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Mycelial growth and solid-state fermentation of lignocellulosic waste by white-rot fungus *Phanerochaete chrysosporium* under lead stress

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ABSTRACT

Lignocellulosic biomass is an abundant renewable resource difficult to degrade. Its bioconversion plays important roles in carbon cycles in nature, which may be influenced by heavy metals in environment. Mycelial growth and the degradation of lignocellulosic waste by lignin-degrading fungus *Phanerochaete chrysosporium* under lead stress were studied. It was shown that *P. chrysosporium* could grow in liquid media with 400 mg L^{-1} Pb(II), and mycelial dry weight was reduced by 54% compared to the control. Yellow mycelia in irregular short-strip shape formed in Pb-containing media, whereas the control showed ivory-white regular mycelial pellets. Two possible responses to Pb stress were: dense hyphae, and secretion from mycelia to resist Pb. During solid-state fermentation of straw, fungal colonization capability under Pb stress was positively correlated with the removal efficiency of soluble–exchangeable Pb when its content was higher than 8.2 mg kg^{-1} dry mass. Carboxymethyl cellulase activity and cellulose degradation were inhibited at different Pb concentrations, whereas low Pb concentrations increased xylanase and ligninolytic enzyme activities and the hemicellulose and lignin degradation. Cluster analyses indicated that Pb had similar effects on the different microbial indexes related to lignin and hemicellulose degradation. The present findings will advance the understandings of lignocellulose degradation by fungi under Pb pollution, which could provide useful references for developing metal-polluted waste biotreatment technology.

1. Introduction

Biodegradation of plant residues rich in lignocellulose is an important process for the carbon cycling in nature. Lignocellulose is a macromolecular complex consisting of lignin, cellulose and hemicellulose. Lignin is a highly irregular and insoluble polymer and chemically bonded by covalent linkages with hemicellulose, and the lignin–carbohydrate complexes enwrap cellulose in plant cell wall (Pérez et al., 2002). This intricate association constitutes a barrier to the lignocellulose transformation, so the degradation process of plant residues is often slow in nature (Malherbe and Cloete, 2002; Kumar et al., 2006). A large number of microorganisms has attracted particular attention for their potential ability of lignin degradation (Hatakka, 2001). White-rot fungi are known as the most efficient lignin degraders, in which the representative species *Phanerochaete chrysosporium* has been most extensively studied due to the ability to degrade a wide range of organic substrates (Dorado et al., 1999; Fragoeiro and Magan, 2005; Wen et al., 2009; Yu et al., 2009).

Since heavy metals can affect the microbial reproduction and cause morphological and physiological changes (Rossbach et al., 2000; Pagès et al., 2007), the biodegradation processes might be influenced by toxic heavy metals in environment. Pb is a non-essential but highly toxic metal widespread in the biosphere. The primary sources of Pb-contamination come from mining and smelting activities, combustion of leaded gasoline, land application of sewage sludge, battery disposal and Pb-bearing products (Dollar et al., 2001; Rodrigues et al., 2010). There are Pb mine areas and Pb-contaminated soils in many countries, as a result the plants nearby are polluted to some extent. High concentrations of Pb are found in the leaves, stems and other parts of the plants in Pb-contaminated soils (Río-Celestino et al., 2006; Rotkittikhun et al., 2006). Microorganisms have to cope with toxic Pb during their growth in the Pb-contaminated substrates, and the exposure of microorganism to metals always inhibits microbial growth and

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activity (Baldrian et al., 2000; Singhal and Rathore, 2001). Microbial enzymes might be affected by heavy metals due to the potential inhibition to both enzymatic reactions and complex metabolic processes (Tuomela et al., 2005). Therefore, the *in situ* degradation of lignocellulose in contaminated soils is disturbed, and the presence of metals is a serious limitation to the application of *in situ* biotreatment technologies. Previous researches focused on the fungal response to essential metals such as copper and zinc, but the studies concerning the interaction of Pb with white-rot fungi have drawn attention in recent years (Say et al., 2001; Iqbal and Edyvean, 2004). It was confirmed that *P. chrysosporium* could grow at the Pb(II) concentrations of 5–30 mg L^{-1} and remove Pb from wastewater with its mycelium (Yetis et al., 2000; Baldrian, 2003). We also found that *P. chrysosporium* could effectively degrade the organic matter at the total Pb concentrations of 105 and 400 mg kg^{-1} (dry weight) in composting (Huang et al., 2006; Zeng et al., 2007). However, the effects of Pb on the growth, mycelial morphology and lignocellulolytic enzyme activities of *P. chrysosporium* are still not completely clear. No details on the adaptation responses and mechanisms of *P. chrysosporium* under Pb stress have been reported in literature so far. It is of particular interest from environmental point of view to understand how *P. chrysosporium* reacts to toxic Pb, which would provide useful information for the development of fungi-based technologies to improve the degradation of metal-polluted lignocellulosic wastes.

Therefore, the main objective of this study was to gain insight into the growth, activities and degradation ability of *P. chrysosporium* in treating lignocellulosic waste under Pb stress. We studied the characteristics of growth and mycelial morphology of *P. chrysosporium* at different concentrations of Pb to simulate different levels of Pb pollution, and discussed the mechanisms of fungal response to Pb. Lignocellulolytic enzyme activities and lignocellulose degradation in Pb-contaminated lignocellulosic waste treatment by *P. chrysosporium* were also investigated.

2. Materials and methods

2.1. Fungal strain and spore suspension preparation

P. chrysosporium strain BKMF-1767 was obtained from China Center for Type Culture Collection (Wuhan, China). Fungal cultures were maintained on potato dextrose agar (PDA) slants at 4 °C, and then transferred to PDA plates at 37 °C for several days. The spores on the agar surface were gently scraped and blended in the sterile distilled water as spore suspension. The spore concentration was assessed by microscope with a blood cell counting chamber and adjusted to 2.0×10^6 spores mL^{-1}.

2.2. Liquid cultures with different Pb concentrations

P. chrysosporium was cultured in the sterile potato dextrose liquid media supplemented with $Pb(NO_3)_2$ by adding total Pb(II) 0, 30, 200 and 400 mg L^{-1}, respectively. The initial pH of liquid media is 6.8 in each culture flask. The culture flasks were shaken at 150 rpm for 20 d, and the initial spore concentration in each flask was 5.0×10^5 spores mL^{-1}. The cultures of *P. chrysosporium* were kept at 37 °C during the whole experimental process. All the chemicals used in this work were of analytical reagent grade. All experiments were performed in three replicates.

2.3. Mycelial morphology analysis

After 20 d of liquid culture, mycelial shape and color of *P. chrysosporium* in each flask were observed and recorded. The mycelium from each flask was filtrated through pre-weighted Whatman No. 1 filter papers and thoroughly washed with deionized water. The mycelium retained by filter paper was freeze-dried and weighed. For mycelial morphology comparison, the freeze-dried mycelium from Pb-free media and that from Pb-containing media were respectively coated with gold and examined by a JEOL-5600LV (Japan) scanning electron microscope (SEM).

2.4. Solid-state fermentation and Pb tolerance

Straw was air-dried and ground to pass through a 2-mm nylon screen, and the content of total Pb in the straw was 1.8 mg kg^{-1}. Then 35 g of straw powder, contained in each 500-mL fermentation flask labeled as A(control), B(30), C(200) and D(400), was supplemented and mixed thoroughly with $Pb(NO_3)_2$ solutions by adding total Pb(II) 0, 30, 200 and 400 mg kg^{-1} straw (dry weight), respectively. Each flask was stoppered and autoclaved for 30 min at 121 °C, and then the substrate was inoculated by 2.1 mL spore suspension of *P. chrysosporium*. The initial pH of straw media is 6.9 in each flask. Solid-state fermentation (SSF) of straw was performed in a constant-temperature incubator for 45 d. During the whole fermentation process, the temperature was kept at 37 °C and the humidity was maintained at the initial level (75%). To make a better comparison, the non-inoculated control flasks were used.

Three sub-samples were periodically taken from the top, middle and bottom depths in the straw substrates in SSF flask, respectively. The three sub-samples were combined for the analyses. All analyses were performed in three replicates. For every straw sample, microbial biomass, lignocellulolytic enzyme activities, polysaccharides (separately, hemicelluloses and cellulose), and lignin content, as well as content of soluble-exchangeable Pb, were determined.

2.5. Microbial biomass and enzyme activities assay

Fungal biomass carbon was measured by the method introduced by Wu et al. (1990). The fresh sample was divided into two equal portions. One portion was fumigated for 24 h at 25 °C with ethanol-free chloroform containing 20 μL 2-methyl-2-butene L^{-1}. Following fumigant removal, the sample was extracted with 100 mL 0.5 M K_2SO_4 by 30 min rotary shaking (200 rpm) and then filtered. The non-fumigated portion was extracted similarly. Organic C in the extraction was measured by American OI 1010 TOC instrument. Then biomass C was estimated by the organic C extracted from fumigated sample subtracting that from non-fumigated sample. During the process of fermentation, fresh samples taken under sterile conditions were extracted with deionized water at a ratio of 1:10 (w/v). Each extraction was performed under rotary shaking (200 rpm) for 1 h. Then the homogenate was centrifuged at 4 °C for 20 min, and the supernatant was filtered through filter papers (Whatman No. 1). The filtrate was analyzed for activities of enzymes. Lignin peroxidase (LiP) was measured with a UV–vis spectrophotometer according to Tien and Kirk (1988). One unit (U) of LiP activity was defined as the amount of the enzyme required to produce 1 μmol veratryl aldehyde from the oxidation of veratryl alcohol per min. Manganese peroxidase (MnP) was analyzed by monitoring the change in absorbance of reaction mixture at 290 nm (Hiroyuki et al., 1992). 1 mL of reaction mixture contained a final concentration of 50 mM tartaric acid, 0.4 mM H_2O_2 and 0.1 mM $MnSO_4$ and 0.2 mL of culture filtrate. MnP unit activity was defined as the amount of enzyme required for the production of 1 μmol Mn^{3+} per min. Activity of carboxymethyl cellulase (CMCase) was estimated using carboxymethyl cellulose according to the method recommended by Ghose (1987). Xylanase activity was determined according to Saha et al.

(2005). Specific activities of these enzymes were expressed as units per gram of dry medium.

2.6. Analysis of lignocellulose degradation and Pb content

To determine the lignocellulose degradation ability of *P. chrysosporium*, the contents of lignin, cellulose and hemicellulose were analyzed. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to the procedures outlined by Van Soest et al. (1991). Hemicellulose was estimated as the difference between NDF and ADF. Cellulose was estimated as the difference between ADF and ADL content. Lignin was estimated as the difference between ADL and ash content. Selective index was calculated as the ratio of lignin/lignocellulose degradation efficiency. The content of soluble–exchangeable Pb was measured as introduced by our previous study (Huang et al., 2006). Degradation efficiencies of lignin, cellulose or hemicellulose at the *n*th day (D_n) were calculated by the following formula:

$$D_n = \frac{m_p - m_n}{m_p} \times 100\% \quad (1)$$

where m_p and m_n represent the total amount of lignin, cellulose or hemicellulose in fermentation substrate at the previous sampling time and that at the *n*th day, respectively.

2.7. Fungal colonization capability and removal efficiency of soluble–exchangeable Pb

Fungal colonization capability under Pb stress at the *n*th day was estimated by the ratio of fungal biomass in Pb-containing medium to that in Pb-free control at the *n*th day. The removal efficiency of soluble–exchangeable Pb at the *n*th day (R_n) was calculated by the following formula:

$$R_n = \frac{C_p - C_n}{C_p} \times 100\% \quad (2)$$

where C_p and C_n represent the content of soluble–exchangeable Pb at the previous sampling time and that at the *n*th day, respectively. In B(30), C(200) and D(400), removal efficiency of soluble–exchangeable Pb and fungal colonization capability under Pb stress were analyzed at the *n*th day, which were all used for the subsequent correlation analysis.

2.8. Statistical analysis

The results to be presented were the mean values of three replicates, and the standard deviations were used to analyze experimental data. Statistical analyses were performed to obtain more comprehensive and useful information, using the software package SPSS 13.0 for Windows (SPSS, Germany). ANOVA was performed for mycelial dry weight in liquid culture. Correlation analysis was used to determine relationships between fungal colonization capability under Pb stress and removal efficiency of soluble–exchangeable Pb. Cluster analysis was used to classify experimental treatment and control groups based on the calculation of distance measures between the values of microbial indexes (xylanase, hemicellulose degradation, LiP, lignin degradation, selective index, MnP, microbial biomass, cellulose degradation and CMCase) in one group and those in another group. And cluster analysis was also carried out to identify the effects of Pb on the above nine microbial indexes based on the calculation of similarity measures between different microbial indexes. Between-groups linkage and furthest neighbor were used as cluster method for the above two clustering respectively.

3. Results and discussion

3.1. Effect of Pb ions on fungal growth and hyphal morphology in liquid culture

It was observed that the range of pH values was between 6.7 and 7.2 during the culture process. The higher initial Pb(II) concentration resulted in the greater inhibition to growth of *P. chrysosporium* in liquid medium, and the mycelial dry weight was reduced by 38% and 54% at the Pb(II) concentrations of 200 and 400 mg L^{-1}, respectively, as compared to the control (Table 1). There was insignificant difference ($P = 0.056$) between the fungal growth at 30 mg L^{-1} of Pb(II) and that at 0 mg L^{-1}. However, 200 and 400 mg L^{-1} of Pb(II) significantly inhibited fungal growth ($P = 0.007$ and 0.002, respectively). The finding that *P. chrysosporium* could survive and grow even in the potato dextrose liquid medium with 400 mg L^{-1} of Pb(II) provided evidence for the fungal resistance to Pb. It indicated that Pb mainly had fungistatic effect but not fungicide effect on *P. chrysosporium* at lower than 400 mg L^{-1} Pb(II). Falih (1997) found that *P. chrysosporium* could grow in Czapek-Dox medium with 400 mg L^{-1} Pb. These findings confirmed the capability of *P. chrysosporium* to survive in different Pb-contaminated substrates.

The inhibition of fungal growth caused by Pb ions was accompanied by the morphological changes of the growing mycelium (Table 1). The mycelia with irregular short-strip shape were found in liquid media with high concentrations (200 or 400 mg L^{-1}) of Pb(II), whereas the regular mycelial pellets were observed at the concentrations of 0 or 30 mg L^{-1} of Pb(II). Short-strip mycelia in the media with high concentrations of Pb(II) had smoother surface than mycelia pellets in Pb-free media due to the shortening appearance of surface hypha. The mycelia cultivated in Pb-containing media turned yellow or dark yellow, while the controls were ivory white (Table 1). Such behaviors have been previously reported for other white-rot fungi in Cd-containing cultures (Gabriel et al., 1996; Baldrian, 2003). The interaction of fungi with heavy metals causes severe changes in the physiological processes, and at high concentrations, it can even kill the mycelium. Therefore, fungi evolved active defense mechanisms to alleviate toxicity of metals. The defense is usually based on the immobilization of heavy metals by the extracellular chelating compounds, and the metal chelates precipitated on the cell wall surface of fungal hyphae (Baldrian, 2003). *P. chrysosporium* could produce extracellular metal chelators, and the changes in mycelial color exposed to metals might be indicative of the formation of metal chelates (Machuca et al., 2001; Say et al., 2001). It provides a means to immobilize the soluble metal ions or complexes as insoluble precipitates, thus decreases the bioavailability and toxicity of metals.

Mycelium of *P. chrysosporium* was observed by SEM to reveal the fungal response. The mycelia from Pb-free liquid media exhibited sparse and loosely entangled hyphae, whereas densely entangled hyphae were found after exposure to Pb (Fig. 1). This

Table 1
Mycelial characteristics of *Phanerochaete chrysosporium* grown in liquid culture media with different Pb concentrations after 20 d of culture.

Pb concentration (mg L^{-1})	Mycelial characteristics			
	Shape	Surface	Color	Dry weight (mg)[a]
0	Pellet	Rough	Ivory white	107 ± 4
30	Pellet	Rough	Yellow	94 ± 3
200	Pellet, short strip	Smooth	Dark yellow	66 ± 3
400	Short strip	Smooth	Dark yellow	49 ± 1

[a] Values of dry weight are means ($n = 3$) with standard deviations.

coincided with the finding of the earlier study on the effect of Hg on the mycelial morphology of *Pycnoporus cinnabarinus* (Mandal et al., 1998). Baldrian (2003) reported that the addition of Cd led to the formation of dense hyphae caused by the increase of lateral number per branch point and a decrease of the distance between branch points. From our findings and the known interaction mechanism (Baldrian, 2003), two possible responses of fungi were hypothesized to play key roles in resisting the metal toxicity: (i) the formation of peculiarly dense hyphae to limit metal ions touch on fungal cell wall, and (ii) the secretion of chelators from mycelia to reduce metal toxicity by chelation. Similar responses induced by heavy metals were found in other microorganisms as already noticed by several researchers (Cánovas et al., 2003; Declerck et al., 2003).

3.2. Relationship between fungal colonization and soluble–exchangeable Pb

In this study, a pH range between 6.5 and 7.3 suitable for fungal colonization was recorded during SSF of straw by fungus *P. chrysosporium*. Fungal growth is mainly influenced by the content of soluble–exchangeable metal during SSF, because the soluble–exchangeable metal is more toxic to microorganisms than the inactive metal. The correlation between fungal colonization capability under Pb stress and the removal efficiency of soluble–exchangeable Pb was analyzed (Fig. 2). Fungal colonization capability under Pb stress was positively correlated with the removal efficiency of soluble–exchangeable Pb, when the content of soluble–exchangeable Pb in culture media was higher than 8.2 mg kg^{-1} dry mass. However, no obvious relationship was established between fungal colonization capability under Pb stress and the removal efficiency of soluble–exchangeable Pb, when its content was lower than 8.2 mg kg^{-1} dry mass. With the decrease of high content of soluble–exchangeable Pb, the fungi thoroughly spread on solid medium in fermentation flask. The reason might be that the inhibition of Pb turned weak due to the decrease of soluble–exchangeable Pb by *P. chrysosporium*. The decrease of soluble–exchangeble Pb might be attributed to the adsorption and cation exchange on hypha and the chelation by extracellular metabolite (Baldrian, 2003; Zeng et al., 2007), which could provide favorable conditions for fungal growth and complete colonization. Baldrian et al. (2000) reported that high concentrations of soluble metal caused the weakening of colonization capability or incomplete colonization of *Pleurotus ostreatus*. The tolerance of *P. chrysosporium* to Pb toxicity might be another reason for the successful colonization and vigorous growth.

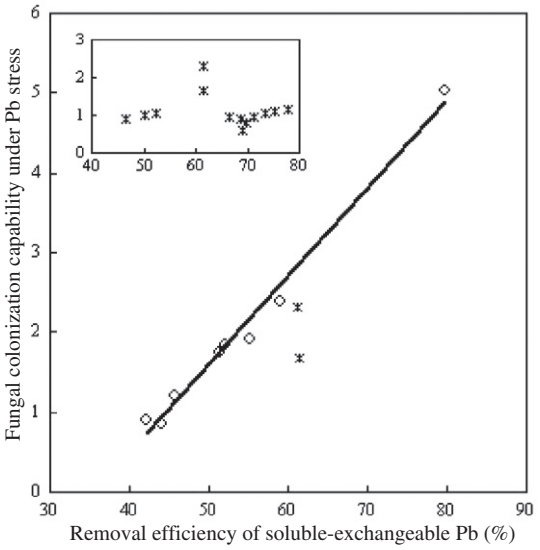

Fig. 2. Relationships between fungal colonization capability under Pb stress (i.e., the ratio of fungal biomass in Pb-containing medium to that in Pb-free control) and the removal efficiency of soluble–exchangeable Pb during solid-state fermentation of Pb-contaminated straw by *P. chrysosporium*. The inner graph shows the nonlinear relationship among the above two variables at low content of soluble–exchangeable Pb (less than 8.2 mg kg^{-1} dry straw). (o): samples with soluble–exchangeable Pb content higher than 8.2 mg kg^{-1}, (*): those less than 8.2 mg kg^{-1}.

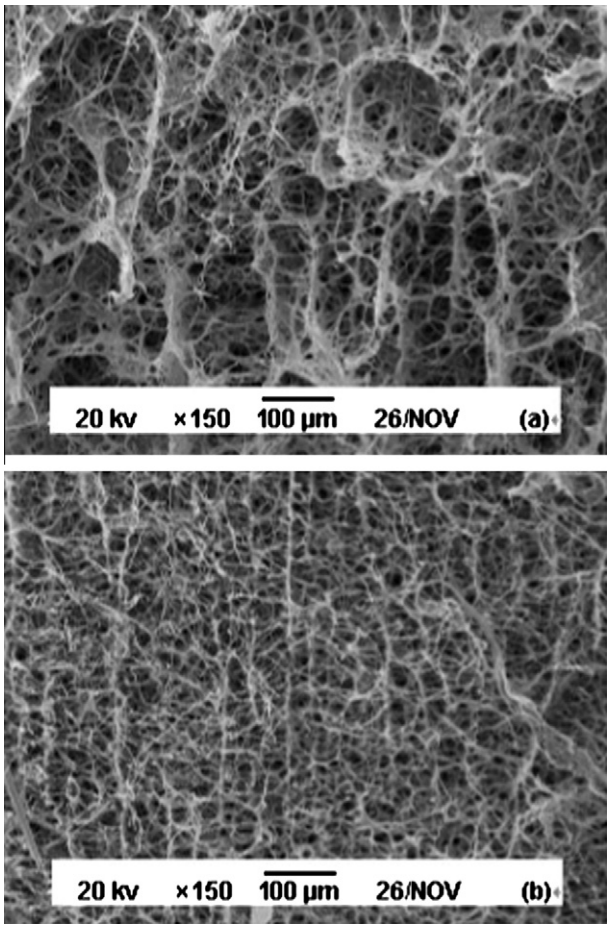

Fig. 1. Scanning electron microscope images of (a) mycelium of *P. chrysosporium* from Pb-free medium and (b) that from Pb-containing medium (200 mg L^{-1}) after 20 d of culture.

3.3. Dynamic changes of enzyme activities

The changes of LiP, MnP, xylanase and CMCase activities during SSF of straw with different initial Pb concentrations are presented in Fig. 3. This is the first report concerning lignocellulolytic enzymes of *P. chrysosporium* under Pb stress in SSF. Low activity of LiP was observed in D(400) at the initial stage of SSF. The higher LiP activities were found in B(30) and C(200) than those in A(control) from days 9 to 21. LiP activities in B(30) and C(200) showed the maximum values (0.92 and 0.97 U g^{-1}, respectively) on day 21, whereas that in D(400) did not reach 0.88 U g^{-1} until day 33. The maximal levels for LiP activity were not significantly different, though significant differences in time to reach the peak were found ($P < 0.05$). A(control) and B(30) exhibited high MnP activity (11.9 and 22.1 U g^{-1}, respectively) on day 18, whereas in C(200) and D(400), peak values of 15.8 and 9.3 U g^{-1} were present on day 21. Both the maximal MnP activities and the time to reach the peak were significantly different ($P < 0.05$). These results indicated that

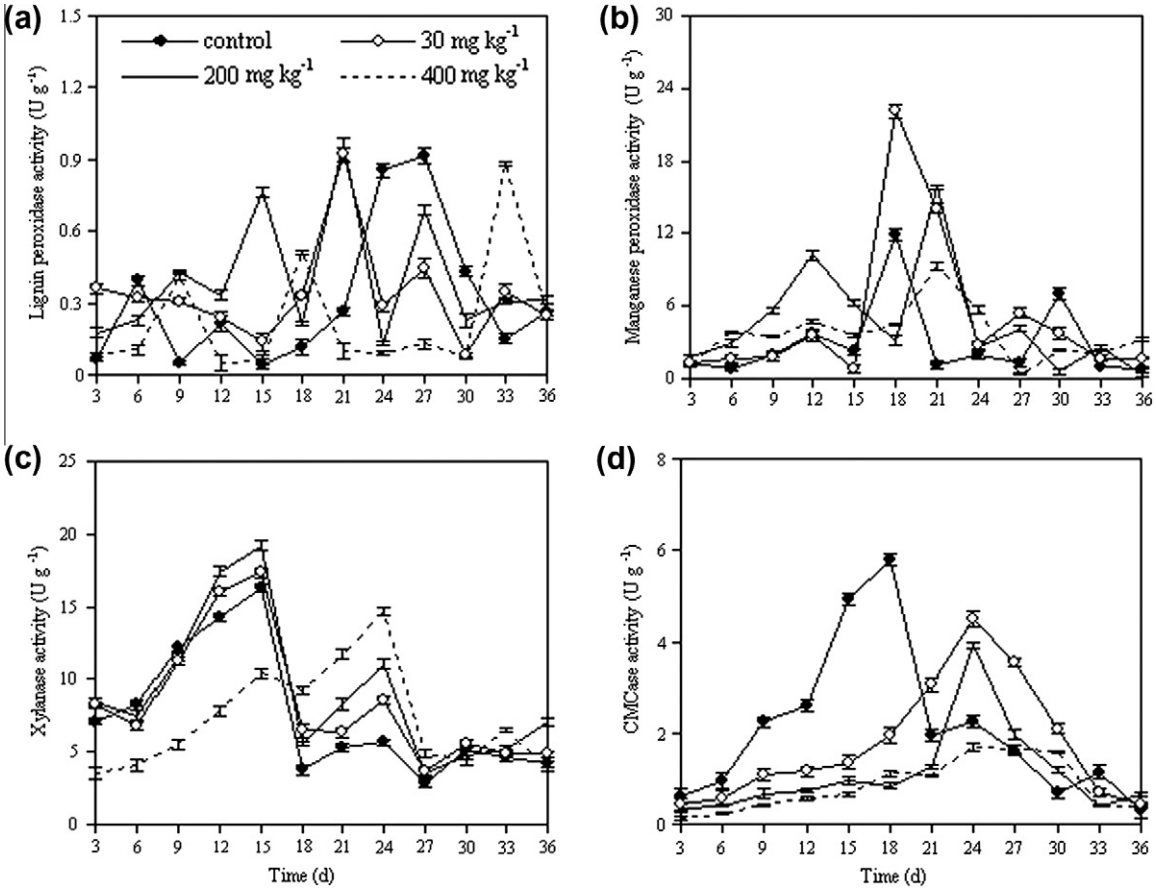

Fig. 3. Activities of lignin peroxidase, manganese peroxidase, xylanase and CMCase during solid-state fermentation by *P. chrysosporium* in the control without Pb addition and in treatments with Pb addition (30, 200 and 400 mg kg^{-1} dry mass, respectively). The bars represent the standard deviations of the means ($n = 3$).

P. chrysosporium could maintain good activities of ligninolytic enzymes (LiP and MnP) under Pb stress. This observation was in good agreement with the Pb-resistant character of *P. chrysosporium*. It is also in agreement with previous findings reporting that laccase and MnP activities of other white-rot fungus *P. ostreatus* increased in Pb-supplemented medium (Baldrian et al., 2005). Addition of 400 mg kg^{-1} of Pb(II) decreased the activities of LiP and MnP (Fig. 3), which might be due to the adjustment of cell physiology after excessive Pb exposure. The resuming of enzyme activities in D(400) might be attributed to the decrease of Pb toxicity after several days of SSF by *P. chrysosporium*. Moreover, the ligninolytic enzymes of white-rot fungi with metal tolerance could degrade various hazardous xenobiotics, so the fungi are expected to treat complex wastes contaminated with xenobiotics and heavy metals (Hatvani and Mécs, 2003).

Xylanase, involved in the hemicellulose degradation, was promoted in B(30) and C(200) and inhibited in D(400) (Fig. 3). The relative difference of peak value of xylanase activity between control and D(400) was only 1.7 U g^{-1}, although the time reaching the peak was significantly ($P < 0.05$) delayed from day 15 in control to day 24 in D(400). Results indicated that *P. chrysosporium* could still excrete highly active xylanase in the presence of Pb. Low concentrations of Pb stimulated the production of ligninolytic enzymes and xylanase. This might be attributed to the enhancement of fungal metabolism to maintain energy in response to Pb stress. However, the mechanism was not completely clear and needed further studies. CMCase activity showed a peak in A(control) on day 18, whereas in other treatments it reached a peak on day 24. The maximum activity of CMCase in B(30), C(200) and D(400) was significantly ($P = 0.016$, 0.001 and 0.001, respectively) lower than that in control (Fig. 3). CMCase was restrained by Pb toxicity, and the higher initial Pb concentration resulted in the greater inhibition. It might be because the metals entering fungal cell disturbed the production of enzyme on the levels of transcriptional and translational regulation (Baldrian, 2003; Baldrian et al., 2005). CMCase activities in all Pb-supplemented treatments were resumed after 18 d of SSF, which might be because of the lowering of soluble–exchangeable Pb.

3.4. Lignocellulose biodegradation in the presence of Pb

Lignocellulose was degraded effectively by *P. chrysosporium*, even at the concentration of 400 mg kg^{-1} dry mass of Pb (Fig. 4). The highest degradation efficiency of lignin in A(control) was lower than that in B(30) and C(200), and the time reaching the highest degradation efficiency in A(control) was longer. Hemicellulose degradation was stimulated in B(30) and C(200) during SSF, whereas cellulose degradation was inhibited obviously at the initial stage of SSF because of exposure to Pb. The cellulose degradation efficiency decreased with the increasing initial Pb concentrations in the medium. Results indicated that Pb discriminatingly affected lignin and cellulose degradation, which might be due to the different effects of Pb on ligninolytic enzymes and CMCase activities, as described above. It was also found that the degradation efficiency of lignocellulose components increased from days 6 to 24 and then decreased with fermentation time. The reason might be that the growth and activity of *P. chrysosporium* turned weak after 24 d of fermentation due to the continual decrease of nutriment. They coincided with the changes of enzyme activities as shown in Fig. 3.

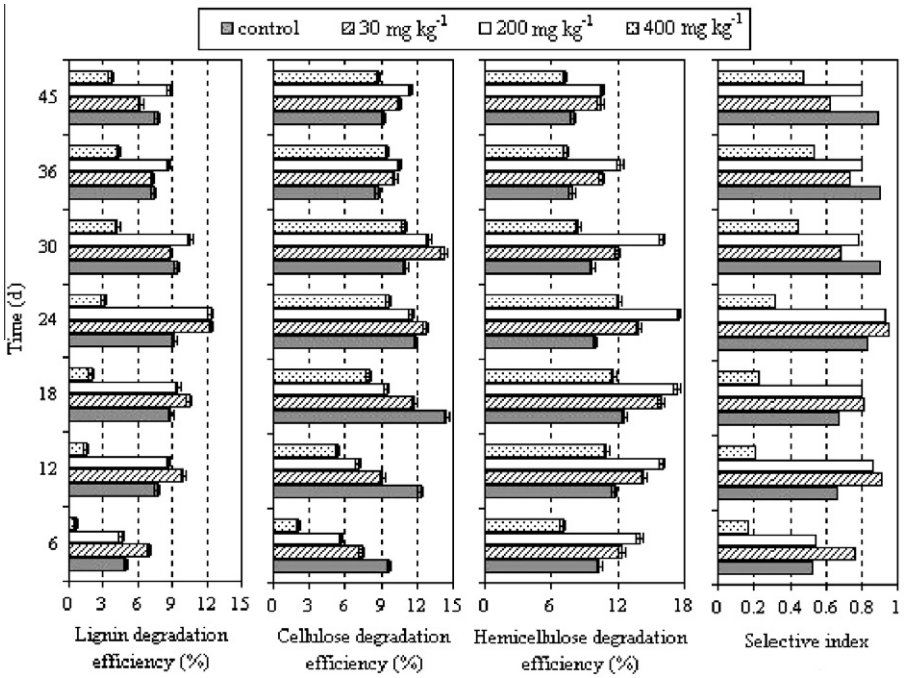

Fig. 4. Degradation of lignocellulose components during solid-state fermentation by *P. chrysosporium* in the control without Pb addition and in the treatments with Pb addition (30, 200 and 400 mg kg^{-1} dry straw, respectively). The bars represent the standard deviations of the means ($n = 3$).

3.5. Classification of different effects of different Pb levels

Classification of experimental and control groups was carried out by cluster analysis according to the growth, enzyme activities and degradation ability of *P. chrysosporium* in SSF (Fig. 5a). They were classified as three groups. B(30) and C(200) were classified as one group, while A(control) and D(400) were identified as two different groups. It indicated that the characteristics of fungal growth and activities in B(30) and C(200) were similar, whereas they were different from A(control) and D(400). These results confirmed that the presence of Pb influenced the growth and activities of *P. chrysosporium*, and the effect of Pb concentration of 30 mg kg^{-1} on these microbial indexes was similar to the effect of Pb concentration of 200 mg kg^{-1}.

To understand the effect of Pb on different microbial indexes, nine indexes related to fungal growth and activities were classified as shown in Fig. 5b. Three groups of indexes were obtained. Xylanase, hemicellulose degradation efficiency, LiP, lignin degradation efficiency and selective index belonged to Group 1, which showed that the effect of Pb on these indexes were similar. Lignin and hemicellulose degradation were promoted when LiP and xylanase activities were increased at low concentration of Pb, and the negative effects of Pb on the four indexes were simultaneously observed at high concentration (Figs. 3 and 4). These results also indicated that the effect of Pb on lignocellulolytic enzyme activities might be mainly responsible for the response of lignocellulose biodegradation to Pb stress. Since lignin is bonded by covalent linkages with hemicellulose, the hemicellulose transformation is limited by the lignin fraction in the wood cell wall. The hemicellulose degradation efficiency was high when lignin was decomposed effectively in previous researches (Lopez et al., 2006). It was also found in this study that the hemicellulose degradation was inhibited when lignin degradation efficiency decreased at high concentration of Pb. This might explain the slow lignocellulose degradation process observed in metal-contaminated soils. MnP belonging to Group 2 was separated from other eight indexes, which showed that the Pb effect on MnP was different from that on other indexes. Group 3 included microbial biomass, cellulose degradation and CMCase, which were all inhibited in the presence of Pb. The negative effect of Pb on cellulose degradation might be attributed to the inhibition of Pb to microbial biomass and CMCase.

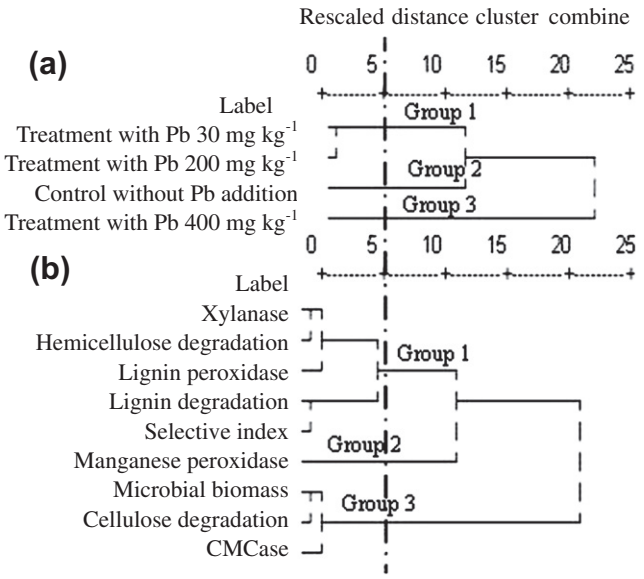

Fig. 5. (a) Classification of control and treatments with different initial Pb concentrations by cluster analysis based on the distance measures between the values of microbial indexes (xylanase, hemicellulose degradation, lignin peroxidase, lignin degradation, selective index, manganese peroxidase, microbial biomass, cellulose degradation and CMCase) in different groups; (b) classification of the above nine microbial indexes based on the similarity measures between different microbial indexes. Between-groups linkage and furthest neighbor were used as cluster method respectively.

4. Conclusions

P. chrysosporium could grow in liquid medium with 400 mg L^{-1} Pb(II), which might be attributed to the densely aggregated hyphae and the secretion from mycelia to resist metal toxicity. Colonization capability of *P. chrysosporium* increased with the decrease of soluble–exchangeable Pb content during SSF of Pb-contaminated substrates. CMCase activity and cellulose degradation were inhibited in the presence of Pb. However, *P. chrysosporium* could maintain good activities of ligninolytic enzymes (LiP and MnP) under Pb stress. Ligninolytic enzymes and xylanase activities were increased at low initial Pb concentrations, as a result the higher lignin and hemicellulose degradation were found. Cluster analyses also indicated that there were similar effects of Pb on the different indexes related to lignin and hemicellulose degradation. The present findings could be used as references for developing the biotreatment technology with *P. chrysosporium* to promote lignocellulosic wastes bioconversion and carbon cycle under heavy metal pollution.

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