Pollutant Characteristics from Wastewater of Poplar Pre-conditioning Refiner Chemical Alkaline Peroxide Mechanical Pulping Pretreated with *Phanerochaete chrysosporium*

Zong-Zheng Yang,[a] Ya-Nan Guo,[a] Ai-Lin Liu,[b] Jie Zhang,[b] Ming-Yue Wang,[b] Ying Liu,[a] Xiao-Le Cao,[a] Zhi-Guo Wu,[a] Li-Ya Wu,[a] Chun-Hu Wang,[a] and Jing-Guo Cao [b,*]

The compositions of the organic pollutants of chemi-mechanical pulping wastewater were studied using Fourier transform infrared spectra and gas chromatography-mass spectrometry. The chemical oxygen demand (COD) was approximately 18110 mg/L, and the ratio of biological oxygen demand to COD (BOD_5/COD) was approximately 0.17, due to low biodegradability. In this study, *Phanerochaete chrysosporium* was selected and the changes in bacterial culture time required to transform pulping wastewater and the metabolites present before and after pretreatment in the wastewater were identified. A decline in the components of the pulping wastewater was observed. Initially, 43 kinds of organic compounds were detected in the raw water. After 3 days of treatment, only 21 organic compounds were detected. Then, 7 days later, the organic pollutants in the waste amounted to only 7 organic compounds. Through the analysis of analysis of P-RC APMP pulping wastewater and that treated by *Phanerochaete chrysosporium* using GC-MS, the relative content of organic acids was 12.4%, in which phenylpropionic acid had the highest concentration of 11.3%. So, in the culture medium, phenylpropionic acid as the single carbon source can be completely degraded within 96 h.

Keywords: Poplar; Pulping wastewater; Phanerochaete chrysosporium

Contact information: a: College of Chemical Engineering and Materials Science, Tianjin University of Science and Technology, Tianjin 300457, China; b: College of Marine and Environmental Sciences, Tianjin University of Science and Technology, Tianjin 300457, China;
** Corresponding author: cjg@tust.edu.cn*

INTRODUCTION

With the rapid development of the paper industry, the output of pulping waste liquid and the content of pollutants are increasing year by year. Pulp and paper has become one of the industries with the largest emissions of organic pollutants in China. Therefore, the treatment of slurry waste liquid has become an issue of great concern. There are many kinds of treatment technologies for pulping waste liquid and they are developing continuously. Chemi-mechanical pulping has high yield, and the waste liquid has low calorific value and solid content. The acid precipitation method and advanced oxidation method consume excessive amounts of chemicals and cost too much to operate. In contrast, the biological method has high efficiency and low cost, so it is widely used at present.

Pulp wastewater contains a large number of refractory organic pollutants, such as lignin, hemicelluloses, fine cellulose, and fine cellulose derivatives. The degradation of these organic materials is the result of the interaction of fungi, bacteria, actinomycetes, or corresponding microbial communities, in which the fungi play a leading role (Cacchio *et al.* 2001). The ability of the actinomycetes to degrade is followed by that of the fungi, and the bacteria has the weakest capacity for degradation. Several fungi, such as white rot fungi, brown rot fungi, and soft rot fungi, can degrade lignocellulose. However, white rot fungi are the most effective and most important microorganisms that can completely degrade lignin into CO_2 and H_2O. The main enzymes of degradation are H_2O_2 oxidase, peroxidase with H_2O_2 as a receptor, lactase, reductase, and other enzymes (Wu *et al.* 2005).

The rapid development of cutting-edge analysis technology provides a powerful tool for the detection and identification of toxic organic pollutants in wastewater. Four kinds of white rot fungi were screened to degrade poplar lignin; in order to elucidate the degradation mechanism of poplar lignin by white rot fungi, gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) were employed to detect the changes of functional groups in lignin (Han *et al.* 2008). The fermentation product made from cotton pulp and alkali lignin was degraded by *Phanerochaete chrysosporium* (*Pleurotus ostreatus*) and the degradation products were then systematically analyzed by means of infrared spectroscopy and GC-MS (Lihong *et al.* 2013). Pre-conditioning liquor from refiner chemical alkaline peroxide mechanical pulp (P-RC APMP) has complex components and high organic matter content.

The present work attempts to select *Phanerochaete chrysosporium* as a pretreatment strain for pulping waste and use GC-MS and other methods to determine the composition of organic pollutants in poplar P-RC APMP pulping wastewater during biological treatment. Additionally, this study aimed to use a single specific organic substance to analyze metabolites during degradation. It provides theoretical basis and practical guidance for the deep treatment of poplar chemical mechanical pulping waste liquid and the removal of refractory organic matter in wastewater. At the same time, a new approach was explored for the research of resource utilization

EXPERIMENTAL

Materials

The samples were collected during the preimpregnation, defibrination, and screw extrusion processes from poplar P-RC APMP in a paper factory in the Shandong province in Yanzhou, China.

Phanerochaete chrysosporium (No. 40719) was purchased from the China Industrial Microbial Culture Collection Management Center (Beijing, China).

Chemicals

Potato dextrose agar (PDA) medium contained: potato extract: 1.0 L; glucose: 20.0 g; dipotassium hydrogen phosphate (KH_2PO_4): 3.0 g; magnesium sulfate ($MgSO_4$): 1.5 g; yeast extract: 0.1 g; and agar: 20 g.

Fermentation medium contained: sterile pulping waste (100 mL) and sterilized waste liquid (100 mL). These were stored in cone-shaped bottles, respectively, until use.

The mineral salts medium (MSM) contained (g/L): ammonia sulfate [$(NH_4)_2SO_4$]:1; sodium chloride (NaCl):1; dipotassium hydrogen phosphate (K_2HPO_4):1.5; dipotassium hydrogen phosphate (KH_2PO_4):0.2; magnesium sulfate ($MgSO_4$): 0.2; and phenylpropionic acid (carbon source):0.5.

The above three kinds of medium had a pH of 7.0. Then high-pressure steam sterilization was done for 20 min under the condition of 121 ℃. For solid media, another 2% of agar was used. All chemicals used in this study were of the highest analytical grade available and supplied by Sinopharm Chemical Reagent Co., Ltd. (SCRC; Beijing, China).

Methods

The compositions and transformed products of wastewater were analyzed *via* gas chromatography with mass spectrometry (GC-MS). The pulping wastewater was extracted using ethylene chloride and diethyl ether. The organic phase was then extracted using quantitative ethyl acetate and dried in a vacuum dryer. Finally, the extracted samples were analyzed using a MS4000 GC-MS device (Varian/Agilent, Santa Clara, CA, USA; Gao *et al.* 2002).

Cultivation and domestication of strains

Phanerochaete chrysosporium was stored on a refrigerated slope under the condition of 4 ℃. It was taken out and inoculated in the PDA solid medium. The bacteria were activated by culturing them at 30 ℃. After that, the bacteria were inoculated in the liquid PDA medium to prepare the seed solution.

In order to enhance the tolerance and degradation ability of *Phanerochaete chrysosporium* to pollutants contained in pulping waste liquor, the *Phanerochaete chrysosporium* was inoculated in a 250 mL conical bottle containing ten times diluted 100 mL waste, which was cultured at 30 ℃ constant temperature in a rocker. Four days later the bacteria grown in the liquid were inoculated into the solid medium, then cultured again at 37 ℃ for 4 days. The above operation was repeated, gradually increasing the concentration of pulping waste liquor, and finally obtaining the acclimated strain.

The bacteria were washed 3 to 4 times with sterile water, then filtered to retain the sediment. The sediment was then added to the fermentation medium and cultured for 4 days in the condition of 30 °C, 150 revolutions per minute. The bacteria after cultivation can be used to evaluate the characteristics of the waste liquor after biological treatment.

Component analysis of pulping wastewater treated and untreated by Phanerochaete chrysosporium.

The water samples were centrifuged at 6500 r/min for 15 min, and the supernatant was used to detect the indexes of pollutant load in the wastewater. The chemical oxygen demand (COD), biochemical oxygen demand (BOD), amount of total soluble solids, amount of soluble organic matter, amount of ammonia nitrogen, total phosphorus, total sugar, and reducing sugar content were measured using the analytical methods in *Water and Wastewater Monitoring and Analysis Methods* (4^{th} Edition) (Guo *et al.* 2002). The acid-soluble lignin content was measured at 250 nm (Duan 2010). The SiO_2 content was investigated using the weight method (Ke *et al.* 1992), and the extracted hemicellulose was measured using ethanol precipitation (Jiying and Huiren 2010).

Degradation of phenylpropionic acid by Phanerochaete chrysosporium

The cultured strain was added into a mineral medium to evaluate the ability of the *Phanerochaete chrysosporium* to degrade phenylpropionic acid during 6 days time, and samples were taken to analyze the degradation of phenylpropionic acid with *Phanerochaete chrysosporium* at 0 h, 24 h, 72 h, 96 h, and 144 h, respectively.

Following degradation, the solution (10 mL) was taken and centrifuged at 6500 r/min for 15 min. An Agilent 1260 Infinity liquid chromatograph (Kunjianjiecheng, Beijing, China) with a UV detector (Puxi, Beijing, China) was used to analyze the phenylpropionic acid qualitatively and quantitatively.

Detection of the products transformed from phenylpropionic acid

The fermentation broth was collected from the culture of the sole carbon source of phenylpropionic acid to detect the metabolites of the fungi.

The liquid methyl esterification and extraction steps were as follows (Liu *et al.* 2014): The filtered sample (40 mL) was dried *via* rotary evaporation. The residue was dissolved in 5 mL of methyl esterification reagent (methanol (CH_3OH) and sulfuric acid (H_2SO_4); volume ratio = 10:1), mixed vigorously for 2 min, heated in a water bath for 4 h at 55 °C, and then cooled for 12 h. A 5 mL quantity of n-hexane was then added, and the contents were vortexed for 2 min. The materials were then allowed to stand until divided into two phases. The organic phase extracts were collected, and the residual water was removed and dried over anhydrous sodium sulfate. Finally, GC-MS analysis was conducted (Arora and Dandhu 1984).

RESULTS AND DISCUSSION

Analysis of Pulp Wastewater Composition

Comparison of treated and untreated pulp wastewater

Table 1 shows the change in the P-RC APMP pulping wastewater treated by *Phanerochaete chrysosporium*, in which COD declined 62% and BOD_5 slightly increased after biological treatment. This can be caused by the fact that when high amounts of phenols were present in the wastewater, the environment was toxic. Not many microbes could survive and consume the carbon sources, leading to lower BOD. When white rot fungi consume the phenols, they not only convert phenols into other compounds, but they also reduce the toxicity of the wastewater. Thus, the microbes could thrive, and the BOD was higher (Jönsson *et al.* 2013). After the biological treatment, the content of various components in the wastewater decreased to different extents. Among them, the degradation rate of reducing sugar was the highest at 42%, which indicated that the reducing sugar in the wastewater was used as a carbon source during the growth of the *Phanerochaete chrysosporium*. Moreover, the reduction rate of total sugar consumption was 26%, which suggests that *Phanerochaete chrysosporium* could make full use of wastewater monosaccharides and oligosaccharides. The removal rate of lignin in wastewater was 31%, and the concentration of hemicelluloses also slightly decreased. It has been demonstrated that *Phanerochaete chrysosporium* also secrete hemicelluloses-degrading enzymes while secreting lignin-degrading enzymes (Xuezhi *et al.* 2005). In addition, the concentration of ammonia nitrogen in the wastewater also decreased, showing that the pulp wastewater could be used as a nitrogen source for the growth of *Phanerochaete chrysosporium*. Finally, the results indicated that the organic matter of P-

RC APMP pulping wastewater had been removed to some extent after treatment by *Phanerochaete chrysosporium*.

Table 1. Component Analysis of Pulping Wastewater Treated and Untreated by *Phanerochaete chrysosporium*

Parameter (mg/L)	Untreated	Treated	Parameter (mg/L)	Untreated	Treated
COD (mg/L)	8728	3266	Reducing sugar (mg/L)	519.68	300.6
BOD5 (mg/L)	2800	2960	Total sugar (mg/L)	710.32	514.62
B/C	0.32	0.90	Acid-soluble lignin (g/L)	0.42	0.29
Total solid (g/L)	9.8	7.24	Hemicelluloses (g/L)	10.13	7.47
Inorganic (g/L)	3	2.86	Total phosphorus (mg/L)	27.92	39.2
SiO_2 (g/L)	0.05	0.04	Ammonia nitrogen (mg/L)	44.58	29.75

Analysis of Treated and Untreated Pulping Wastewater Composition with *Phanerochaete chrysosporium*

Gas chromatography mass spectrometry was used to analyze the organic compositions of the P-RC APMP pulping wastewater treated and untreated with *Phanerochaete chrysosporium*. Figure 1 shows the total ion chromatogram of the organic matter extracted from the wastewater by liquid-liquid extraction with dichloromethane and ether. The structure and formulas of the components were confirmed by the GC-MS processing system. The results are shown in Table 2.

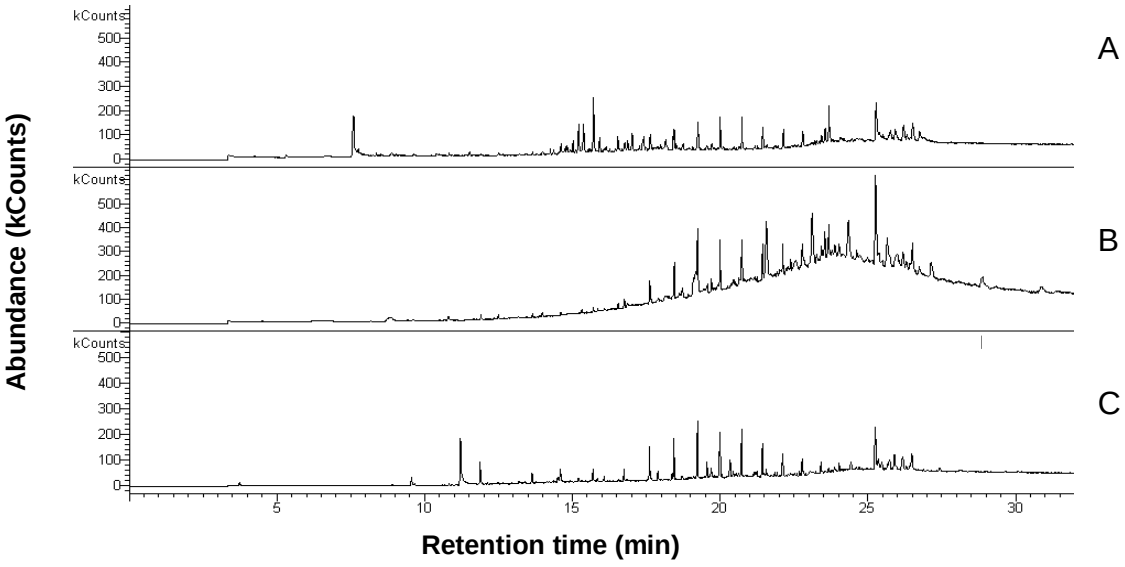

Fig. 1. GC-MS chromatogram of Poplar P-RC APMP pulping wastewater treated and not treated by *Phanerochaete chrysosporium*: A: Pulping wastewater; B: Pulping wastewater treated for 3 days by *Phanerochaete chrysosporium*; and C: Pulping wastewater treated for 7 days by *Phanerochaete chrysosporium.*

Table 2. Analysis of P-RC APMP Pulping Wastewater and that Treated by *Phanerochaete chrysosporium* Using GC-MS

Chemical Type	Stay Time (min)	Organic Formula	Organic Name	Relative Content (%)		
				Pulping Waste	Biotransformation 3 d	Biotransformation 7 d
Acid	5.314	C7H14O2	N-heptanoic acid	0.706	-	-
	7.586	C9H10O2	Phenylpropionic acid	11.29	-	-
	10.391	C12H16O2	2, 5-dimethylbenzene butyric acid	0.373	-	-
	16.262	C21H28O2	1, 2, 3, 4, 4a, 10a-hexahydro-1,4a-dimethyl-7- (1-methyl) -1-rosin acid	0.354	-	-
	26.209	C17H26O3	(4-nonylphenoxy) acetic acid	4.46	4.461	0.471
	26.755	C29H46O	Cardamom-3,5-dien-7-one	2.33	3.353	-
Ketone	8.370	C9H8O2	3-isochromanone	0.332	-	-
	16.872	C16H20O	3, 6, 7, 8-tetrahydro-3, 3, 6, 6-tetramethyl-1(2H) -one	1.641	-	-
	25.924	C30H48O	Feather ketene	2.015	0.738	0.257
Phenols	9.551	C10H15NO	4- (2-dimethylaminoethyl) phenol	-	-	1.603
	10.926	C16H26O2	2-methyl-5-nonyl-1, 3-benzenediol	-	-	0.067
	14.760	C18H24O	4-[(1E,3S)-3- vinyl -3,7- dimethyl -1,6- octadiene] - phenol	0.421	-	-
	15.925	C18H22O	4-sec-butyl-2- (A-methyl benzyl) phenol	1.744	-	-
	17.416	C18H20O2	3,5,5 (10), 9 (11) -tetraene-17-carbonyl-phenol	0.225	-	-
	18.159	C19H24O	2-(1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl) phenol	1.062	0.317	0.094

Chemical Type	Stay Time (min)	Organic Formula	Organic Name	Relative Content (%)		
				Pulping Waste	Biotransformation 3 d	Biotransformation 7 d
Furan	11.528	C15H26O	Dihedron-incense furan	0.852	0.062	-
	17.016	C17H16O2	3-(4- methoxyphenyl)-2,6- dimethyl - benzofuran	2.779	-	-
Amine	11.205	C12H11N	Diphenylamine	-	-	16
	17.898	C22H39N	4-hexadecylaniline	-	-	1.712
	22.689	C28H43N	4, 4'-dioctyldiphenylamine	-	-	0.947
Alkene	14.254	C19H28	Androst-2, 16-diene	0.718	-	-
	15.377	C19H26	Heptyl -3- phenyl -1,4-cyclohexadiene	3.219	-	-
	19.249	C22H44	1-dodecylene	3.926	8.595	10.75
	20.331	C20H40	(E) -3-ethylenes	-	-	4.296
Ester	15.026	C21H30O2	Dehydroabietic acid methyl ester	1.445	-	-
	15.541	C21H32O2	Methyl phthalate	0.324	-	-
	15.719	C21H28O2	6,8,11,13-Abietatetrene-18-oic acid methyl ester	9.122	0.894	2.789
	16.101	C17H16O4	1,4-dihydro-3(3-methyl-2-butenyl)-1,4-dicarbonyl-2-naphthoic acid methyl ester	0.267	-	1.576
Phenanthrene	14.363	C19H28	7β-dimethyl-1-methylene-7α-ethyl-alkenyl-phenanthrene	0.398	-	0.217
	15.601	C19H28	7-vinyl- , 2, 3, 4, 4a, 5, 6, 7, 8, 10, 10a dodecahydro-4a, 7-dimethylphenol	0.356	-	0.107
	16.144	C18H22	1, 7-dimethylphenol	0.356	-	-
	17.275	C18H18	1-methyl-7- (1-methylethyl) - phenanthrene	0.59	-	-

Chemical Type	Stay Time (min)	Organic Formula	Organic Name	Relative Content (%)		
				Pulping Waste	Biotransformation 3 d	Biotransformation 7 d
Alkane	16.456	C18H22	3, 3 ', 4,4'-tetramethyldiphenyl ethane	0.409	-	-
	17.630	C17H36	2, 6, 10-trimethyl tetrad cane	2.832	3.269	5.619
	20.010	C17H36	Heptadecane	4.47	8.831	9.959
	20.737	C18H38	Octadecane	3.542	5.608	8.659
	21.440	C20H42	Eicosane	3.135	4.974	6.782
	22.136	C27H56	Heptadecane	2.977	4.767	5.145
	22.793	C28H58	Octadecane	2.284	3.043	2.807
Alcohol	18.517	C20H30O	1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-(1S,4aS,10aR)1-docosene	0.797	0.6	-
	23.554	C27H44O	Cholesta-4, 6-diene-3B-alcohol	2.502	4.428	0.098
	23.682	C27H44O	(3B) -cholesta-4, 6-dien-3-ol	6.505	8.457	0.921
	24.426	C28H44O	Ergo sterol	-	-	0.309
	25.277	C29H50O	(3b, 24S) -standoster-5-en-3-ol	7.839	23.69	13.03
	26.526	C31H52O	24-methylene-,(3b)-9,19-Cyclolanostan-3-ol	4.586	6.821	0.432
Alse	11.886	C10H15NO2	Ethyl benzene	-	-	4.86
	15.217	C18H24	1, 2, 3, 7, 8, 9, 10, 11, 12, 12a dodecahydrofen	3.052	-	0.155
	16.542	C17H17N	9-butylacridine	1.818	0.861	0.081
	17.389	C18H20O2	Fenaproxone	0.59	-	-
	17.487	C18H18	(Cyclopentylphenylmethyl) - benzene	0.366	0.107	-
	18.415	C20H26	1,2,3,3a,4,5,6,7,8,9,9a,10,11,12-tetradecylperylene	0.989	6.122	0.257

As shown by Fig. 1 and Table 2, the composition of the wastewater was very complex, containing 43 organic substances, before treatment with *Phanerochaete chrysosporium*. The main organic pollutants included esters, phenols, alkenes, ketenes, alcohols, and organic acids, in which the relative content of alkenes was 19.2%. Three Environmental Protection Agency (EPA)-specific pollutants were detected (Guan *et al.* 2010), namely octadecane, eicosane, and octacosane. Additionally, the relative content of aromatic compounds calculated from the peak area reached 56.5%, and the relative content of the three phenols detected from aromatic compounds was 1.71%. The relative content of organic acids was 12.4%, in which phenylpropionic acid had the highest concentration of 11.3%. The content of alcohols was 23.1%. In addition, benzene, ketenes, furan, olefins, acridine or its derivatives, and other organic pollutants were also detected.

The biological treatment was expected to considerably reduce organic matter content. After 3 days of biotransformation, the number of organic species in the raw water decreased from 43 to 21. Additionally, the area and intensity of the chromatographic peak also decreased, which indicated that the biological treatment effectively removed the organic matter from the wastewater. The relative content of aromatic compounds after treatment was remarkably reduced by 41.2%. The relative contents of organic acids and phenols were completely degraded. The relative content of alcohols was 44.1%, which was 21.0% higher than that of raw water. Furthermore, the contents of (3b) -penta-4,6-dien-3-ol, and (3b, 24S) -ostan-5-en-3-ol obviously increased. In addition, the relative content of alkenes and olefins increased, with the relative content of alkenes increasing from 19.2% to 30.5%.

After 7 days of biotransformation, the number of organic pollutants detected in pulping wastewater was 14, which was lower than the amount detected prior to biological treatment and higher than the amount detected after 3 days of biotransformation. Compared to raw water, the compound peak area decreased 13.5%. Two additional effluent phenol compounds and aniline were found, with the contents of 6.5% and 18.7%, respectively. This occurrence was probably caused by incomplete organic extraction or an inappropriate detection boundary. Moreover, the aniline organic matter was not detected in the original wastewater but was detected in the wastewater after the biological treatment. Three aniline substances were detected after 7 days of transformation. Additionally, a 19.7% increase in alkenes was also detected due to the degradation of aromatic compounds in waste liquid by laccase secreted by white-rot fungi (Chagas and Durrant 2001; Bajpai 2012), so that the groups on the benzene ring or side chain during the degradation process may be ring-opened to form alkenes or smaller molecular weight organic compounds such as alcohols, aldehydes, olefins, *etc*. The relative content of organic acids was greatly reduced, and some of the fatty acids and aromatic acids were lower than the detection range of the instrument. The relative contents of ketones, esters, and alcohols were slightly decreased, while the relative contents of olefins were increased. This indicated that the *Phanerochaete chrysosporium* greatly reduced the organic pollutants in wastewater treatment, which reduced the pollution load. Furthermore, the macromolecules in the degradation process of organic matter were degraded to other small organic molecules or converted into other types of organics.

Degradation of Phenylpropionate Acid by *Phanerochaete chrysosporium*

According to results of the GC-MS analysis, phenylpropionic acid was a major substance in all of the components, which has rarely been reported in previous literature on the degradation and metabolism of phenylpropionic acid. Therefore, further study of the metabolic process of phenylpropionic acid could provide a theoretical basis for the future treatment of *Phanerochaete chrysosporium* with pulping waste.

The degradation of phenylpropionic acid by *Phanerochaete chrysosporium* with phenylpropionic acid as the sole carbon source was examined, and the results are shown in Fig. 2.

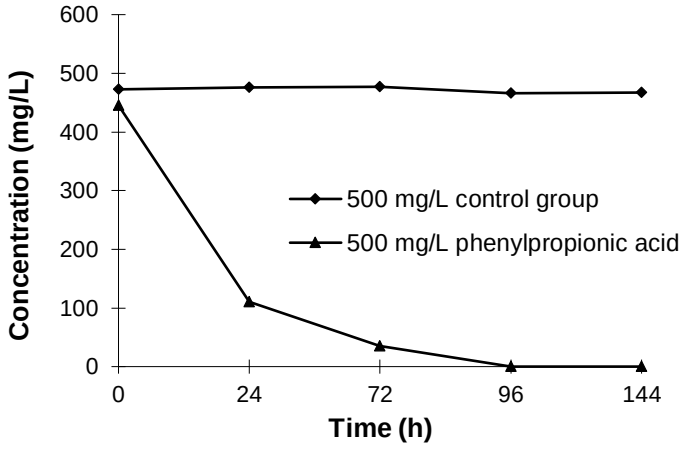

Fig. 2. Biodegradation of phenylpropionic acid by *Phanerochaete chrysosporium*

Figure 2 shows that the carbon source was completely consumed during the treatment. After a reaction duration of 96 h, the concentration of phenylpropionic acid was beneath the detection limit, indicating that it had completely degraded. During the first 24 h, the concentration of phenylpropionic acid rapidly declined, and then it declined slowly. Finally, phenylpropionic acid was totally consumed after 96 h, which showed that *Phanerochaete chrysosporium* completely degraded the phenylpropionic acid.

Identification of Transformed Product from Phenylpropionic Acid

To understand the different steps involved in the degradation of phenylpropionic acid, it was essential to determine the product formed during the degradation process. Therefore, the authors analyzed the metabolites of a phenylpropionic acid fermentation broth under different cultivation times. The results of the GC-MS are shown in Fig. 3.

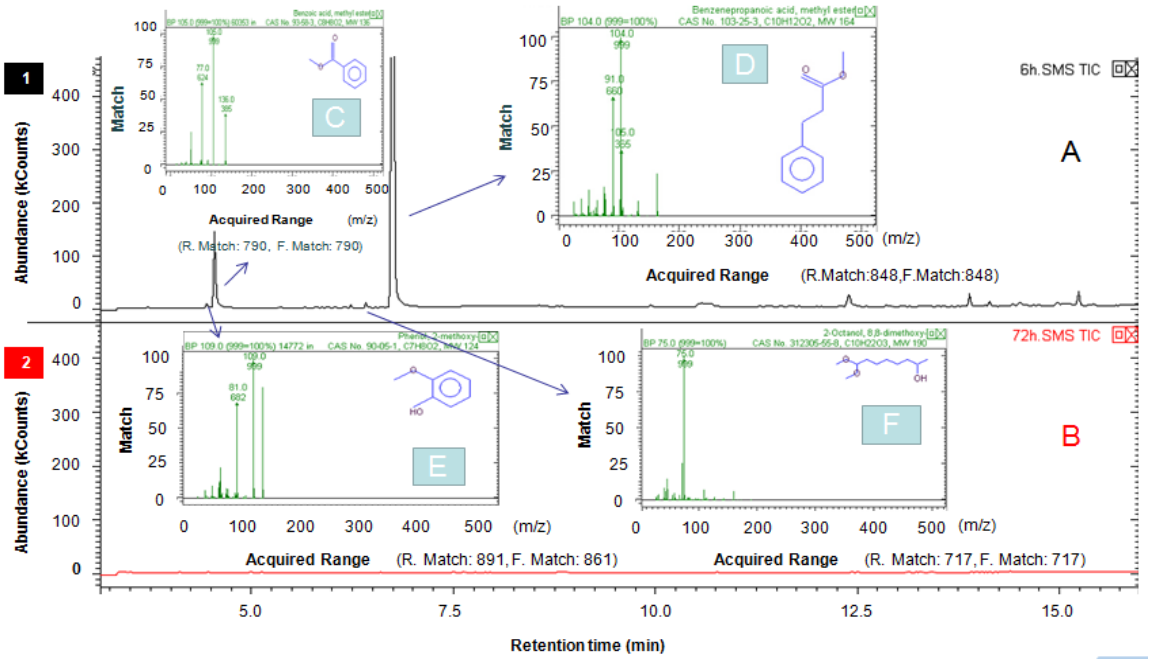

Fig. 3. GC-MS analysis of phenylpropionic acid treated and untreated by phanerochaete chrysosporium; (A: phenylpropionic acid was treated 6 h; B: phenylpropionic acid was treated 96 h; C: Methyl benzoate; D: Methyl phenylpropionic acid; E: 2-methoxy-phenol; and F: 8, 8-dimethoxy-2-Octanol)

As shown in Fig. 3, after culturing for 6 h, three major degradation products were revealed. The retention times were 4.44 min, 4.62 min, and 6.24 min, and the corresponding mass peaks were 124, 136, and 190, respectively. The authors speculated that the product could be 2-methoxy-pheno, benzoic acid, or 8,8-dimethoxy-2-octanol according to the mass spectrum. The similarities of the three organic matters with the standards in the GC-MS mass spectrum were 90%, 80%, and over 70%, respectively. In addition, the retention time of phenylpropionic acid was determined as 6.762 min. When the reaction time was extended to 96 h, these metabolites were completely degraded. Combining the knowledge that dioxygenase catalyzes the ortho-metabolism of benzoic acid (Kirk and Shimada 1985; Shimoni *et al.* 2003), the degradation process of *Phanerochaete chrysosporium* with phenylpropionic acid as the sole substrate was analyzed. It can be speculated that phenylpropionic acid was converted to benzoic acid *via* side chain cleavage, then p/o-methoxyphenol was formed next, which was followed by the conversion of p/o-methoxyphenol to p/o-hydroxyphenol *via* demethylation. Then, 8,8-dimethoxy-2-octanol or another similar straight chain alcohol was formed by the ring-opening reaction of benzene ring, and the tricarboxylic acid cycle finally began. The benzene ring is known to open due to catechol cracking (Hongman *et al.* 2003). This metabolic process occurred through the *Phanerochaete chrysosporium* first catalyzing the benzene ring side chain and open-loop cracking into benzene-free organic matter until the degradation was completed (Krings *et al.* 2001; Chen *et al.* 2011).

CONCLUSIONS

1. The *Phanerochaete chrysosporium* treatment effectively removed various components in pulping wastewater. The total removal rate of COD in the wastewater reached 62%. The rate of reducing sugar degradation, the total sugar consumption, and the acid-soluble lignin in waste liquid was approximately 42%, 26%, and 31%, respectively, and the hemicellulose content was also slightly reduced. In addition, the content of ammonia and nitrogen in the wastewater decreased, whereas the total phosphorus content increased 12.6 mg/L compared to the content of the raw water. These findings were explained by the fact that the organ phosphorus in the wastewater was converted to inorganic phosphorus after *Phanerochaete chrysosporium* treatment, increasing the total phosphorus content.
2. As indicated by the GC-MS results, the composition of the pulping water was quite complicated. The organic matter content decreased from 43 to 21 after 3 days of treatment. After 7 days, the type of organic pollutants in the wastewater was reduced by 14 before treatment. After the biological treatment of pulping spent water, the organic species were largely removed. The relative content of the aromatic compound peak area was 43.0%, which was 13.5% less than before treatment, and the relative content of the alkenes increased 19.7%. The relative content of ketenes, esters, and alcohols also slightly decreased and the relative content of olefins increased. It can be seen that the treatment of *Phanerochaete chrysosporium* effectively removed the organic pollutants in the wastewater and reduced the pollution load. Further, organic matter with macromolecules was degraded into other small molecular organic compounds or converted into other kinds of organic matter during the degradation process.
3. As the single carbon source, the phenylpropionic acid was completely degraded within 96 h during the *Phanerochaete chrysosporium* treatment because the phenylpropionaic acid was converted to benzoic acid *via* side chain cleavage and then formed p/o-methoxyphenol. Afterwards, p/o-methoxyphenol became p/o-hydroxyphenol *via* demethylation. Then, 8, 8-dimethoxy-2-octanol or other straight chain alcohols were produced *via* the ring-opening reaction of the benzene ring and eventually started the tricarboxylic acid cycle.

ACKNOWLEDGMENTS

The authors are grateful for the financial support of the National Science and Technology Support Foundation of China (2011BAC11B04).

REFERENCES CITED

Arora, D. S., and Dandhu, D. K. (1984). "Lactase production and wood degradation by teammates hirsute," *Folia Microbiology* 29(4), 310-315. DOI: 10.1007/bf02875963

Bajpai, P. (2012). "Brief description of the pulp and paper making process," in:

Biotechnology for Pulp and Paper Processing, Springer US, Boston, USA, pp. 7-14. DOI: 10.1007/978-1-4614-1409-4_2.

Cacchio, P., Ercole, C., and Veglio, F. (2001). "Cellulose enzymatic hydrolysis of wheat straw after soild-state pre-treatment by *Trametes trogii*, a factorial study," *Annals of Microbiology* 51, 215-224.

Chen, Y., Peng Y., Dai, C. C., and Ju, Q. (2011). "Biodegradation of 4-hydroxybenzoic acid by phomopsis liquidambar," *Applied Soil Ecology* 51, 102-110. DOI: 10.1016/j.apsoil.2011.09.004

Chagas, E. P., and Durrant, L. R. (2001). "Decolorization of azo dyes by white rot fungi and *Pleurotus sajorcaju*," *Enzyme and Microbial Technology* 29(8-9), 473-477. DOI: 10.1016/s0141-0229(01)00405-7

Duan, X. L. (2010). *Utilizable Treatment of Effluent from High Yield Pulping of Poplar by Aspergillums niger*, Master's Thesis, Tianjin University of Science and Technology, Tiajin, China.

Guan, X. Y., Dai, C. C., and Xu, Y. F. (2010). "Enhancement of polyunsaturated fatty acid production by selenium treatment in polyunsaturated fatty acid-producing fungus," *Journal of the American Oil Chemists' Society* 87(11), 1309-1317. DOI: 10.1007/s11746-010-1610-1

Guo, J., Huan, J., Bao, H., Zong, J., and Wei, H. (2002). *Water and Wastewater Monitoring and Analysis Methodology*, China Environmental Science Press, Beijing, China, pp. 105-107, 200-284.

Gao, Y., Yan, B., Zhang, Z., Chen, G., and Xie, G. H. (2002). "Biodegradation of pollutants in CTMP effluent by white-rot fungi *Phanerochaete chrysosporium*," *Transactions of China Pulp and Paper* 17, 44-47.

Jiying, Z., and Huiren, H. (2010). "The separation and application of the hemicelluloses from the waste liquor of APMP," *Transactions of China Pulp and Paper* 25(1), 33-38. DOI: 10.1021/om500888d

Jönsson, L. J., Alriksson, B., and Nilvebrant, N.-O. (2013). "Bioconversion of lignocellulose: Inhibitors and detoxification," *Biotechnology for Biofuels* 6(1), 16.

Ke, Z., and Siyi, Z. (1992). "The physicochemical properties of cooking waste liquid," in: *Comprehensive Utilization of Cooking Waste Liquor in Paper Industry and Its Pollution Control Technology*, China Light Industry Press, Beijing, China, pp. 400-437.

Kirk, T. K., and Shimada, M. (1985). "Lignin biodegradation: The microorganisms involved and the physiology and biochemistry of degradation by white rot fungi," *Biosynthesis and Biodegradation of Wood Components* 3(1), 579-605. DOI: 10.1016/b978-0-12-347880-1.50025-8

Krings, U., Pilawa, S., Theobald, C., and Berger, R. G. (2001). "Phenyl propenoic side chain degradation of ferule acid by *Pycnoporus cinnabarinus*-elucidation of metabolic pathways using [5-2H]-ferule acid," *Journal of Biotechnology* 85(3), 305-314. DOI: 10.1016/S0168-1656(00)00396-5

Li, X. Z., Zhao, J., and Yan, F. (2005). "Study on treatment of pulp and papermaking wastewater by white rot fungi," *Journal of China Pulp and Paper* 20(1), 88-91. DOI: 10.3321/j.issn:1000-6842.2005.01.022

Lihong, Z., Wenli, Y., and Quanli, L. (2013). "Mechanism and research progress of lignin removal from papermaking wastewater by white rot fungi," *Journal of*

Liaoning University of Technology 3(6), 375-382. DOI: 10.3969/j.issn.1674-3261.2013.06.006

Liu, S., Wang, E. W., Li, M. L., and Liu, Y. (2014). "Research progress of fatty acid methyl esterification method," *Chinese Pharmacy* 37, 3535-3537. DOI: 10.6039/j.issn.1001-0408.2014.37.27.

Shimoni, E., Baasov, T., Ravid, U., and Shoham, Y. (2003). "Biotransformation of propenylbenzenes by an *Arthrobacter sp*. and its t-anethole blocked mutants," *Journal of Biotechnology* 105(1-2), 61-70. DOI: 10.1016/s0168-1656(03)00141-x.

Wu, J., Xiao, Y. Z., and Yu, H. Q. (2005). "Degradation of lignin in pulp mill wastewaters by white-rot fungi on biofilm," *Bioresource Technology* 96(12), 1357-1363. DOI: 10.1016/j.biortech.2004.11.019.

Xuezhi, L., Jian, Z., and Fei, Y. (2005). "Study on treatment of pulp and papermaking wastewater by white rot fungi," *Journal of China Pulp and Paper* 20(1), 88-91. DOI: 10.3321/j.issn:1000-6842.2005.01.022

Article submitted: December 11, 2017; Peer review completed: August 18, 2018; Revisions received: December 28, 2018; Revisions accepted: February 28, 2019; Published: April 29, 2019.
DOI: 10.15376/biores.14.2.4792-4805