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ORIGINAL PAPER

Biodelignification of rice straw by *Phanerochaete chrysosporium* in the presence of dirhamnolipid

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Abstract Lignin degradation by white-rot fungi has received considerable attention as a means for reducing accumulation of lignocellulosic wastes in the environment. The stimulatory effect of surfactants on fungal lignocellulose bioconversion also has attracted wide interest. In this study the influence of dirhamnolipid biosurfactant on biodegradation of rice straw by *Phanerochaete chrysosporium* was investigated. It was shown that the biodelignification process of rice straw can be significantly enhanced by the presence of dirhamnolipid biosurfactant. In particular, the dirhamnolipid at the concentration of 0.007% increased the peak activity of lignin peroxidase (LiP) by 86% without affecting the manganese peroxidase (MnP) activity. The water-soluble organic carbon (WSOC) contents in the straw substrates as well as the microbial growth and activity were effectively improved by dirhamnolipid, while the degradation rate of lignin increased by 54% with dirhamnolipid of 0.007%. Observed chemical structural and morphological changes showed that the straw substrates were delignified in the presence of dirhamnolipid with the formation of terrace-like fragments separated from the inner cellular fibers and the release of simple compounds. Variation partitioning analysis revealed that the dirhamnolipid addition induced a significant straw biodelignification which explained 22.1% ($P = 0.013$) of the variance.

Keywords Lignin · Biodelignification · Dirhamnolipid · Biosurfactant · *Phanerochaete chrysosporium*

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Introduction

The increasing development of agro-industrial activity has led to the production of a large quantity of lignocellulosic residues from wood, herbaceous, agricultural, forestry, municipal solid wastes and various industrial wastes all over the world (Sánchez 2009). Lignin is a highly irregular and insoluble polymer and chemically bonded by covalent bonds with hemicellulose, and the lignin-carbohydrate complexes enwrapping cellulose in plant cell wall (Pérez et al. 2002). This intricate association constitutes accessibility barriers to the lignocellulose transformation, so the lignocellulosic wastes are difficult to degrade, and the degradation process is

often slow in nature (Malherbe and Cloete 2002; Huang et al. 2008). Up to date, lots of cellulose, hemicellulose and lignin produced as by-products in agriculture or forestry are used, but in many developing countries, still large amounts of them are considered as wastes.

Many microorganisms are capable of degrading and utilizing cellulose and hemicellulose as carbon and energy sources. However, a much smaller group of filamentous fungi has evolved with the ability of breaking down lignin. This is known as white-rot fungi, which possesses the unique ability of efficiently degrading lignin to CO_2 (Hofrichter 2002). This degradative ability of white-rot fungi is due to the strong oxidative activity and low substrate specificity of their ligninolytic enzymes. Therefore, much attention has currently been drawn to the development of biodelignification with white-rot fungi for the efficient treatment of lignocellulosic wastes (Stepanova et al. 2003; Kumar et al. 2006; Tang et al. 2006). The findings that white-rot fungus *Phanerochaete chrysosporium* (*P. chrysosporium*) could efficiently bridge the lignin barrier has attracted attention in particular. Therefore, *P. chrysosporium* was studied as a characteristic species (Zeng et al. 2007b; Tang et al. 2009).

In a previous research on the inoculation of white-rot fungi into agricultural waste fermentation, the degradation of lignin was enhanced mainly in the catalysis of extracellular lignin peroxidase (LiP, EC 1.11.1.14) and manganese peroxidase (MnP, EC 1.11.1.13) (Dorado et al. 1999). Improving the production and activity of lignin-degrading peroxidases in biodelignification became of significance. Recently, intensive researches have found that the surfactants have stimulatory effects on promoting the production of cellulase, xylanase and LiP et al., thus may improve the decomposition of lignocellulosic waste (Ahuja et al. 2004; Liu et al. 2006; Liu et al. 2008). However, chemically-synthesized surfactants are unbiodegradable and toxic. Due to the high effectiveness and low toxicity to microorganisms and environmental compatibility, the unique ability of biosurfactant to enhance the organic contaminant biodegradation and bioremediation has gained more and more attention and will undoubtedly lead to application in pollution control. The stimulative effect of rhamnolipid biosurfactant on cellulose and hemicellulose degradation has been reported by Shi et al. (2006). However, few reports are available on the biosurfactant addition for the lignin degradation with inocula of *P. chrysosporium*.

The aim of this work was to investigate the biodelignification of rice straw in the presence of dirhamnolipid biosurfactant by *P. chrysosporium* under solid state fermentation (SSF) condition. Variation partitioning analysis was conducted to evaluate the mutual influence of the biosurfactant addition and the biodelignification process parameters. This research helps to understand complex aspects of combined effects of *P. chrysosporium* and dirhamnolipid during lignin degradation, and give an insight into biosurfactants' influence on natural lignocellulosic residues biodegradation processes occurring in the environment.

Materials and methods

Strain and chemicals

The fungus used for lignocellulose biodegradation, *P. chrysosporium* CCTCC BKMF-1767, and the biosurfactant production strain, *Pseudomonas aeruginosa* CCTCC AB93066, were purchased from the China Center for Type Culture Collection (Wuhan, China). Fungal cultures for *P. chrysosporium* were maintained on potato dextrose agar (PDA) slants at 4°C, and then transferred to PDA plates and maintained at 37°C for 4 days. The spores on the agar surface were gently scraped and blended in the sterile distilled water as spore suspension. The spore concentration was measured and adjusted to 2.0×10^6 spores/ml. The *P. aeruginosa* strain was maintained as slants on peptone agar medium at 4°C and transferred monthly until use. All the chemicals used in this work were of analytical reagent grade.

Biosurfactant production, separation and purification

The rhamnolipid-producing strain used in this study was *P. aeruginosa* AB93066. For seed-culture, the strain from frozen stock was activated at 37°C for 24 h. After that, a single colony was taken from the slants, and transferred into 40 ml beef extract-peptone

liquid medium. The cultivation condition for the seed culture was 37°C, 200 rpm. After 24 h incubation, the seed culture (3% inoculum) was inoculated into four 1,000 ml flasks with 200 ml mineral salt medium (MSM) for liquid fermentation, respectively. The MSM contained 18.0 g l^{-1} glucose as carbon source. The composition of MSM was as follows: $NaNO_3$ (0.20%), KH_2PO_4 (0.15%), $Na_2HPO_4{\cdot}12H_2O$ (0.15%), $MgSO_4$ (0.10%), $FeSO_4{\cdot}7H_2O$ (0.001%), and pH was adjusted to 6.5 with NaOH. The culture temperature and agitation rate was 37°C and 200 rpm. After 48-h fermentation, the crude surfactant was extracted from the culture medium as described by Liu et al. (2006), and purified by column chromatography using the method of Yuan et al. (2007). The effluents containing the dirhamnolipid component as determined by thin layer chromatography (TLC) (Zeng et al. 2007a) were collected, and the dirhamnolipid was obtained after removing the solvent by vacuum evaporation at 40°C. The CMC of the dirhamnolipid was determined from a semilog plot of surface tension versus dirhamnolipid concentration, and it was calculated to be 0.0075%.

Solid state fermentation (SSF) conditions

Rice straw was obtained from Changsha, central south of China. It was air-dried and coarsely crushed into a size of 15 mm in length, and then milled to pass through 40-mesh screen. For SSF experiment, 15 g straw powder was used for each of the four treatments. One was moistened by substrate solution and designed as control, while the other three were moistened by substrate solutions containing 0.007, 0.021, and 0.042% dirhamnolipid, respectively. The substrate solution consisted of VB_1 (0.001 g l^{-1}), ammonium tartrate (0.2 g l^{-1}), $MgSO_4{\cdot}7H_2O$ (0.05 g l^{-1}), KNO_3 (1.5 g l^{-1}), glucose (1.0 g l^{-1}) and microelement solution (70 ml l^{-1}). The composition of microelement solution was as follows (in 1 l): NaCl 1.0 g, $CoCl_2{\cdot}6H_2O$ 0.18 g, $Na_2MoO_4{\cdot}2H_2O$ 0.01 g, $ZnSO_4{\cdot}7H_2O$ 0.1 g, $CaCl_2$ 0.1 g, $CuSO_4{\cdot}5H_2O$ 0.01 g, $MnSO_4{\cdot}H_2O$ 0.5 g, $FeSO_4{\cdot}7H_2O$ 0.1 g, $AlK(SO_4)_2{\cdot}12H_2O$ 0.01 g, $MgSO_4{\cdot}7H_2O$ 3.0 g, H_3BO_3 0.01 g, and 1.5 g of nitrilotriacetate. Each flask was autoclaved, and then 1.5 ml spore suspension was inoculated. The initial humidity was 80%. The fermentation experiments were performed at 30°C for 32 days.

Chemical and enzymes assay

The pH value was measured in 1:10 (w:v) sample-H_2O suspension with a PHSJ-3F laboratory pH meter (Leici Instrument, Shanghai, China). The suspension was shaken on a reciprocal shaker at 200 rpm for 60 min, and then centrifugated (8,000 rpm, 10 min). The supernatant was filtered through a 0.45 μm Millipore fillter paper (Millipore Corporation Bedford, MA 01730, USA). Then the filtrate was analyzed for water-soluble organic carbon (WSOC) using an infrared C analyser (TOC Analyzer 1010, Germany).

The LiP activity in the extracellular medium was assayed by monitoring the oxidation of veratryl alcohol to veratraldehyde at 25°C as indicated by an increase in $A_{310\ nm}$ (Liu et al. 2008). The 3.0 ml assay mixture contained 1.8 ml sodium tartarate (250 mmol l^{-1}, pH 3.0), 0.6 ml veratryl alcohol (10 mmol l^{-1}) and 0.6 ml enzyme sample. The reaction was initiated by the addition of 0.03 ml H_2O_2 (40 mmol l^{-1}). One unit of LiP activity was defined as the amount of the enzyme which led to the production of 1 μmol veratryl aldehyde from the oxidation of veratryl alcohol per minute. The activity of MnP was measured by monitoring the change in oxidation state of Mn^{2+} to Mn^{3+} at $A_{240\ nm}$ (Rogalski et al. 2006). The final reaction mixture contained 2.7 ml $MnSO_4$ (40 mmol l^{-1}) which was mixed with sodium tartarate buffer (at pH 4.5, 50 mmol l^{-1}) and 0.3 ml of enzyme sample. The reaction was initiated by adding 0.03 ml H_2O_2 (40 mmol l^{-1}). One unit of MnP activity was expressed as the amount of enzyme which led to the production of 1 μmol Mn^{3+} from the oxidation of Mn^{2+} per minute. Spectrophotometric measurements were performed with a UV–visible light spectrophotometer (model UV-2550, Shimadzu Company, Tokyo, Japan).

Fungal biomass estimation

One gram of SSF substrate was weighed and dispersed in 5 ml sterile distilled water. The samples were completely homogenized so that the whole fungal biomass got separated from lignocellulose material. The homogenized samples stood undisturbed so that the higher molecular weight lignocellulose material could be settled, while the fungal biomass in the supernatant was collected separately

by centrifugation at 8,000 rpm for 10 min. After that, the fungal biomass dried under vacuum condition and weighed.

Determination of lignin content

The lignin contents of the substrates were analyzed with Van Soest's method (Van Soest et al. 1991) by Foss Fibertec 2010 (Sweden). The lignin was estimated as the difference between aciddetergent lignin (ADL) and ash content. Lignin degrading ratio was calculated by the following formula:

$$R_n = \frac{m_0 - m_n}{m_0} \times 100\%$$

where R_n is the degrading ratio for the nth sampling, %; m_0 is the initial content of lignin, g; m_n is the nth sampling content of lignin, g.

Scanning electron microscopy (SEM) analysis

The treated straw samples with 0.007% dirhamnolipid and without biosurfactant from SSF after 32 days of biodelignification were initially dried using a vacuum freezing dryer. Scanning electron micrographs (SEM) of the selected samples were obtained with a JSM-6700F field emission scanning electron microscope (JEOL Ltd., Japan).

Fourier transform infrared (FTIR) spectroscopy

Perkin–Elmer infrared spectrophotometer (Spectrum 2000, Germany) was used to investigate the change in surface functional groups of the lignocellulosic substrate after SSF. The substrate samples were powdered and the measurements were carried on thin films between KBr plates. The spectral range varied from 4,000 to 450 cm^{-1}.

Statistical analysis

The effects of dirhamnolipid addition as well as time course of rice straw biodelignification data were subjected to variation partitioning analysis using the Canoco 4.5 software (Canoco for Windows 4.5, Netherlands). Variation partitioning analysis (Borcard et al. 1992) helps to display the variability of patterns, constrained by the factors of interest. It is proposed as a method based on Canonical Correspondence Analysis (CCA) for partitioning of the variation in a species-sample data matrix onto different sets of explanatory variables. This method does not only allow statistical testing, but it also provides a quantification of the variation explained by the different sets of variables. In this research, the results of detrended correspondence analysis (DCA) for total inertia in species indicated that the length of gradient was less than 3.000 (the first axes length of gradient was 0.590). Therefore, the data obtained from biodelignification of rice straw were submitted to redundancy analysis (RDA). The significance of the result was tested with the Monte Carlo permutation (using a maximum number of iterations of 999) test.

Results and discussion

Physicochemical change during biodelignification

The initial pH values were nearly 6.5 for all samples. It was shown by Fig. 1a that the similar patterns were found for pH values varying with time at four dirhamnolipid concentrations. In the first 12 days of biodelignification, pH of all samples increased sharply to about 8.5, and then, the pH values decreased after Day 12. The pH values of all samples are in the range of 5.5–9.0, which was in the optimum range for lignin degradation by *P. chrysosporium*. During the initial phase, the sharp increment of pH may attribute to the readily degradable organic nitrogen being decomposed and ammonia releasing. However, during the later phase, the pH values of all samples declined to 6.0 approximately, due to depletion of the readily degradable organic nitrogen and formation of oxalic acid (Chi and Yin 2007). The results indicated that the addition of biosurfactant did not affect pH values.

Meanwhile, it was shown by Fig. 1b that the WSOC contents of the four treatments all increase in the first 24 days and then decrease slightly. Treatments with dirhamnolipid were of higher WSOC contents than the control. 0.007% dirhamnolipid treatment got the highest WSOC content, which was 83.6% higher than that in the control. However, higher concentration of dirhamnolipid did not result in a higher increase of WSOC released.

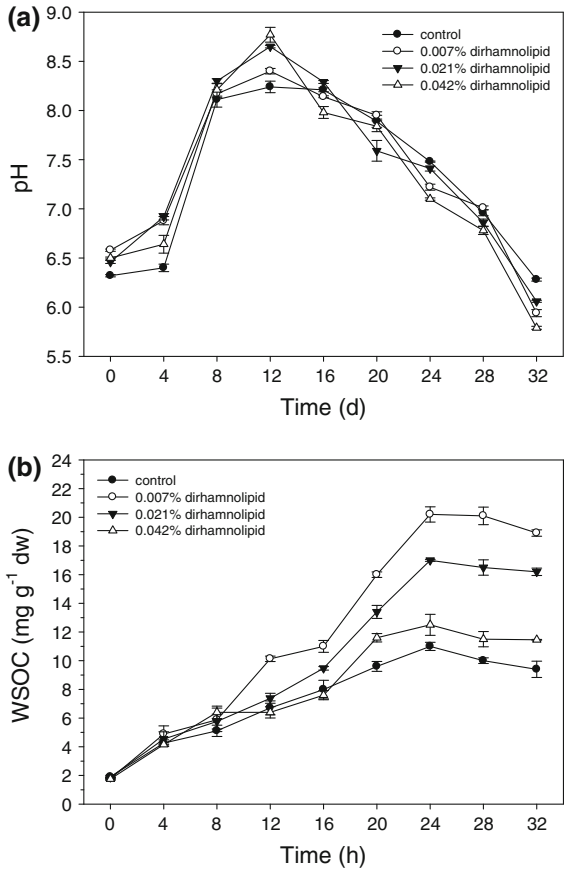

Fig. 1 Changes of pH (**a**) and water-soluble organic carbon (**b**) during biodelignification of rice straw. The *vertical bars* (may be in the symbol) designate the standard deviations for the mean of three replicative tests

Change of biomass during biodelignification

The *P. chrysosporium* biomass during the biodelignification is presented in Fig. 2. It was the lowest for the control during the whole SSF process, while the highest biomass was obtained in the presence of 0.042% dirhamnolipid. Biomass increased markedly during the first 24 days and then showed a declining trend in samples with dirhamnolipid. In the control, the biomass reached its peak value as 0.56 μg g^{-1} dw on Day 20 and decreased thereafter. The results indicated that the growth of *P. chrysosporium* was significantly affected by the addition of biosurfactant. The reason is probably that the biosurfactant had the effect on the water holding capacity of the substrate, which was caused by the biosurfactant wetting ability. Therefore, it could help impend water evaporation in the substrate, and consequently improve the growth of microorganism (Zhong et al. 2006). In addition, it was supposed that the dirhamnolipid was used as an available carbon source by *P. chrysosporium*. As a result, higher growth rate of the microorganism at the beginning was obtained by the increasing concentration of biosurfactant added. However, the *P. chrysosporium* biomass was reduced to different levels after 32 days of SSF due to the moisture content of the substrate decreasing and carbon source deficiency.

Effect of dirhamnolipid on LiP and MnP

The development of LiP activities during the biodelignification process is shown in Fig. 3a. The results indicates that dirhamnolipid of low concentrations could promote the LiP activity. In all samples the LiP activities reached the maximum values on Day 28. The maximum activities of LiP in 0.007% and 0.021% dirhamnolipid runs were 86 and 40% higher than that in the control, respectively. However, 0.042% of dirhamnolipid seemed to have no effect on the LiP activity. In Fig. 3b, the MnP activity of the four treatments all peaked at Day 24. The results indicated that dirhamnolipid had little stimulatory effect on MnP activity, which was in accordance with the results obtained by Liu et al. (2008). The results of the lignin-degrading peroxidases variations can be nevertheless well explained the results in Fig. 1b. As the trends of WSOC content were in accordance with

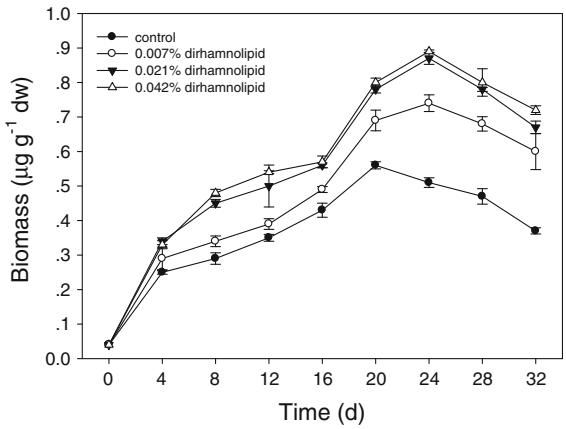

Fig. 2 Changes of biomass during biodelignification or rice straw. The *vertical bars* (may be in the symbol) designate the standard deviations for the mean of three replicative tests

the changes of LiP activity, it is supposed that the promotion of LiP activity might improve the lignin degradation which resulted in extra WSOC release. However, dirhamnolipid in the medium at different concentrations above critical micelle concentration (CMC) might provoke complex responses of the microbial cells (Sotirova et al. 2009). Meanwhile, the stimulatory effect on enzyme excreting might be inhibited at higher concentrations of surfactant. Whereas MnP secretion strongly depended on Mn^{2+} concentration (Brown et al. 1991) and gained no benefit from dirhamnolipid addition.

Change of lignin content

Lignin content decreased slightly in the initial stage (0–8 days), while dirhamnolipid took no effect on lignin degradation in this phase. As the distinct changes in WSOC and LiP in the liquid phase of the substrates, the increase of lignin biodegradation rates in the presence of dirhamnolipid were improved after Day 8 (Fig. 4). In the sample with 0.007% dirhamnolipid the lignin content decreased markedly, and the lignin degradation rate reached 40% on Day 32, which were the highest among the four treatments. Meanwhile, the lowest lignin degradation percentage of 26% was obtained in control sample. Several previous studies (Van der Meer et al. 1992; Fu et al. 2007) revealed that biosurfactant might enable the spreading of water in the pores, and then form the large area of water film covering the substrate surface, which resulted in strengthened mass transfer of oxygen. Elevated oxygen levels thus increase the rate of lignin biodegradation through the production of hydrogen peroxide as the extracellular oxidant and the subsequent induction of ligninolytic activity (Sánchez 2009). However, the results demonstrated that dirhamnolipid at concentrations above 0.007% had a low enhancing effect on the lignin degradation. This might attribute to the enzymes binding with biosurfactant at concentrations above CMC, which inhibited the efficient contact between enzyme and substrate. While the excessive biosurfactant addition makes the cellulose conversion process less economically feasible. As a result, the effect of biosurfactant at concentration of CMC showed the highest efficiency in improving the substrate degradation (Whang et al. 2008).

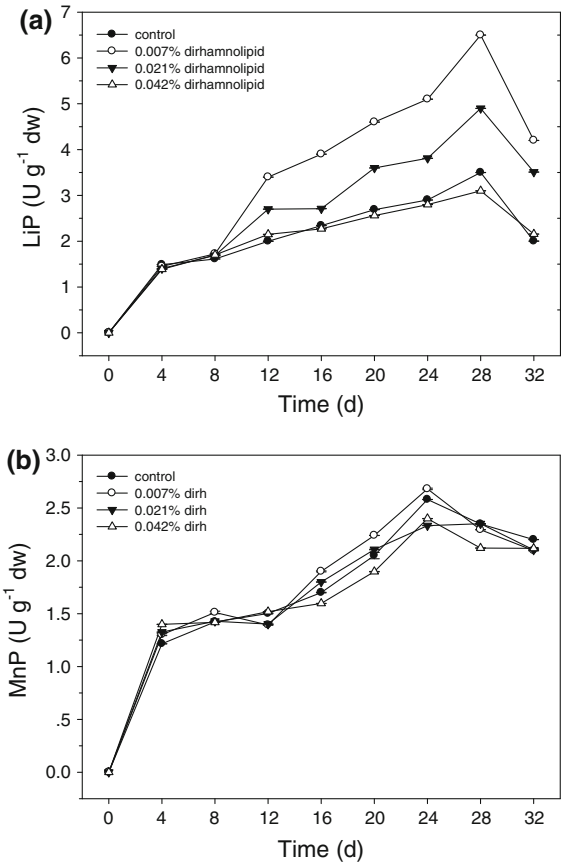

Fig. 3 Variation of LiP (**a**) and MnP (**b**) production during biodelignification of rice straw. The *vertical bars* (may be in the symbol) designate the standard deviations for the mean of three replicative tests

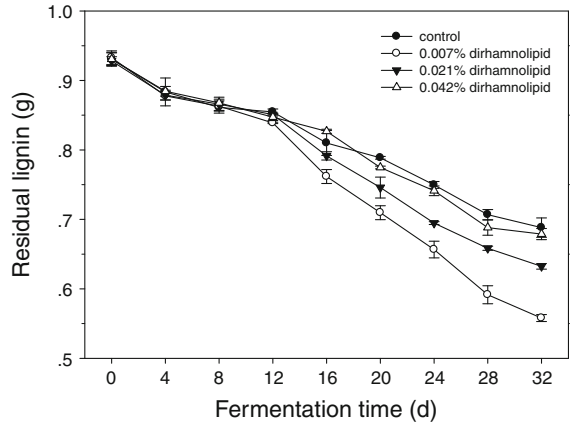

Fig. 4 The residual lignin content in rice straw during biodelignification. The *vertical bars* (may be in the symbol) designate the standard deviations for the mean of three replicative tests

Surface morphology of straw substrate

The SEM images of the substrate surface after 32 days of biodegradation with *P. chrysosporium* in the control sample and with 0.007% dirhamnolipid treatment are given in Fig. 5. There are noticeable concaves on the straw substrate surface in control sample (Fig. 5a), while the cellular fibres adhered to the residual lignin matrix are unfolded as firmly bonded. This is might be caused by the on-site degradation of the lignin layer by the *P. chrysosporium*. In previous studies, rhamnolipid molecules are supposed to reduce the adsorption of microorganism or enzyme onto the substrate (Eriksson et al. 2002; Zhong et al. 2007). Therefore, the mobility of the microorganism or enzyme in the substrate matrix was enhanced, and then the biofilm in deep pores in the substrate was formed. Meanwhile, most of the decomposition sites are supposed to transferred from the surface of the solid substrate to the liquid phase in the medium (Zhong et al. 2006). In the present research, on the surface of straw substrate treated with 0.007% dirhamnolipid, the terrace-like fragments separated from the inner cellular fibres of the substrate were broken (Fig. 5b), indicating that lignin pieces might break away from the surface to the liquid phase of the medium in substrate. As a result, the interrupted surface was observed, and then the detached pieces was supposed to be more easily removed and breakdown.

FTIR spectra

The FTIR spectra of the rice straw before and after biodegradation (without or with 0.007% dirhamnolipid) show the locations of similar peaks but the relative intensities of the absorbance changed during the process (Fig. 6). The interpretation of the recordings is based on the data of many studies (Suhas et al. 2007; Guo et al. 2008; Abouelwafa et al. 2008; Fu et al. 2009).

After biodegradation the band at 3,430 cm^{-1} is significantly narrower toward higher wavenumbers. The vibrancy frequency aliphatic C–H groups (3,000–2,900 cm^{-1}) was significantly decreased during biodegradation, and in the sample with 0.007% dirhamnolipid, it apperanced as a shoulder peak for spectra. The readily visible adsorption peaks at 1,640 and 1,450 cm^{-1} attributed to the aromatic skeletal C=C vibrations (Fu et al. 2009). Concerning the bands between 1,300 and 900 cm^{-1}, the decreases of C–O–C vibrations after biodegradation represented the syringyl and guaiacyl lignin structures may be destroyed. Meanwhile, it should be noticed that the dirhamnolipid at 0.007% caused obvious changes in the fingerprint spectrum (1,300–400 cm^{-1}) of the substrates. In the spectrum of treatment with 0.007% dirhamnolipid, out-of-plane bending vibration of benzene ring (900–650 cm^{-1}) was seen decreasing intensely, while at wavenumbers lower than 550 cm^{-1}, several new peaks were noticed. It is supposed that higher degree of delignification occurred with more simple compounds produced in the substrate with 0.007% dirhamnolipid treated. The qualitative structural modifications are quantitatively confirmed by the variations of the different absorption ratios (Table 1). While the biodelignification with 0.007% dirhamnolipid within the same fermentation time appears to be more efficient in improving the depolymerization than the control. The data

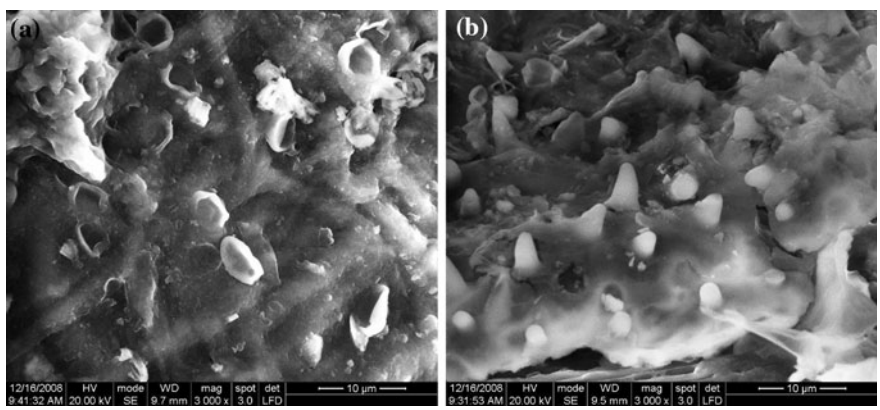

Fig. 5 Electron microscopic image of substrate surface morphology magnified 3000 × : **a** surface of rice straw after 32 days of biodegradation by *P. chrysosporium* without dirhamnolipid; **b** surface of rice straw after 32 days of biodegradation by *P. chrysosporium* in presence of 0.007% dirhamnolipid

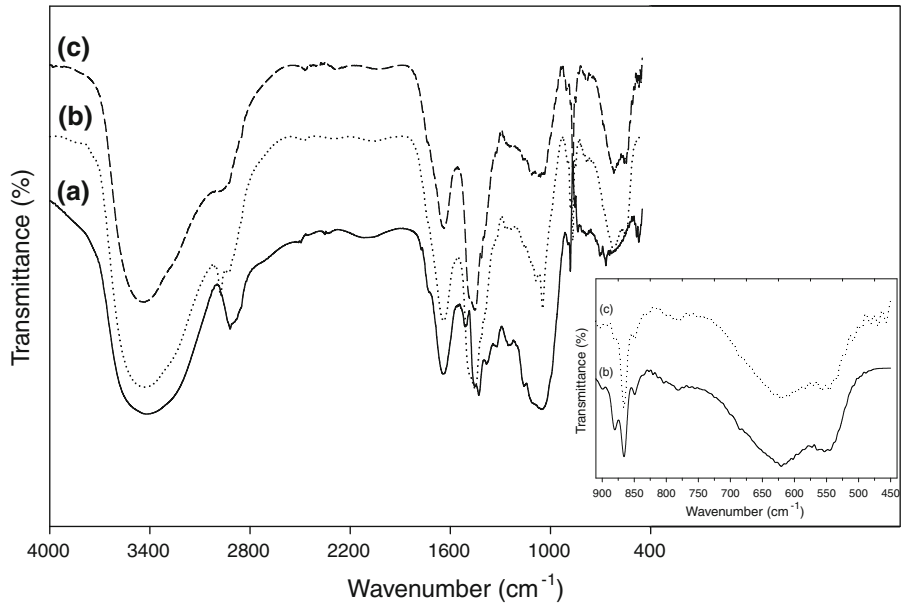

Fig. 6 FTIR spectra of substrate before and after biodelignification: **a** before biodelignification; **b** after 32 days of biodelignification with *P. chrysosporium* without dirhamnolipid; **c** after 32 days of biodelignification with *P. chrysosporium* in the presence of 0.007% dirhamnolipid

give an explanation for the above summarized suppositions.

Statistical analysis

The rice straw biodelignification efficiency altered with the different concentrations of dirhamnolipid addition. The biodelignification rate changed obviously during the test period, accompanied with the changes of physicochemical, biological and enzymatic parameters during the SSF. In order to investigate the effects of environmental factors on the characteristics of the straw biodelignification, the fermentation process parameters were analyzed by redundancy analysis (RDA), as shown in Fig. 7. The values on the axes indicate the percentage of total variation which they explain, while the variance decomposition of the RDA on the rice straw biodelignification profiles is shown as a bar diagram. The fermentation time actually explained 66.6% ($P = 0.001$) of the variance in the biodelignification parameters, while the different concentrations of dirhamnolipid addition explained 22.1% ($P = 0.013$), with a shared variance of 0.0% ($P = 0.001$). In addition, the differences in biodelignification parameters were less pronounced between 8 and 16 days, whereas the parameters at Day 32 differed strongly from that of the earlier sampling dates.

Table 1 Variations of the different absorption ratios from FTIR

Samples	I_{3430}/I_{1450}	I_{2920}/I_{1450}	I_{1640}/I_{1450}	I_{1385}/I_{1450}	I_{1326}/I_{1450}	I_{1250}/I_{1450}	I_{1160}/I_{1450}	I_{1085}/I_{1450}	I_{1040}/I_{1450}	I_{881}/I_{1450}
a	1.53	0.45	0.74	0.66	0.55	0.54	0.87	1.30	1.33	0.24
b	0.97	0.28	0.36	0.32	0.15	0.13	0.16	0.23	0.32	0.10
c	0.81	0.19	0.31	0.30	0.13	0.12	0.14	0.17	0.17	0.06

The values in the table correspond to the relative ratios of the intensity of indicated FTIR absorption peaks compare with the adsorption at 1,450 cm^{-1} wavenumbers of each

a, Straw before biodegradation

b, Straw after biodelignification without surfactants

c, Straw after biodelignification with 0.007% dirhamnolipid

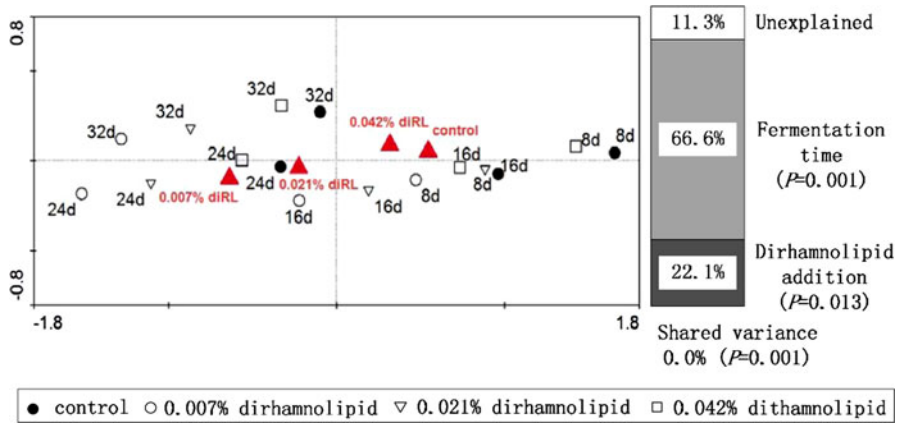

Fig. 7 Redundancy analysis (RDA) of rice straw biodelignification profiles for different contentration of dirhamnolipid addition and solid state fermentation time during the experiment. Time 8, 16, 24, 32 days and dirhamnolipid at 0.0, 0.007, 0.021, 0.042% concentration are used as qualitative explanatory variables (*centroids*)

However, the significance of all dirhamnolipid affecting canonical axes summaried by 999 permutations of Monte Carlo test revealed that $P = 0.013$, which indicated that the effect of dirhamnolipid was significant. From the RDA biplots, it is shown that 0.007% dirhamnolipid played the most important role among the three concentrations of which significantly affected the variation of the straw biodelignification. However, 11.3% of the variance in the biodelignification parameters data remained unexplained. Further research in more microenvironment of lignin biodegradation conditions needs to be undertaken in order to determine these other factors.

Conclusions

The dirhamnolipid speeded up the degradation of lignin, and improved the microbial biomass, LiP activity and WSOC release during the SSF. While the peak activity of LiP was increased by 86% and the degradation rate of lignin was increased by 54% with dirhamnolipid of 0.007%. The addition of dirhamnolipid improved the growth of *P. chrysosporium*, and resulted in extra WSOC release. However, the dirhamnolipid did not affect the pH system and the MnP activity. In the presence of dirhamnolipid, the rice straw substrates were supposed to be delignified with the formation of terrace-like fragments separated from the inner cellular fibres. The enhanced biodelignification may result in the production of more simple compounds. Though the fermentation time strongly affected the process characteristics, dirhamnolipid at 0.007% was statistically significant as explaining the variance of straw biodelignification parameters.

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