J. Microbiol. Biotechnol. (2016), 26(10), 1765–1773
http://dx.doi.org/10.4014/jmb.1602.02014

jmb

Kraft Lignin Biodegradation by *Dysgonomonas* sp. WJDL-Y1, a New Anaerobic Bacterial Strain Isolated from Sludge of a Pulp and Paper Mill S

Jing Duan[1,2], Jidong Liang[1,2*], Yiping Wang[1], Wenjing Du[1], and Dongqi Wang[3]

[1]*Department of Environmental Engineering, Xi'an Jiaotong University, Xi'an 710049, P.R. China*
[2]*State Key Laboratory of Loess and Quaternary Geology, Institute of Earth Environment, Chinese Academy of Sciences, Xi'an 710061, P.R. China*
[3]*State Key Laboratory of Eco-Hydraulic Engineering in Arid Area, Xi'an University of Technology, Xi'an 710048, P.R. China*

Received: February 11, 2016
Revised: June 22, 2016
Accepted: July 1, 2016

First published online
July 6, 2016

*Corresponding author
Phone: +86-13609128415;
E-mail: jidongl@mail.xjtu.edu.cn

Supplementary data for this paper are available on-line only at http://jmb.or.kr.

pISSN 1017-7825, eISSN 1738-8872

Copyright© 2016 by
The Korean Society for Microbiology and Biotechnology

Wastewater containing kraft lignin (KL) discharged from pulp and paper industries could cause serious environmental contamination. Appropriate effluent treatment is required to reduce the pollution. Investigations on anaerobic bacteria capable of degrading KL are beneficial to both lignin removal and biofuel regeneration from the effluent. In this paper, an anaerobic strain capable of degrading KL was isolated from the sludge of a pulp and paper mill and identified as *Dysgonomonas* sp. WJDL-Y1 by 16S rRNA analysis. Optimum conditions for KL degradation by strain WJDL-Y1 were obtained at initial pH of 6.8, C:N ratio of 6 and temperature of 33°C, based on statistical analyses by response surface methodology. For a 1.2 g/l KL solution, a COD removal rate of 20.7% concomitant with biomass increase of 17.6% was achieved after 4 days of incubation under the optimum conditions. After the treatment by strain WJDL-Y1, KL was modified and degraded.

Keywords: Kraft lignin, *Dysgonomonas* sp., anaerobic biodegradation, bacteria isolation, optimization

Introduction

Kraft lignin (KL) is a waste byproduct formed during alkaline sulfide treatment of lignocelluloses in pulp and paper industries [6, 13]. KL and its derivatives, as major coloring and polluting constituents, contribute almost 90-95% of the total pollution load to alkaline pulp and paper mill effluent [5, 8]. If KL-containing effluents are not treated properly, they will cause serious environmental pollution. Hence, it is urgent to develop appropriate treatment methods that decrease the KL impact in effluents. However, KL and its derivatives are complex and composed of chemically heterogeneous polymers that are recalcitrant to biological and chemical degradation [19]. Various physical and chemical treatment methods such as physical coagulation, ultrasound, acid precipitation, and ozonation, have been reported for the degradation and detoxification of KL-containing wastewater [6]. Unfortunately, these methods are less desirable than biological treatment from an environmentally friendly and economic standpoint [38]. Among lignin biodegradation studies, white-rot and brown-rot fungi have been well-documented for their powerful enzymatic systems [36]. The stability of fungi, however, is not good during practical treatment under extreme environmental and substrate conditions, such as higher pH, oxygen limitation, and high lignin concentrations [1, 3]. Therefore, bacteria have attracted increasing attention for their productivity and versatile environmental fitness in recent years. Previously reported lignin-degrading bacteria have been mainly isolated from soil (such as *Bacillus pumilus* and *Bacillus atrophaeus*) [19], sludge of pulp and paper mill (such as *Bacillus* sp., *Aneurinibacillus aneurinilyticus*, *Citrobacter freundii,* and *Citrobacter* sp.) [26–29], the guts of wood-feeding insects (such as Actinomycetes,

Alpha-proteobacteria, and Gamma-proteobacteria) [2, 19], and the steeping fluid of erosive bamboo slips (such as *Novosphingobium* sp. B-7, *Pandoraea* sp. B-6, *Comamonas* sp. B-9 and *Acinetobacter* sp. B-2) [8, 9, 31]. Whereas most of these bacteria are aerobic, pulp and paper industrial wastewater treatment for high organic loads is primarily anaerobic, necessitating the evaluation of anaerobic KL degraders. Anaerobic biotechnological processes have competitive advantages over aerobic ones, such as lower energy consumption for unnecessary oxygen demand, less sludge production, and biofuel regeneration. Although the biodegradation effectiveness of some organics by anaerobic bacteria is poorer than that by aerobes, bacteria concentration enhancement through anaerobic sludge granulation could overcome this drawback. An additional reason to study anaerobic processes is that KL is of great interest as a starting material for second-generation biofuel production through fermentation [19].

The present work investigated anaerobic bacteria capable of degrading KL. A potential novel anaerobic KL-degrading bacterial strain, *Dysgonomonas* sp. WJDL-Y1, was isolated from the sludge of a pulp and paper mill. Environmental parameters for strain WJDL-Y1 to degrade KL were optimized through a statistical technique of response surface methodology (RSM). Furthermore, its ability to degrade KL to simple metabolites was evaluated by COD, UV_{280}, SEM, FTIR, and GC-MS analyses.

Materials and Methods

Inocula and Basal Medium

The inoculum was as same as our previous publication [12]. The volatile suspended solids of the sludge was 3.84% (based on wet weight).

The basal mineral medium amended with KL (KL-MM) contained the following (in g/l): KL, 1.2; KH_2PO_4, 1; Na_2HPO_4, 0.2; NH_4Cl, 1; $MgSO_4$, 0.2; $CaCl_2$, 0.015; $FeSO_4{\cdot}7H_2O$, 0.002; $CuSO_4{\cdot}5H_2O$, 0.0004; $MnSO_4{\cdot}H_2O$, 0.004; $CoCl_2{\cdot}6H_2O$, 0.001; and $NiCl_2{\cdot}6H_2O$, 0.002. HCl or NaOH was used to adjust the pH of the medium to 7.0.

Bacteria Isolation and Identification

Bacteria isolation and identification techniques were conducted following the method described in a previous study [12]. According to KL-MM degradation based on COD decrease, the purified WJDL-Y1 colony was separated as a KL degrader and stored as inoculum for further evaluation of its KL degradation. Through 16S rRNA gene sequencing and aligning with other bacterial species in the GenBank database, the bacterial species was identified. By using the neighbor-joining method with MEGA 5.0 software, a phylogenetic tree was drawn.

Biodegradation and Optimization

Using the same optimization method as in our previous publication [10], the experiment was designed by RSM adopting the Box-Behnken design (BBD). Temperature (A), initial pH (B), and C:N ratio (C) were selected as independent variables, fluctuating at three levels. Based on the results of preliminary experiments, the values of the variables and their variation limits were confirmed. The COD removal rate (Y) was selected as the dependent variable (response). Seventeen experimental points with five central replications were conducted (Table S1). Data were analyzed by the software Design-Expert 8.0.5 to obtain polynomial regression equations and the variance (ANOVA).

Biodegradation assays were carried out in glass serum flasks (250 ml) containing 100 ml of KL-MM. The difference of C:N ratios was realized by adjusting the NH_4Cl concentrations in the KL-MM. Three milliliters of the inoculum (about 1.0×10^6 CFU/ml) of the pure strain WJDL-Y1 was inoculated in each flask. In order to create the anaerobic condition, a butyl rubber stopper and an aluminum crimp cap were used to seal each flask, and then we purged N_2 to the culture medium and the headspace for 5 min. Finally, the flasks were put in a shaking (100 rpm) incubator for 6 days under different experimental conditions (Table S1). COD reduction of the samples was measured after the incubation.

Finally, an additional biodegradation test under the optimal conditions was carried out to verify the results estimated by the RSM equation and also to analyze the degradation metabolites.

Analytical Methods

The bacterial biomass was quantified with an optical density at 600 nm (OD_{600}) by a UV-vis spectrophotometer (TU-1801; Beijing Persee General Instrument Co., Ltd., China) with KL-MM as the blank. Biomass was removed by being centrifuged at 5,000 ×*g* for 30 min, and then COD and UV_{280} (the characteristic absorption peak for lignin) were measured to evaluate lignin removal. COD was measured using the dichromate method (APHA 2005, USA). UV_{280} was monitored by an UV-Vis spectrophotometer (Agilent 8453; Agilent, USA). The pH was determined with a digital pH meter (PB-10; Beijing Sartorius, China).

Cell-free supernatants of the untreated and the treated samples were freeze-dried. Then, the dried powder samples were examined by field-emission scanning electron microscopy (FE-SEM; JEOL, JSM-6700F, Japan) and Fourier-transform infrared spectroscopy (Bruker Tensor 37 FTIR; Bruker, Germany).

Lignin biodegradation metabolites were identified by gas chromatography coupled to mass spectrometry (GC-MS) (Agilent Technologies, Inc., USA). The procedure of the analysis was according to the method described in a previous study [12].

Results and Discussion

Isolation and Identification of a Bacterial Strain

The strain WJDL-Y1 was identified by molecular genetic analyses. The 16S rRNA gene sequences were analyzed by

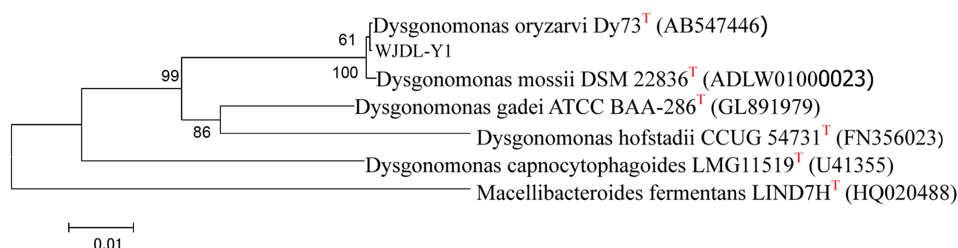


Fig. 1. Phylogenetic tree based on the results of 16S rRNA sequence comparison.

The tree was obtained using neighbor-joining with *Macellibacteroides fermentans* LIND7H[T] as the outgroup.

BLAST using the GenBank database of the National Center for Biotechnology Information. Strain WJDL-Y1 was found to closely match the species *Dysgonomonas* sp. (Fig. 1).

The sequence data of strain WJDL-Y1 gave 99% homology with *Dysgonomonas mossii* (Accession No. ADLW01000023). Therefore, strain WJDL-Y1 was designated as *Dysgonomonas* sp. WJDL-Y1 (Accession No. KF176996). According to relative references [22, 25, 40], *Dysgonomonas* sp. belongs to the Bacteroidetes class. Within the same class, our group isolated an aerobic lignin-degrading strain *Sphingobacterium* sp. HY-H [35] that had been reported to degrade lignin by others [33].

Several reports mentioned strains belonging to *Dysgonomonas* that have been found in microbial electrolysis cells [20, 22, 25, 39, 40]. They were detected in the anode chamber, degrading organic matter to produce volatile fatty acids. A few reports [14, 18, 24, 30, 34] indicated some of these strains were found in the process of denitrification and sulfate reduction. Previous studies also found that the bacteria of *Dysgonomonas* genus exhibited biodegradation activity on a various range of substrates, such as wheat-straw [39], alginate, Acid Red B dye [21], compounds in drug production [22], starch [25], and acetate [40]. To the best of our knowledge, the ligninolytic activity of *Dysgonomonas* has not been reported.

Biodegradation Optimization

Analysis of variance. BBD of RSM was employed to optimize the biodegradation conditions of KL by strain WJDL-Y1. Random sequences were performed in the experiments to minimize the effects of uncontrolled factors [23].

Based on the experimental results (Table S1), a quadratic function of the COD removal rate (Y) changing with temperature (A), initial pH (B), and C:N ratio (C) was achieved as shown in Eq. (1).

$$Y = 21.34 + 0.10A + 2.53B + 0.60C + 1.35AB + 1.15AC + 0.30BC - 3.65A^2 - 6.65B^2 - 3.35C^2 \quad (1)$$

The results of the Fisher test (*i.e.*, F-test) and ANOVA for the quadratic model's statistical importance evaluation are in Table 1. It shows that the model was significant to predict the response values, with *p*-values less than 0.0001. Model and term *p*-values <0.05 indicate the model is significant for 95% confidence intervals [17, 32]. The *p*-value for the "lack of fit" test was larger than 0.05 and the value of adequate precision was larger than 4, suggesting the model is desirable [37]. An adequate precision of 25.583 for Y indicates the adequacy of the model. The predicted value of the determination coefficient ($R^2 = 0.9907$) indicated that 99.07% of the total variation could be explained by the model. In addition, the difference of 0.2 between the predicted R^2 and the adjusted R^2 also confirmed the high significance of the model .

Effect of parameters on COD removal rate. A perturbation plot (Fig. 2) describes the response variation with one

Table 1. ANOVA result of the COD removal rate (Y) quadratic model.

Source	Sum of squares	Degrees of freedom	Mean square	F-value	*p*-Value Prob > F
Model	385.17	9	42.80	83.29	<0.0001[a]
Residual	3.60	7	0.51		
Lack of fit	2.97	3	0.99	6.26	0.0544[b]
Pure error	0.63	4	0.16		

a, significant; b, insignificant; $R^2 = 0.9907$, $R^2adj = 0.9789$, $R^2pred = 0.8754$, Adeq precision = 25.583.

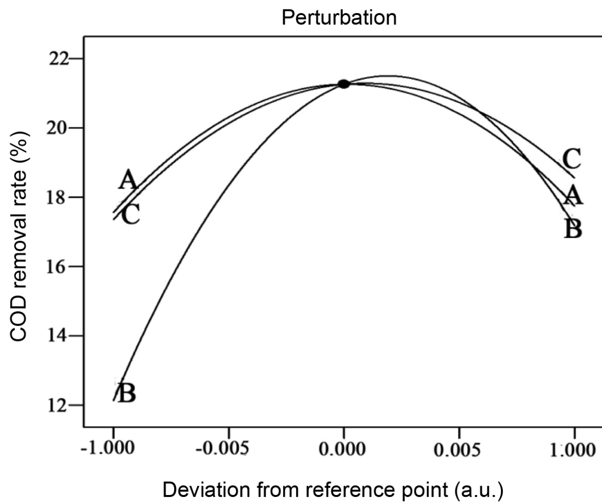
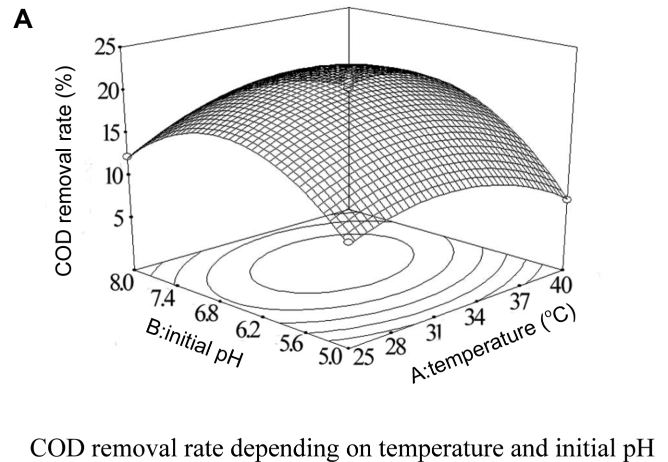


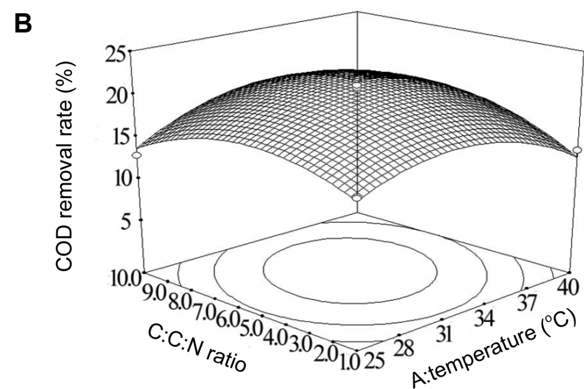
Fig. 2. Perturbation plot of COD removal rate (A: temperature (25°C, 32.5°C, 40°C); B: initial pH (5, 6.5, 8); C: C:N ratio (1, 5.5, 10).

independent variable over the experimental range while other independent variables are fixed at a central point [17]. A sharper factor curvature indicates a more important effect on the response. According to Fig. 2, the initial pH showed more significant effects on the COD removal rate (Curve B) than temperature and C:N ratio did. It is in accordance with the values of coefficients of variables in the model (Eq. (1)), in which both the linear and quadratic effects of initial pH (Curve B) make more contribution to COD removal rate than the other effects do.

With one variable at its optimum level (temperature: 32.5°C; initial pH: 6.5; C:N ratio: 5.5) and the other two variables fluctuating across the experimental range, 3D response surfaces and two-dimensional contour lines based on the model were obtained (Fig. 3). According to Fig. 3A, the COD removal rate initially increased and then declined with the enhancement of temperature and initial pH. The COD removal rate peaked at the middle range of temperature and a high C:N ratio (Fig. 3B). As illustrated in Fig. 3B, the maximum COD removal was reached in the high range of C:N ratio and initial pH. According to the contour lines in Fig. 3, there are significant interactions between each two of the three parameters. The effects of these three parameters on COD removal can be ranked in an order of C:N ratio > pH > temperature. That means C:N ratio serves as an important indicator to control the biodegradation of KL by strain WJDL-Y1. It also confirms that the initial pH is also an important factor for KL biodegradation [8].



COD removal rate depending on temperature and initial pH



COD removal rate depending on temperature and C:N ratio

Fig. 3. Contour plot and response surface for COD removal rate depending on two of the parameters.
(**A**) COD removal rate depending on temperature and initial pH. (**B**) COD removal rate depending on temperature and C:N ratio.

Optimization parameters. In this study, all factors were optimized "within the predetermined range" to maximize the COD removal rate through the analysis of Expert 8.0.5 software. The temperature of 31.5°C, initial pH of 6.8, and C:N ratio of 6.5 were determined as the optimum variables, and the maximum predicted COD removal rate was 21.6%. The predicted value was in a good agreement with the verification experiment result of 20.8% under the predicted optimum conditions. It indicate that the quadratic model developed by BBD is reliable.

KL Biodegradation and Its Metabolite Analysis

Bacterial growth and KL biodegradation. During the first 3 days of the optimal incubation, remarkable bacterial growth concurred and a significant COD and UV_{280} decrease

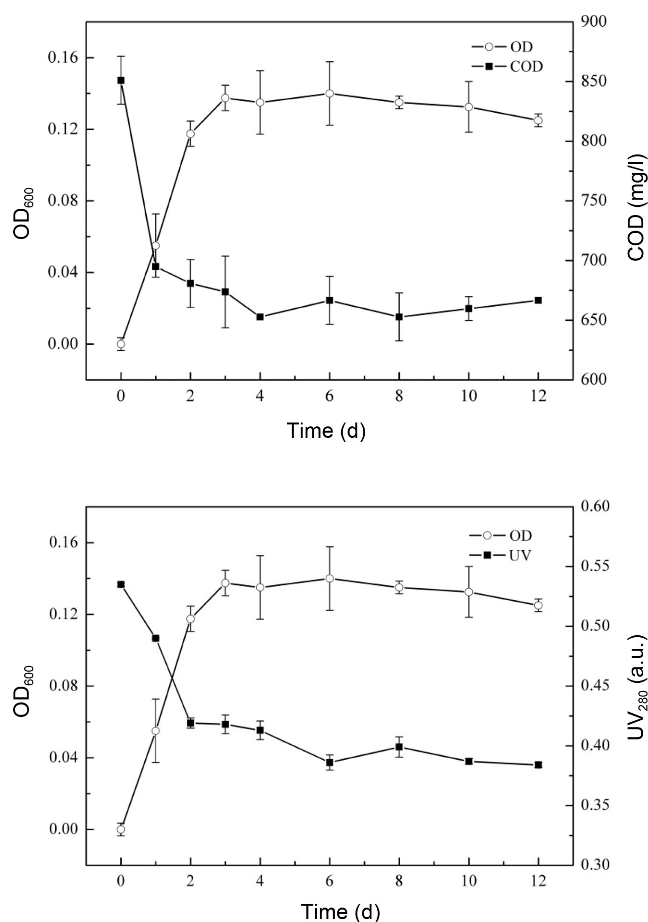


Fig. 4. Growth of strain WJDL-Y1 (○) and reduction of COD and UV_{280} (■) under optimal incubation conditions.

was observed (Fig. 4). Then, COD removal and UV_{280} decrease steadied off. The COD removal rate and biomass increase rate (based on OD_{600}) peaked at 20.7% and 17.6%, respectively, after 4 days incubation. The UV_{280} decrease rate peaked at 28.1% after 6 days of incubation. KL as the sole carbon source in the medium contributed to almost the entire COD load. Furthermore, the characteristic absorption peak value of lignin was found at 280 nm. Hence, KL degradation can be reflected by the reduction of COD and UV_{280}.

The breakdown and degradation of lignin by bacteria have attracted evergrowing interests in recently published studies [2]. A range of bacteria have been proved to break down lignin. Raj *et al*. [7, 26–29] isolated several lignin-degrading bacteria from sludge of a pulp and paper mill, such as *Bacillus* sp., *Aneurinibacillus aneurinilyticu*, and *Paenibacillus* sp. These bacteria have been reported to degrade KL (500 mg/l) by 32–43% after 6 days of incubation under

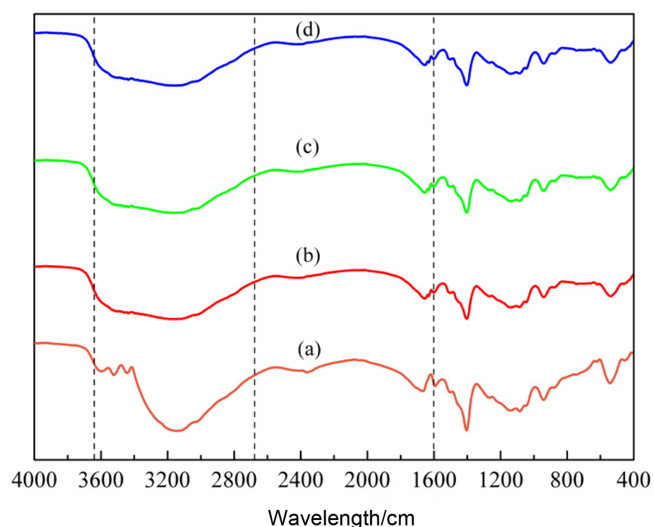


Fig. 5. FTIR spectra of KL samples before and after degradation by strain WJDL-Y1 (a: before degradation; b, c, and d: after degradation for 4, 9, and 16 days, respectively).

the conditions of pH 7.6, temperature around 30°C, and shaking at 120 rpm. However, these bacteria need low-molecular-weight compound such as glucose or peptone as co-substrate. Chai *et al*. [4, 8, 9, 31, 41] published several articles to discuss new lignin-degrading bacteria *Novosphingobium* sp. B-7, *Pandoraea* sp. B-6, *Comamonas* sp. B-9, and *Acinetobacter* sp. B-2. These strains were isolated from eroded bamboo slips. When these strains used KL with initial concentration of 3.0 g/l as the sole source of carbon and energy, the COD removal rate of KL ranged from 32% to 38% after 7 days of incubation under the conditions of pH 7.0, temperature at 30°C, and shaking at 120 rpm.

However, all the aforementioned strains were aerobic bacteria. Recently, two anaerobic strains named *Bacillus pumilus* and *Bacillus atrophaeus* were isolated from rainforest soils for their high laccase activity. *Bacillus pumilus* and *Bacillus atrophaeus* were confirmed with the ability to degrade KL (1 g/l), with removal rate of 27.5% and 35.1%, respectively, after 7 days of treatment under the conditions of pH 7.0, temperature of 28°C, and shaking at 120 rpm [19]. Compared with these strains, strain WJDL-Y1 exhibited faster growth (3 days) with KL (1.2 g/l) as a sole carbon source and energy source. Thus, it is meaningful to evaluate the industrial application potential of strain WJDL-Y1.

Lignin and its degradation metabolites. To further verify KL biodegradation by strain WJDL-Y1, metabolic products were analyzed by FTIR, SEM, and GC-MS.

The FTIR spectra of KL samples before and after degradation by strain WJDL-Y1 are shown in Fig. 5. In

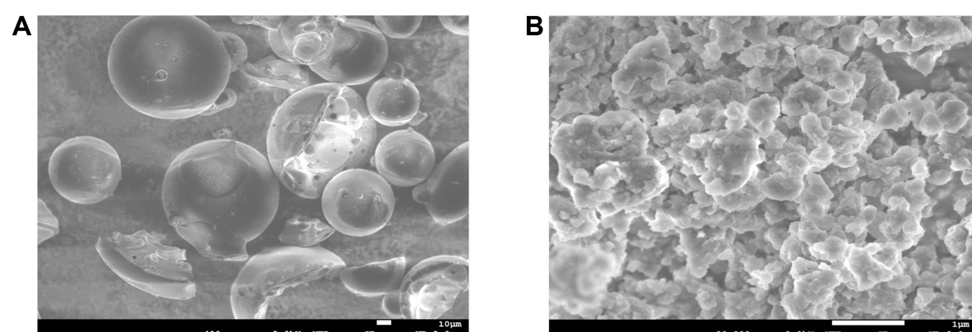


Fig. 6. SEM of KL samples before and after degradation by strain WJDL-Y1.
(**A**) Before degradation. (**B**) After degradation.

Table 2. Metabolites identified by GC-MS during KL degradation by strain WJDL-Y1.

No.	RT (min)	Indentified compounds	Molecular formular	Time (days) 0	4	6	9	12	16	20
1	6.728	Ethanol	C_2H_6O	+	+	+	+	+	+	+
2	6.819	Propanoic acid	$C_3H_6O_2$	-	+	+	+	+	+	+
3	9.729	Phenol	C_6H_6O	-	+	+	+	+	+	+
4	10.031	Lactic acid	$C_3H_6O_3$	-	-	+	-	+	-	+
5	10.357	Glycolic acid	$C_2H_4O_3$	-	+	+	-	-	+	+
6	11.266	2-Hydroxybutanoic acid	$C_4H_8O_3$	+	+	+	+	+	+	+
7	12.866	Guaiacol	$C_7H_8O_2$	+	+	+	-	-	-	-
8	14.204	Butane diacid	$C_4H_6O_4$	-	+	+	+	+	+	+
9	14.387	Dimethyl succinic acid	$C_5H_8O_4$	-	+	+	-	-	-	-
10	15.032	*p*-Hydroxybenzaldehyde	$C_7H_6O_2$	+	+	+	-	-	-	-
11	15.615	Hydrocinnamic acid	$C_9H_{10}O_2$	-	+	+	+	+	+	-
12	15.627	Benzenediol	$C_6H_6O_2$	-	+	+	-	+	+	+
13	16.769	Ethylvanillin	$C_9H_{10}O_3$	-	+	+	+	-	-	-
14	16.861	4-Hydroxypentenoic acid	$C_5H_8O_3$	-	+	+	-	-	-	+
15	17.534	Vanillin	$C_8H_8O_3$	+	+	+	+	+	+	+
16	17.936	Vanillic acid	$C_8H_8O_4$	+	+	+	+	+	+	+
17	18.770	Vanillic alcohol	$C_8H_{10}O_3$	+	+	+	+	+	+	+
18	18.253	*p*-Hydroxybenzoic acid	$C_7H_6O_3$	+	+	-	+	+	+	+
19	19.159	Syringaldehyde	$C_9H_{10}O_4$	+	-	-	+	+	+	+
20	20.113	*p*-Hydroxyphenylpropanoic acid	$C_9H_{10}O_3$	-	-	-	-	+	-	+
21	20.433	Acetosyringone	$C_{10}H_{12}O_4$	+	-	-	-	-	-	-
22	20.805	Dibutyl phthalate	$C_{16}H_{22}O_4$	+	+	+	+	-	-	-
23	21.371	Methyl palmitate	$C_{17}H_{34}O_2$	-	+	-	-	-	-	-
24	21.588	Syringic acid	$C_9H_{10}O_5$	+	+	+	+	-	-	+
25	21.548	Ferulic acid	$C_{10}H_{10}O_4$	-	+	+	+	+	+	+
26	22.559	Hexadecanoic acid	$C_{16}H_{32}O_2$	+	+	+	+	+	+	+
27	24.131	Octadecanoic	$C_{18}H_{36}O_2$	+	+	+	+	+	+	+

RT: Retention time; "+"present; "-"absent.

general, the patterns of the peaks in the spectra were similar, except for some changes in peak intensities. Peaks around 3,400/cm corresponding to the band of O-H increased after biodegradation, implying the hydroxyl

units in lignin were oxidized to alcohols or acids. Both the peaks around 1,600/cm, ascribed to the aromatic skeletal vibration of the phenylpropane unit, and the peaks around 2,900/cm, corresponding to a methyl unit, showed a minor decease in peak intensities, indicating the cleavage of the aromatic ring and demethylation during the degradation process of KL by strain WJDL-Y1.

Scanning electron microscopy analysis of the surface morphology of KL before and after being treated by strain WJDL-Y1 is shown in Fig. 6. The untreated KL was spherical with a smooth surface, and the diameter varied from 40 to 120 μm (Fig. 6A). The surface of the treated KL was rough and full of loose and small pieces of broken fragments as shown in Fig. 6B (×20,000). These structure changes confirmed the degradation of KL by strain WJDL-Y1.

The main identified compounds by GC-MS are listed in Table 2. As in our previous article, some low-molecular-weight (LMW) aromatic compounds (*i.e.*, guaiacol, p-hydroxybenzaldehyde, vanillin, vanillic acid, vanillic alcohol, *p*-hydroxybenzoic acid, syringaldehyde, acetosyringone, and syringic acid) and certain high-molecular-weight fatty acids (*i.e.*, hexadecanoic acid and octadecanoic acid) were detected in the control sample. These compounds may result from the KL degradation and bacterial cells.

After being inoculated with strain WJDL-Y1 for several days, more compounds were identified in the sample than before. Some LMW aromatic compounds deriving from phenol units of lignin, including hydrocinnamic acid, vanillic acid, *p*-hydroxyphenylpropanoic acid, syringic acid, and ferulic acid, were detected after incubation [11]. These metabolites were created by natural lignin polymer units, including the radical polymerization of guaiacyl (G) units from precursor coniferyl alcohol, syringyl (S) units from precursor sinapyl alcohol, and *p*-hydroxyphenyl (H) units from precursor *p*-coumaryl alcohol [2, 5, 12]. Among these compounds, hydrocinnamic acid, *p*-hydroxyphenylpropanoic acid, and ferulic acid were new intermediate metabolites. Many LMW acids (*i.e.*, propanoic acid, lactic acid, glycolic acid, butane diacid, dimethyl succinic acid, and 4-hydroxypentenoic acid) and phenols (*i.e.*, phenol, benzenediol) were also detected as new intermediate products in the inoculated sample. It is interesting to note that ethanol, anillic alcohol, and vanillin were detected in all samples before and after biodegradation. Ethylvanillin, acetosyringone, and *p*-hydroxybenzaldehyde could not be detected after incubation. Consistent with other studies [15, 16, 27, 42], lignin degradation generated more acid-type compounds than aldehyde and ketone-type molecules. Specifically, ferulic acid and vanillic acid were detected in the extract of the KL degradation products in this research, which means that strain WJDL-Y1 has application potential in the food additive and preservative production areas.

In conclusion, *Dysgonomonas* sp. strain WJDL-Y1, as a new anaerobic lignin-degrader, was isolated from the sludge of a pulp and paper mill. Strain WJDL-Y1 has application potential not only in the treatment of wastewater with plenty of lignin-related contaminants but also in producing biofuel and chemicals from lignin-related materials.

Acknowledgments

This work was supported by the Natural Science Foundation of Shaanxi Province in China (Grant No. 2015JQ2052), and Shaanxi Science and Technology Activities Foundation for the Returned Visiting Scholars (2014).

References

1. Abhishek A, Dwivedi A, Tandan N, Kumar U. 2015. Comparative bacterial degradation and detoxification of model and kraft lignin from pulp paper wastewater and its metabolites. *Appl. Water Sci.* 1-11.
2. Bugg TDH, Ahmad M, Hardiman EM, Rahmanpour R. 2011. Pathways for degradation of lignin in bacteria and fungi. *Nat. Prod. Rep.* **28:** 1883-1896.
3. Bugg TDH, Ahmad M, Hardiman EM, Singh R. 2011. The emerging role for bacteria in lignin degradation and bio-product formation. *Curr. Opin. Biotechnol.* **22:** 394-400.
4. Chai L-Y, Chen Y-H, Tang C-J, Yang Z-H, Zheng Y, Shi Y. 2014. Depolymerization and decolorization of kraft lignin by bacterium *Comamonas* sp. B-9. *Appl. Microbiol. Biotechnol.* **98:** 1907-1912.
5. Chandra R, Abhishek A, Sankhwar M. 2011. Bacterial decolorization and detoxification of black liquor from rayon grade pulp manufacturing paper industry and detection of their metabolic products. *Bioresour. Technol.* **102:** 6429-6436.
6. Chandra R, Bharagava RN. 2013. Bacterial degradation of synthetic and kraft lignin by axenic and mixed culture and their metabolic products. *J. Environ. Biol.* **34:** 991-999.
7. Chandra R, Raj A, Purohit HJ, Kapley A. 2007. Characterisation and optimisation of three potential aerobic bacterial strains for kraft lignin degradation from pulp paper waste. *Chemosphere* **67:** 839-846.
8. Chen Y, Chai L, Tang C, Yang Z, Zheng Y, Shi Y, Zhang H, *et al*. 2012. Kraft lignin biodegradation by *Novosphingobium* sp. B-7 and analysis of the degradation process. *Bioresour. Technol.* **123:** 682-685.
9. Chen YH, Chai LY, Zhu YH, Yang ZH, Zheng Y, Zhang H. 2012. Biodegradation of kraft lignin by a bacterial strain *Comamonas* sp. B-9 isolated from eroded bamboo slips. *J.*

Appl. Microbiol. **112:** 900-906.
10. Cui X, Liang J, Wang D. 2015. Optimization of integrated electrochemical oxidation and biodegradation (EO-BD) for kraft lignin. *J. Chem. Technol. Biotechnol.* **90:** 747-753.
11. Deba F, Xuan TD, Yasuda M, Tawata S. 2007. Herbicidal and fungicidal activities and identification of potential phytotoxins from *Bidens pilosa* L. var. *radiata* Scherff. *Weed Biol. Manage.* **7:** 77-83.
12. Duan J, Huo X, Du WJ, Liang JD, Wang DQ, Yang SC. 2016. Biodegradation of kraft lignin by a newly isolated anaerobic bacterial strain, *Acetoanaerobium* sp. WJDL-Y2. *Lett. Appl. Microbiol.* **62:** 55-62.
13. Duan J, Liang JD, Du WJ, Wang DQ. 2014. Biodegradation of kraft lignin by a bacterial strain *Sphingobacterium* sp. HY-H. *Adv. Mater. Res.* **955-959:** 548-553.
14. Gao M, Li L, Liu J. 2011. Simultaneous removal of hydrogen sulfide and toluene in a bioreactor: performance and characteristics of microbial community. *J. Environ. Sci.* **23:** 353-359.
15. Gupta VK, Minocha AK, Jain N. 2001. Batch and continuous studies on treatment of pulp mill wastewater by *Aeromonas formicans*. *J. Chem. Technol. Biotechnol.* **76:** 547-552.
16. Hemandez M, Hernandez-Coronado MJ, Montiel MD, Rodriguez J, Arias ME. 2001. Analysis of alkali-lignin in a paper mill effluent decolourised with two *Streptomyces* strains by gas chromatography-mass spectrometry after cupric oxide degradation. *J. Chromatogr. A* **919:** 389-394.
17. Hosseinpour V, Kazemeini M, Mohammadrezaee A. 2011. Optimisation of Ru-promoted Ir-catalysed methanol carbonylation utilising response surface methodology. *Appl. Catal. A Gen.* **394:** 166-175.
18. Hu J, Li D, Liu Q, Tao Y, He X, Wang X, *et al*. 2009. Effect of organic carbon on nitrification efficiency and community composition of nitrifying biofilms. *J. Environ. Sci.* (*China*) **21:** 387-394.
19. Huang X-F, Santhanam N, Badri DV, Hunter WJ, Manter DK, Decker SR, *et al*. 2013. Isolation and characterization of lignin-degrading bacteria from rainforest soils. *Biotechnol. Bioeng.* **110:** 1616-1626.
20. Kircheva N, Outin J, Perrier G, Ramousse J, Merlin G, Lyautey E. 2015. Bio-electrochemical characterization of air-cathode microbial fuel cells with microporous polyethylene/silica membrane as separator. *Bioelectrochemistry* **106:** 115-124.
21. Li H, Tan L, Ning S, He M. 2015. Reactor performance and microbial community dynamics during aerobic degradation and detoxification of Acid Red B with activated sludge bioaugmented by a yeast *Candida tropicalis* TL-F1 in MBR. *Int. Biodeterior. Biodegradation* **104:** 149-156.
22. Liu R, Gao C, Zhao Y-G, Wang A, Lu S, Wang M, *et al*. 2012. Biological treatment of steroidal drug industrial effluent and electricity generation in the microbial fuel cells. *Bioresour. Technol.* **123:** 86-91.
23. Marcos M, Garcia-Cubero MT, Gonzalez-Benito G, Coca M, Bolado S, Lucas S. 2013. Optimization of the enzymatic hydrolysis conditions of steam-exploded wheat straw for maximum glucose and xylose recovery. *J. Chem. Technol. Biotechnol.* **88:** 237-246.
24. Matos M, Pereira MA, Parpot P, Brito AG, Nogueira R. 2014. Influence of tetracycline on the microbial community composition and activity of nitrifying biofilms. *Chemosphere* **117:** 295-302.
25. Montpart N, Rago L, Baeza JA, Guisasola A. 2015. Hydrogen production in single chamber microbial electrolysis cells with different complex substrates. *Water Res.* **68:** 601-615.
26. Raj A, Chandra R, Krishna Reddy MM, Purohit HJ, Kapley A. 2007. Biodegradation of kraft lignin by a newly isolated bacterial strain, *Aneurinibacillus aneurinilyticus* from the sludge of a pulp paper mill. *World J. Microbiol. Biotechnol.* **23:** 793-799.
27. Raj A, Reddy MMK, Chandra R. 2007. Decolourisation and treatment of pulp and paper mill effluent by lignin-degrading *Bacillus* sp. *J. Chem. Technol. Biotechnol.* **82:** 399-406.
28. Raj A, Reddy MMK, Chandra R. 2007. Identification of low molecular weight aromatic compounds by gas chromatography-mass spectrometry (GC-MS) from kraft lignin degradation by three *Bacillus* sp. *Int. Biodeterior. Biodegradation* **59:** 292-296.
29. Raj A, Reddy MMK, Chandra R, Purohit HJ, Kapley A. 2007. Biodegradation of kraft-lignin by *Bacillus* sp. isolated from sludge of pulp and paper mill. *Biodegradation* **18:** 783-792.
30. Sarti A, Pozzi E, Chinalia FA, Ono A, Foresti E. 2010. Microbial processes and bacterial populations associated to anaerobic treatment of sulfate-rich wastewater. *Process Biochem.* **45:** 164-170.
31. Shi Y, Chai L, Tang C, Yang Z, Zheng Y, Chen Y, Jing Q. 2013. Biochemical investigation of kraft lignin degradation by *Pandoraea* sp. B-6 isolated from bamboo slips. *Bioprocess Biosyst. Eng.* **36:** 1957-1965.
32. Soltani M, Moghaddam TB, Karim MR, Baaj H. 2015. Analysis of fatigue properties of unmodified and polyethylene terephthalate modified asphalt mixtures using response surface methodology. *Eng. Fail. Anal.* **58:** 238-248.
33. Taylor CR, Hardiman EM, Ahmad M, Sainsbury PD, Norris PR, Bugg TDH. 2012. Isolation of bacterial strains able to metabolize lignin from screening of environmental samples. *J. Appl. Microbiol.* **113:** 521-530.
34. Viviantira E, Wan C, Wong B-T, Lee D-J. 2012. Denitrifying sulfide removal with methanogenic culture. *J. Taiwan Inst. Chem. Eng.* **43:** 374-385.
35. Wang D, Lin Y, Du W, Liang J, Ning Y. 2013. Optimization and characterization of lignosulfonate biodegradation process by a bacterial strain, *Sphingobacterium* sp. HY-H. *Int. Biodeterior. Biodegradation* **85:** 365-371.
36. Wang Y, Liu Q, Yan L, Gao Y, Wang Y, Wang W. 2013. A novel lignin degradation bacterial consortium for efficient pulping. *Bioresour. Technol.* **139:** 113-119.

37. Wu J, Zhang H, Oturan N, Wang Y, Chen L, Oturan MA. 2012. Application of response surface methodology to the removal of the antibiotic tetracycline by electrochemical process using carbon-felt cathode and DSA (Ti/RuO_2-IrO_2) anode. *Chemosphere* **87:** 614-620.
38. Xiong XQ, Liao HD, Ma JS, Liu XM, Zhang LY, Shi XW, *et al*. 2014. Isolation of a rice endophytic bacterium, *Pantoea* sp. Sd-1, with ligninolytic activity and characterization of its rice straw degradation ability. *Lett. Appl. Microbiol.* **58:** 123-129.
39. Zhang Y, Angeliclaki I. 2012. Innovative self-powered submersible microbial electrolysis cell (SMEC) for biohydrogen production from anaerobic reactors. *Water Res.* **46:** 2727-2736.
40. Zhang Y, Min B, Huang L, Angelidaki I. 2011. Electricity generation and microbial community response to substrate changes in microbial fuel cell. *Bioresour. Technol.* **102:** 1166-1173.
41. Zheng Y, Chai L-Y, Yang Z-H, Tang C-J, Chen Y-H, Shi Y. 2013. Enhanced remediation of black liquor by activated sludge bioaugmented with a novel exogenous microorganism culture. *Appl. Microbiol. Biotechnol.* **97:** 6525-6535.
42. Zheng Y, Chai L, Yang Z, Chen Y, Shi Y, Wang Y. 2014. Environmentally safe treatment of black liquor with *Comamonas* sp. B-9 under high-alkaline conditions. *J. Basic Microbiol.* **54:** 152-161.