical and chemical properties of composting materials were shown in Table 1.

An experimental composting system with a weight of about 15 kg (dry wt) was set up. The soil waste, which consist of PCP-spiked soil, rice straw, vegetables and bran were mixed at a ratio of 5.3:6:1.7:1 (fresh wt), and then packed loosely in an open box with dimension of 0.65 m × 0.45 m × 0.42 m (length × width × height). The mixture had good heat preservation. The organic matter (OM) content of this mixture was 58%, while the initial C/N ratio was about 30:1. The PCP concentration in the mixture was equivalent to 50 mg (kg dry wt)$^{-1}$. Moisture was monitored and adjusted to about 60% during the first fermentation phase and about 45% during the second fermentation phase by the addition of sterile deionized water, respectively. To provide some aeration, the mixture was turned twice a week during the first 2 weeks and then once a week afterwards. Three runs, each in triplicate, were set up. Run A was the control without *P. chrysosporium* inoculants. Run B was inoculated with 0.5% *P. chrysosporium* mycelium (fresh wt) in each kg dry mixture during the first fermentation phase (day 0). Run C was inoculated with the same amount of *P. chrysosporium* mycelium during the second fermentation phase (day 15).

The experiment was conducted for 60 days and compost samples were collected every 3 days. At each sampling occasion, three subsamples for parameter analysis were taken from different places of the composting material (about 0.2 m in depth). Samples for total DNA extraction were stored immediately at −20 °C until use.

2.3. Analysis of composting parameters

The temperature in the compost piles was monitored every day. The moisture content was measured after drying the samples overnight at 105 °C. The dried samples were ground and analyzed for total organic carbon (TOC) by dry combustion [22]. The total nitrogen (TN) was measured using the Kjeldahl's method [23]. The germination index (GI) was determined using seed germination and root length tests of *Lepidium sativwm* L. according to the method described by Jiang et al. [6]. The aqueous compost extracts were obtained by mechanically shaking the samples with distilled water at the solid: liquid ratio of 1:10 (w/v) for 1 h. The suspensions were centrifuged at 13,000 × *g* for 20 min and filtered through 0.45 μm membrane filters. The filtrates were used for the enzyme activity analyses with an ultraviolet spectrophotometer (UV-2250, SHIMADZU Corporation, Japan).

MnP activity was estimated by monitoring the oxidation of phenol red spectrophotometrically at 610 nm [12]. One unit of enzyme activity was defined as the amount of enzyme required to oxidize 1 μmol of phenol red per minute. LiP activity was estimated by monitoring the oxidation of veratryl alcohol to veratryl aldehyde spectrophotometrically at 310 nm [24]. One unit of enzyme activity was defined as the amount of enzyme required to produce 1 μmol veratryl aldehyde per minute. Lac activity was determined by the oxidation of 2,2′-azino-bis-[3-ethyltiazoline-6-sulfonate] (ABTS) spectrophotometrically at 436 nm [25]. One unit of enzyme activity was defined as the amount of enzyme required to oxidize 1 μmol of ABTS per minute.

2.4. PCP extraction and analysis

PCP was extracted from compost samples as described by Scelza et al. [26] with a few modifications. Ten grams samples (fresh wt) were solvent extracted by shaking at 200 rpm with 20 ml acetone and *n*-hexane (1:1, v/v) for 2 h. The solvent was decanted after centrifugation at 10,000 × *g* for 10 min. Isolated samples were allowed to dry at 30 °C for 24 h and subsequently extracted with 10 ml acetone and *n*-hexane (1:1, v/v) for 10 min followed by 10 min of centrifugation at 10,000 × *g* [27]. Solvent extracts were dried under a stream of nitrogen and redissolved in acetonitrile for HPLC analysis. The residual PCP in all the samples was quantified by HPLC (Agilent Technologies, USA) using an UVD detector and an Eclipse XDB-C18 (4.6 mm × 150 mm) column with 5 μm particle size. Analysis was conducted using 68% of acetonitrile and 32% of buffered water (1% acetic acid) as mobile phase with a column temperature at 25 °C and 1.0 ml min^{-1} flow rate. Detection was carried out at 220 nm. PCP concentrations and removal percentage were calculated by reference to appropriate standard PCP solutions.

2.5. Bacterial community fingerprinting by PCR-DGGE analysis

The final compost samples (day 60) from the three runs were used for DGGE analysis. DNA extraction from 0.5 g of each sample (fresh wt) was performed based on the method described by Yang et al. [28] followed by removal of humic substances [1]. DNA was purified with BioTeke multifunctional DNA purification kit (BioTeke Corporation, China) following the manufacturers' instructions.

The 16S rDNA genes were amplified with bacterial universal primers 338f/518r [29]. A GC clamp was attached to forward primer to prevent complete separation of the strands during DGGE. The reaction mixture (50 μl) contained 10 pmol each primer, 200 μmol of each deoxynucleoside triphosphate, 5 μl 10× Ex *Taq* buffer, 2 U Ex *Taq* polymerase, 2 μg of bovine serum albumin (BSA) and 1 μl DNA template. Cycle conditions for the amplification were as follows: an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 56 °C for 40 s, extension at 72 °C for 40 s, then a final extension at 72 °C for 10 min included to complete any partial polymerizations with Bio-Rad PCR Thermal Cycler Model (Bio-Rad, USA). The amplified products were analyzed by electrophoresis on a 2% agarose gel, followed by staining with SYBR Green I nucleic acid gel stain, and then were visualized under ultraviolet light.

The DNA fragments of the PCR products were separated on a DGGE gel, which was prepared according to the method of Muyzer et al. [20] with a denaturing gradient from 30 to 65% (where 100% is defined as 7 M urea with 40% deionized formamide). The electrophoresis was performed in an electrophoresis cell D-Code™ System (Bio-Rad, USA) at 60 °C and 120 V for 12 h. After stained with SYBR Green I nucleic acid gel stain for 30 min, the gel was scanned and analyzed for understanding the DGGE profiles.

2.6. Data analysis

All results reported in this study were the means and standard deviations of determinations made on three replicates. Statistical analysis was carried out using SPSS software 16.0. Sample means were compared using Least Significant Differ-

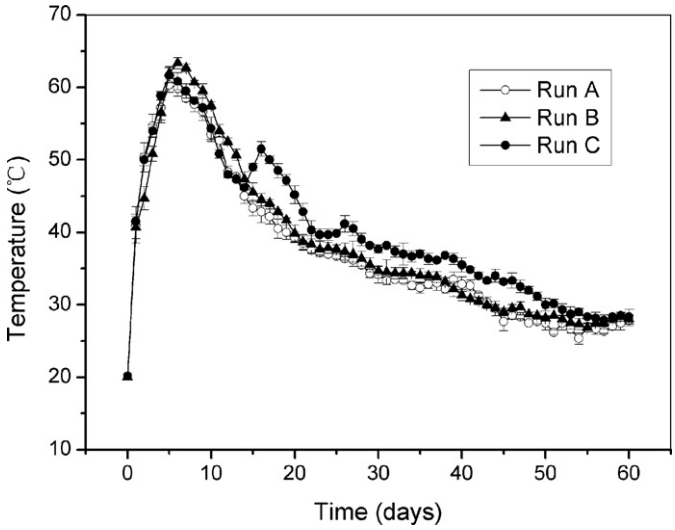

Fig. 1. Temperature evolutions during composting.

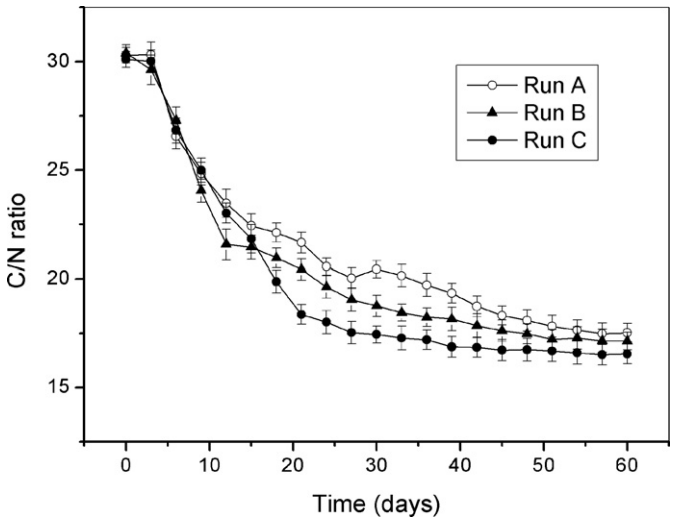

Fig. 2. Changes of C/N ratio during composting.

ence (LSD) test. The DGGE bands were counted using Quantity One-1-D analysis software (version 4.5). Principle component analysis (PCA) based on DGGE data was performed using the Canoco 4.5, with focus on inter-sample distances. The Shannon index (H) was calculated as:

$$H = \sum \left(\frac{Ni}{N}\right) \ln \left(\frac{Ni}{N}\right)$$

where Ni is the height of a peak of each band i, i is the number of bands in each DGGE profile, and N is the sum of all peak heights in a given DGGE profile.

3. Results and discussion

3.1. Evaluation of composting process

Temperature is deemed one of the most important factors affecting the microbial growth and the composting progress [30]. Using a variable temperature profile is required to promote contaminant degradation and microbial activity [16]. According to changes of temperature (Fig. 1), the composting process could be divided into two phases: (1) the first fermentation phase (days 0–14); and (2) the second one (days 15–60). High temperature (>50 °C) was maintained for 10 days in Runs A and C, and 12 days in Run B. These results showed that the temperature was affected by the inoculation during the first fermentation phase. Furthermore, the inoculation during the second fermentation phase increased the temperature in Run C in comparison with Runs A and B. Differences in changes of temperature from the three runs indicated that both of the inoculations carried out during different phases enhanced the biological activity in compost. That suggestion was supported by Abdelmajid et al. [31] who reported that more heat output probably was the result of higher biological activity in compost.

In this study, C/N ratio and GI were used for compost maturity evaluation. Due to its capacity to reflect organic matter decomposition and stabilization during composting, it is necessary to determine the C/N ratio [32]. Results about this ratio showed a strong decrement at the beginning of the composting process (from days 0 to 12), while the final values of C/N ratio in all runs were about 17 (Fig. 2). These results indicated that mature compost products were obtained from Runs A, B and C by the end of the process. Usually, compost is thought to be mature when the C/N has dropped to lower than 20 [33]. The C/N ratio in Run C decreased to 20 after 18 days, while similar values were obtained in Run A after 27 days and in Run B after 24 days, respectively. This observation indicated that the number of composting days for maturation in Runs B and C was less than that in Run A due to the effect of inoculation.

The germination index (GI) is an integrated biological indicator, which is regarded as the most sensitive parameter used to evaluate the toxicity and degree of compost maturity. Ranalli et al. [34] considered it as mature compost with the GI value of 80%. As shown in Fig. 3, the GI in Run B showed higher values than that in Runs A and C during the first fermentation phase. By the end of the composting, the respective GI values of 109, 122 and 136% for Runs A, B and C showed that all composts were almost mature. It was also apparent that the GI in Run A remained above 80% after 33 days, while about 27 days were needed for Runs B and C. These results suggested that the phytotoxicity of composts from Runs B and C was lower than that from Run A.

The successful use of compost depends on its degree of maturity and stability, as the application of an immature product can produce phytotoxic effects [35]. Although the use of inoculants to accelerate the composting process or to improve the compost quality has been a controversial subject for a long time [36], the results indicated by C/N ratio and the GI suggested that both of the inoculations during different phases were available to enhance the composting efficiency. Nevertheless, the effect of inoculation time was not identical, as the inoculation during the second fermentation phase had an advantage over the other in accelerating maturation and eliminating the phytotoxicity of compost. The

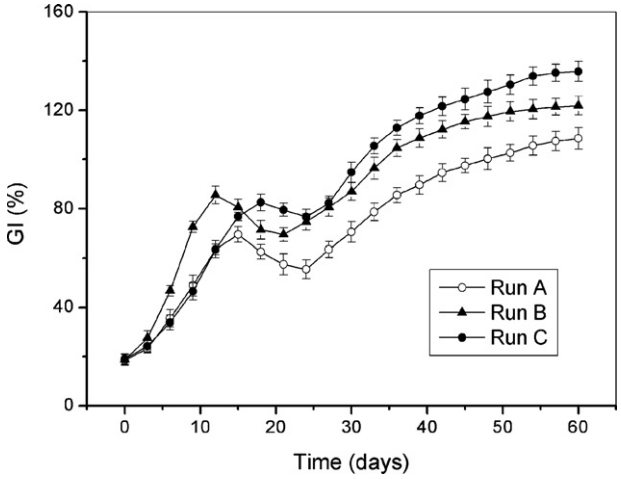

Fig. 3. Changes of germination index during composting.

Table 2
The percentage of PCP removal in the three runs during composting.

Days of composting	Run A	Run B	Run C
6	9.42 ± 3.61 h*	30.52 ± 4.43 g	8.59 ± 2.85 f
12	31.73 ± 2.56 g	49.89 ± 3.54 f	31.12 ± 2.58 e
18	45.37 ± 3.54 f	60.66 ± 3.96 e	57.65 ± 3.15 d
24	55.92 ± 2.98 e	70.69 ± 1.48 d	83.04 ± 2.81 c
30	62.47 ± 2.62 d	77.24 ± 2.58 c	92.31 ± 2.58 b
36	65.11 ± 1.79 cd	81.54 ± 2.46 bc	93.52 ± 3.45 ab
42	68.20 ± 2.35 bc	85.23 ± 3.06 ab	95.33 ± 1.96 ab
48	70.84 ± 3.54 ab	86.96 ± 1.87 a	96.16 ± 3.62 ab
54	74.08 ± 2.85 a	87.94 ± 2.19 a	96.99 ± 2.75 ab
60	74.98 ± 3.54 a	88.62 ± 2.75 a	97.51 ± 2.67 a

* Values within each column followed by the same letter are not significantly different at $p < 0.05$.

effectiveness of inoculation with *P. chrysosporium* depends on the inoculation time.

3.2. PCP removal during composting

It is interesting to note that the decline of PCP in Runs B and C after inoculated with *P. chrysosporium* mycelia was significantly rapider than that in Run A (Table 2). Reasons for these results might be as follows: (1) PCP adsorption by *P. chrysosporium* mycelia [13]; and (2) degradation by extracellular ligninolytic enzymes produced by *P. chrysosporium* after initial uptake by the mycelia [14]. However, difference in PCP removal between Runs B and C was also visible. The removal was much higher in Run C than that in Run B after about 22 days of composting. By the end of the composting process, the PCP removal percentage in all runs reached over 75%. Concerning both of the inoculated runs, the higher PCP removal was observed in Run C (97.5%), followed by Run B (88.6%). The presence of *P. chrysosporium* inoculants led to higher PCP removal than that determined in non-inoculated run. Similar results were obtained by Walter et al., who reported that PCP decline was stronger in soil waste with white-rot fungi than that without it [37]. PCP removal was also directly related to the inoculation time that affected the composting process. The inoculation during the second fermentation phase would be better for PCP removal than that during the first fermentation phase. The reasons may be that this inoculation allowed the inoculants to avoid a thermophilic stage of composting, while moderate composting conditions in the second fermentation phase were important for fungal colonization [38].

3.3. Extracellular ligninolytic enzymatic activities

The activities of three extracellular ligninolytic enzymes including LiP, MnP and Lac, which are related to PCP degradation [11], were assayed. Fig. 4 shows changes of the MnP, LiP and Lac activities during composting of PSW. Some peaks were observable from the changes of MnP activity in the three runs. In particular, two higher peaks were present on days 15 and 36 in Run A without inoculating *P. chrysosporium*. Similar phenomena occurred according to the changes of LiP and Lac activities. Gramss [39] reported that ligninolytic enzymes found in forest litter and soil waste, and their productions sometimes reflect the presence of fungal mycelia. The present study may confirm that some microorganism producing ligninolytic enzymes are inherent in soil waste.

In Fig. 4(a), the significant peaks in MnP activity were found in Run B on days 9, 18 and 36 and in Run C on days 15, 24 and 42. Furthermore, the MnP activity in inoculated runs was apparently higher than that in non-inoculated run from days 18 to 30, while the maximum MnP activity of about 3500 mU g^{-1} was found in Run C on day 24. The results indicated that inoculations during different phases, especially during the second fermentation phase might stimulate the increase of MnP activity. The observation was consistent with the investigation by Zeng et al. [22]. In addition, high level peaks of MnP activity were detected in the three runs at a later stage of composting, while PCP removal increased little as shown in Table 2. Obviously, the present MnP did not decisively result in degradation of PCP.

In Fig. 4(b), the LiP level in Run B increased more sharply than that in Runs A and C before day 21. After inoculated in Run C, the LiP level increased markedly, and peaked at 1169 mU g^{-1} on day 30. The high LiP activity of more than 600 mU g^{-1} was sustained for 2 weeks. The reasons may be that thermophilic temperature was avoided by the inoculants, while the lower C/N ratio in this

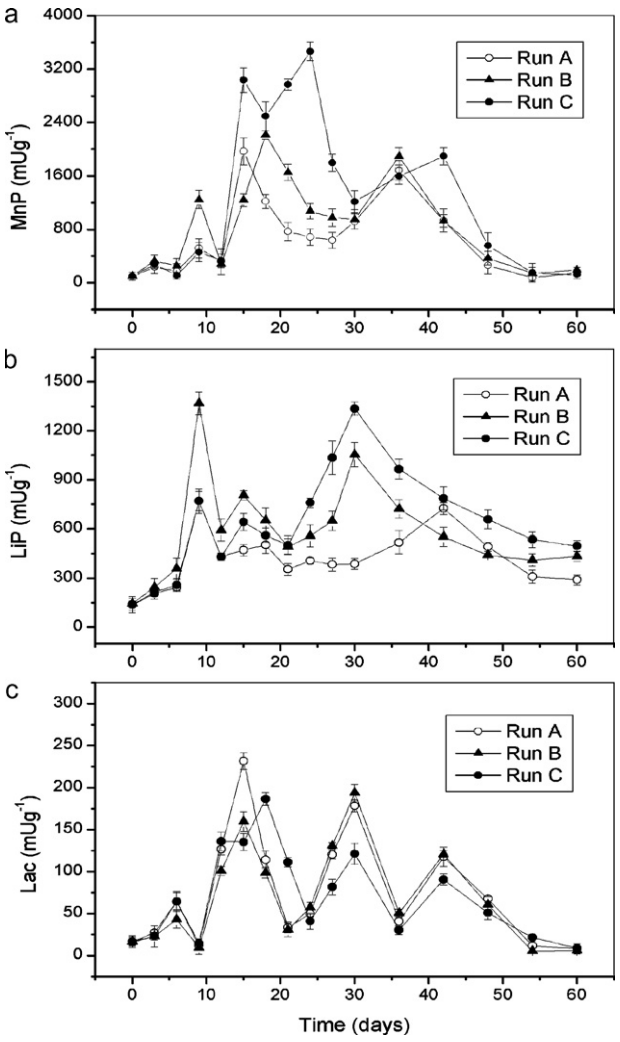

Fig. 4. Changes of MnP (a), LiP (b), and Lac (c) activities during composting.

Table 3
Number of DGGE bands and Shannon diversity index (*H*) of compost samples from Runs A, B and C.

Heaps	Number of DGGE bands	Shannon diversity index (*H*)
Run A	25.00 ± 1.00 a*	2.81 ± 0.01 a
Run B	24.33 ± 0.58 ab	2.80 ± 0.04 a
Run C	23.33 ± 0.58 b	2.66 ± 0.03 b

* Values within each column followed by the same letter are not significantly different at $p < 0.05$.

run could induce the secretion of LiP during composting [40]. Compared with Runs B and C the LiP production was lower in Run A in most cases, which suggested that inoculation with *P. chrysosporium* had a positive effect on LiP production. It is worth noting that the degradation of PCP was quite slow in Run C on day 30 while the LiP activity was extremely high. LiP has been implicated in the process of PCP degradation, since partially purified ligninases preparations can cleave many organic compounds including PCP [10]. However, no direct correlation between the production of LiP and PCP removal was found in our investigation. The different results may be ascribed to the operating conditions and substrate composition, which would affect the production of LiP by *P. chrysosporium* [24].

Fig. 4(c) presents the changes of Lac activity in the three runs during composting. The Lac activity in Runs A and C was higher than that in Run B during the first fermentation phase. The peak Lac activity in Run A was 231 mU g^{-1} on day 15. In contrast, it was lower in the inoculated runs. These results indicated that the Lac activity was inhibited by the inoculants. During the second fermentation phase, similar changes of Lac activity were observed except for Run B, which showed the impact of inoculation on Lac activity was not sustainable. Although exhaustive searches of the *P. chrysosporium* genome provide evidence that conventional Lac are absent [41], the Lac production could be obtained by optimized culture of *P. chrysosporium* [25]. Current research result about the limited Lac activity in the inoculated runs may indicate that composting with PSW does not correspond to the pattern of induced Lac from *P. chrysosporium*, which may displace other Lac producers [22], and therefore lower Lac activity was obtained in inoculated runs.

3.4. Effect of inoculation time on bacterial community composition

All the treated compost samples showed complex DGGE profiles, which were different and reproducible according to the generated profiles in three duplicate experiments. Numerous discrete DGGE bands, resulting from differences between the 16S rDNA gene sequences of different bacterial species, were apparent as shown in Fig. 5. Different banding patterns indicated the differences in compost bacterial community structure and diversity.

Compost bacterial community diversity, as determined by the number of DGGE bands and Shannon diversity index (*H*), was shown in Table 3. The results indicated that the Shannon index of the three runs corresponded to the DGGE bands number, which was used to characterize the richness of the microbial community [42]. Both the number of bands and the Shannon index showed greater difference between Runs A and C than between Runs A and B, and consequently the inoculation during the second fermentation phase had stronger effect on bacterial community structure.

PCA was used for the evaluation of compost bacterial community compositions related to the different runs, as a large amount of data derived from the complex DGGE profiles. In the two-dimensional PCA plot (Fig. 6), 32.2% of the variance was accounted for by the first component, while the second component accounted for 17.0%. In total, 49.2% of the total variance was explained. The

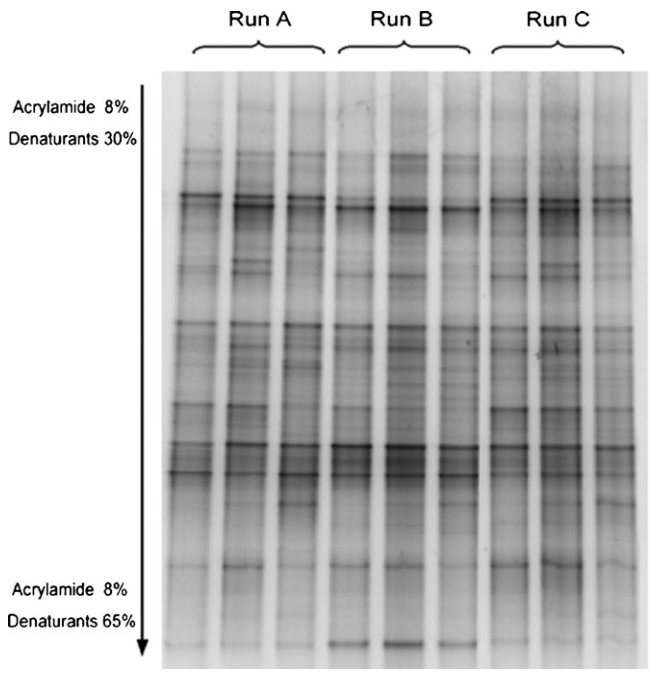

Fig. 5. DGGE profiles of 16S rDNA gene fragments of compost samples from Runs A, B and C.

PCA plot also resolved the nine compost samples into three distinct clusters, which represent the three runs consisting of two inoculated runs and the control, respectively. In each run, the bacterial community compositions of three duplicates were similar. The positions of three clusters were located separately on PCA plot, indicating the differences between three runs in bacterial community composition. These results demonstrated that the inoculations during different phases significantly altered structural composition of bacterial community.

Furthermore, the position relations between each cluster on this plot suggested that the inoculation during the second fermentation phase had a much greater influence on compost bacterial community composition than that during the first fermentation phase. As the microbial community structure and function are commonly used as indicators for compost quality and fertility [43], the authors may suppose that the improvement of composting efficiency is resulted from shifts in microbial community structure, and therefore further study is necessary for inspecting and verifying this explanation.

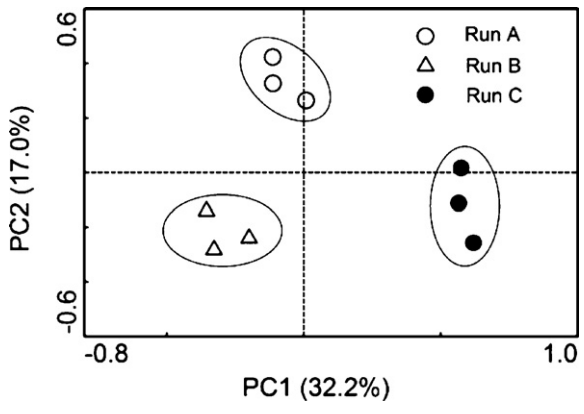

Fig. 6. PCA loading plot from DGGE profiles, depicting the differences in bacterial community composition between Runs A, B and C.

4. Conclusions

It can be concluded that monitoring physico-chemical, biochemical and microbiological properties provide useful information on the effect of different inoculation time on remediation of PSW by composting.

It is evident from the results that the effectiveness of remediation depends on the time when the inoculation is carried out. The presence of the inoculants led to higher PCP removal and better degree of maturity. This is in accordance with the conclusions drawn from extracellular ligninolytic enzymes activities except for laccase activity, though significant correlation between the production of those enzymes and PCP removal was not found in our investigation. Results based on the DGGE profiles indicated significant differences in bacterial community composition between the different treatments, and demonstrated that the inoculations exerted great influences on remediation of PSW with changes in bacterial community composition. The effects were different when the inoculations were carried out during different fermentation phases. The inoculation during the second fermentation phase was more effective than that during the first fermentation phase, since it improved PCP removal and enzyme activities, significantly altered the bacterial community structure, and sped up the composting process.

As a consequence, the inoculation with *P. chrysosporium* during the second fermentation phase has been demonstrated to be a suitable method for remediation of PSW. The differences in bacterial community composition can be considered as support in the impact of the inoculations on microbial community succession.

Acknowledgements

This research was financially supported by the Program for Changjiang Scholars and Innovative Research Team in University (IRT0719), the National Natural Science Foundation of China (50608029, 50808072, 50978088, 51039001), the Hunan Provincial Natural Science Foundation of China (10JJ7005) and the Hunan Key Scientific Research Project (2009FJ1010).

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