Integrated Photocatalytic And Microbial Degradation of Kraft Lignin

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ABSTRACT

We report a novel approach of integrating photochemical oxidation and microbiological degradation of Kraft lignin (KL). Photocatalyst, TiO_2-ZnO-ZrO_2 nanocomposite was synthesized by the sol–gel method and characterized by scanning electron microscopy (SEM), energy-dispersive X-ray (EDX) analysis and X-ray diffraction (XRD). Photocatalyst loadings and exposure times were optimized to obtain between 40-50% oxidation of the free phenolic hydroxyl groups. A facultative anaerobic lignolytic strain isolated from soil microflora further degraded the photocatalyzed kraft lignin to acetyl guiacol and 4-ethoxymethyl-2-methoxy phenol.

***Keywords*:** Kraft lignin, photocatalysis, anaerobic degradation, TiO_2-ZnO-ZrO_2 catalyst, integrated biomass reforming.

1. INTRODUCTION

Lignin is a complex aromatic heteropolymer recalcitrant to chemical and microbial degradation [1]. Kraft lignin (KL) is an alkali soluble byproduct of paper pulping process and constitutes 85% of available technical lignin. During the sulphate (Kraft) process lignin undergoes fragmentation via cleavage of the aryl –alkyl bonds resulting in an increase in the phenolic hydroxyl groups. KL represents a rich non –fossil source of aromatic and cyclohexyl compounds [2]. Currently, KL is used in low impact applications such as fuel and carrier for fertilizers and pesticides [3]. Underutilization of KL is primarily due to the lack of efficient methods to reform lignin into valuable products. Stand-alone biological [4], chemical [5], electrochemical [6] and photochemical [7] have been used to convert lignin into vanillin and vanillic acid. However, lower yields have hindered their utility as industry usable processes. Integrated processes have been widely studied for reforming of the cellulose and hemicellulose biomass components but only a few have been investigated for lignin [8]. In nature, lignin is primarily oxidized by consortia of fungi and bacteria [1]. Here we have attempted to simulate this mechanism by using photooxidized KL as substrate for lignolytic bacteria.

Although TiO_2 is widely accepted as an ideal photocatalyst, metal oxide dopants are known to improve visible light responsiveness [9]. We developed a novel TiO_2-ZnO-ZrO_2 nanocomposite catalyst to degrade KL. This catalyst was coated with platinum and used to depolymerize KL chains and transform its aromatic structure to products amenable to degradation by lignolytic bacteria.

2. EXPERIMENTAL

2.1 Nanocomposite material synthesis

Titanium isopropoxide (5 gms) was added to 10 ml of ethanol acidified with a drop of sulphuric acid. Zinc oxide (0.5% w/w), zirconium oxide (0.25 wt%) and 1.0 gm of Pluronic 123 dissolved in 10 ml ethanol were mixed with the titanium isopropoxide precursor. To the continuously stirred mixture, Ammonium hydroxide (2.0 ml) was added drop wise and the mixture was allowed to gel overnight. The gel was calcined at 600^{o}C for 2 hours. The powdered material thus obtained was coated with platinum using chloroplatinic acid.

2.2 Characterization

The TiO_2-ZnO-ZrO_2 powdered material was characterized using the Rigaku Ultima-Plus X-ray diffractometer. The morphology was studied using Zeiss Supra 40 VP field–emission scanning electron microscope (SEM). BET surface area analyzer Gemini II-2375 from micromeritics was used to determine the specific surface area (SSA) from the adsorption isotherm obtained after degassing the material at 200^{o}C.

2.3 Photocatalytic Reaction Set-Up

Pt/TiO_2-ZnO- ZrO_2 nanocomposite material was dispersed in 10 ml alkaline solution of 0.1mg/ml KL by 30 min sonication. An aliquot of 3 ml was removed and kept in the dark (dark control). The remaining 7 ml was added to a quartz reactor and illuminated by a solar simulator fitted with a 250 W Xenon lamp and AM 1.5G filter. The power density measured at the center of the illuminated window was 100 mW/cm^2. The photocatalysed and dark control samples were centrifuged at 10,000 rpm for 30 min to separate photocatalyst.

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2.4 Microbial Media Preparation

Soil underneath wooden logs lying dormant for a couple of centuries in the deep underground science and engineering laboratory (DUSEL) located in the former gold mines at Homestake, Leeds, SD was collected under aseptic conditions. It was suspended in 200 ml sterile saline and shaken at 200 rpm for 1 hour at 42^0C. Aliquots of 100 µl were used to inoculate 125 ml serum bottles filled with 10 ml mineral salts medium (pH 7.6), supplemented with 0.6 mg/L copper sulfate, 0.05 gm/L yeast extract and 1 gm/L Kraft lignin (MKL) as the sole source of carbon. Reducing media consisting of L-cysteine.HCl and $Na_2S.9H_2O$ at concentrations of 4 mgs each were added prior to the inoculation. The head space was purged with 80%N_2 and 20%CO_2 and the bottles were incubated at 43^0C without shaking. Subsequent enrichments were carried out on fresh MKL media every 4 days for 60 days. The enriched culture was streaked onto LB agar. For integrated process, 200 mg/L of photocatalysed KL was suspended in MKL media and inoculated with loopful of the pure culture.

2.5 UV-Visible Spectroscopy

KL degradation was monitored by UV-Vis spectra and UV-Vis differential ionization [10] spectroscopic scans conducted from 220 nm -500 nm by Cary 50 UV-Visible spectrophotometer. A calibration curve to estimate unconjugated phenolic hydroxyl groups was generated from the absorbance values at 290 nm. Photocatalysis induced free phenol OH degradation was compared with the dark control to calculate percent phenolic OH degradation.

2.6 Gas Chromatography and Mass Spectroscopy

Samples were withdrawn at different time intervals for analysis of degradation products generated following microbial treatment of the photocatalysed samples. An aliquot of 1 µL was injected in a GC-MS (Agilent Technologies, USA) interfaced with a mass spectrometer. The identification of lignin related compounds was done using the NIST (National Institute of Standard and Technology) library, USA.

3. RESULTS AND DISCUSSION

As assessed from SEM images shown in Figure 1, the particle size of platinum coated TiO_2-ZnO-ZrO_2 was found to be less than 100 nm. However, TEM analysis will provide more precise measurements. The EDX analysis of the photocatalytic nanomaterial confirmed the TiO_2:ZnO:ZrO_2 weight ratio to be 5:0.5:0.25.

TiO_2 efficiently mediates photocatalytic degradation of lignin by oxidative chain reaction involving singlet oxygen, hydroxyl and superoxide radicals [11]. Studies have focused on using this catalyst for waste water remediation [12,13]. However, ZnO semiconductor reportedly degraded KL better than TiO_2 [14]. The novel TiO_2-ZnO-ZrO_2 nanocomposite was however found only marginally superior to TiO_2 (data not shown). The photocatalyst and KL in the ratio of 2:1 and exposure time of 3 hours optimally reduced the free phenolic OH groups to between 40%-50%. Photooxidized KL with 43% reduction in phenolic OH groups and untreated KL were used as substrate for microbial degradation.

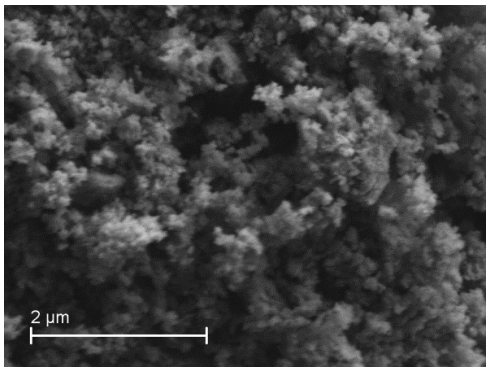


Figure 1: SEM image of sol-gel derived TiO_2-ZnO-ZrO_2 nanocomposite.

The lignolytic capabilities of bacteria isolated from soil microflora obtained from DUSEL were initially tested with untreated KL substrate. Figure 2 shows visible color change in the KL media inoculated with pure culture. It was observed that, in 76 days, the lignolytic bacteria completely degraded untreated lignin to water and carbon dioxide. Figure 3a and 3b show

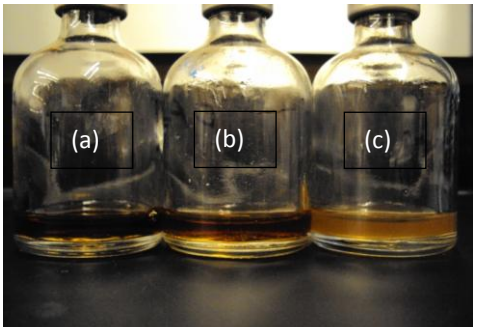


Figure 2: Visible degradation of Kraft lignin, (a) MKL uninoculated media control, (b) media with bacteria incubated for 5 days, and (c) media with bacteria inoculated for 76 days.

UV-Vis spectroscopic confirmation of loss of aromatic rings and free phenolic hydroxyl groups.

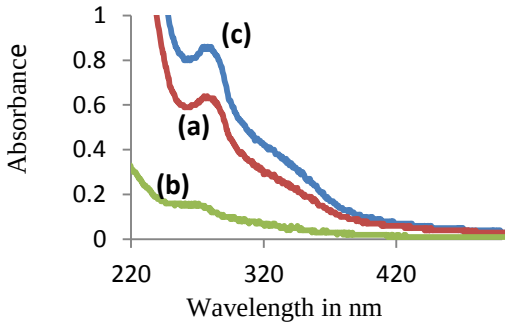


Figure 3a: UV-Vis spectra of MKL media supernatant control with no bacteria (c), MKL media with bacteria after 5 days incubation (a) and 76 days incubation (b).

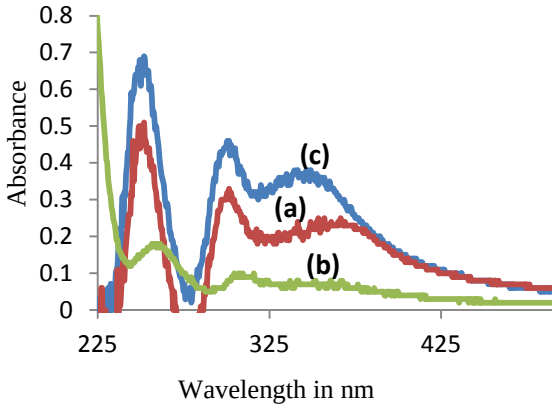
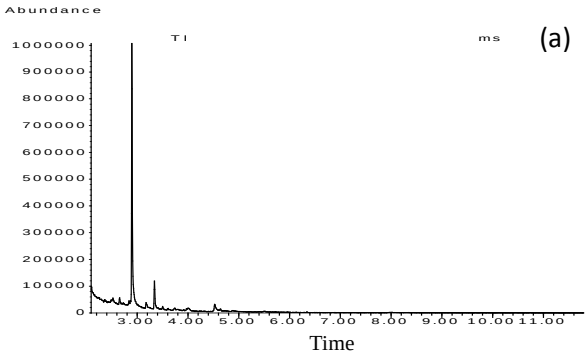


Figure 3b: Differential ionization UV-Vis spectra of MKL media supernatant control no bacteria (c), MKL media with bacteria after 5 days incubation (a) and 76 days incubation (b).

A GC-MS analysis of products performed after 12 hours exposure to these lignolytic bacterial species along with a lignin control is shown in Figures 4a, 4b, and 4c.



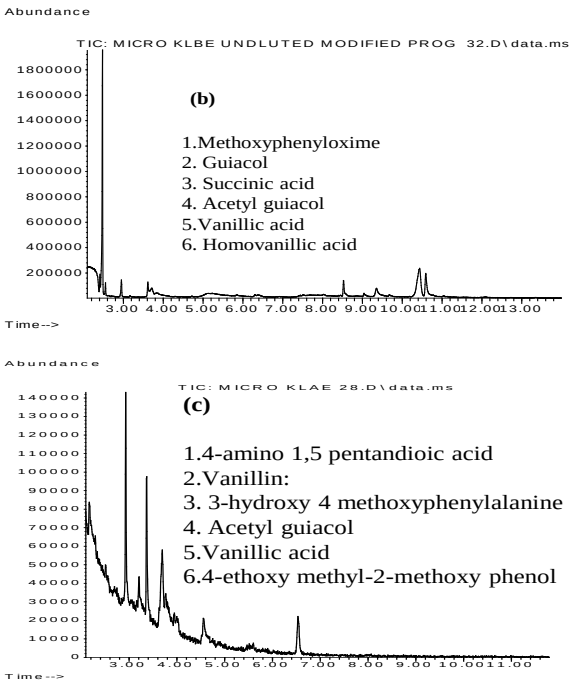


Figure 4: Total ion chromatogram of KL control (a), untreated KL (b) and photooxidised KL after 12 hours of microbial degradation (c).

The control lignin shows absence of degradation products. Minor peaks of vanillic acid and homovanillic acid were seen in untreated KL. Interestingly, although vanillin was detected as one of the major products following photocatalysis, there was no major reduction in the peak height of this value added compound after microbial treatment.

Acetyl guiacol was detected as one of the metabolic end products following 12 hour incubation. The bacteria clearly were not only tolerant to the photooxidation end products, but could use these and the partially degraded KL molecule as a substrate. Amongst various other catalytic methods studied for valorization of lignin [15], photocatalysis using solar energy is the least expensive. Combining the process with microbial or enzymatic degradation could lead to a regulatable integrated process for lignin valorization. Enzymatic hydrolysis of photocatalytically pretreated rice straw occurred at a higher rate due to degradation of the lignin component making hemicelluloses and cellulose accessible to the enzyme [15]. It will be interesting to see if biomass photocatalysed products could also be used as substrates by these bacteria. Lignin degradation has been primarily studied using aerobic bacteria [4,17]. However, in natural ecosystem, anaerobes have been suggested to play a major roles in lignin degradation [18,19,20]. Identification of anaerobes or facultative anaerobes would potentially progress to lignin valorization for biofuels and other value added compounds. Analysis of the 16SrRNA gene of the

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lignolytic bacteria isolated from DUSEL has been undertaken to identify the bacterial species and understand its metabolic pathways.

4. CONCLUSIONS

A novel TiO_2.ZnO-ZrO_2 nanocomposite catalytic material was synthesized by the sol-gel method. In presence of this catalyst, the Kraft lignin was photocatalytically oxidized to vanillin, propyl guiacol, vanillacetic acid and other oxidization products. A facultative anaerobic soil bacterial species enriched in the presence of kraft lignin demonstrated lignolytic abilities. It could completely mineralize the recalcitrant KL macromolecule in 76 days. In a short 12 hour incubation time these bacteria biochemically modified the photooxidised KL to form value added products namely acetyl guiacol, but did not significantly degrade vanillin produced in the photooxidation step. Additionally the bacteria also fragmented KL to another phenolic monomer, 4-ethoxymethyl-2-methoxy phenol.

REFERENCES

[1] F. Ruiz-Duenas and A. Martinez, Microbial Biotechnology, 2 (2), 164, 2009.

[2] A.Vishtal and A. Kraslawski, Bioresources, 6 (3) 3547, 2011.

[3] J. Holladay, J. Bozell, J. White and D. Johnson in Report by the Pacific Northwest National laboratory and The National Renewable Energy Laboratory, Vol II, 2007.

[4] R. Chandra, S. Singh, M. Krishna Reddy, D. Patel, H. Purohit and A. Kapley, Journal of General and Applied Microbiology, 54, 399, 2008.

[5] G. Gellerstedt, A. Majtnerova and L. Zhang, Competes Rendus Biologies, 327, 817, 2004.

[6] C.S.A Antunes, M. Bietti, M. Salamone, N. Scione, Journal of Photochemistry Photobiology, 163, 453, 2004.

[7] M. Kaneko, J. Nemoto, H. Ueno, N. Gokan, K.Ohnuki. Electrochemistry Communications, 8, 336, 2006.

[8] M. Tian, J. Wen, D. MacDonald, R. Asmussen and A. Chen, Electrochemistry Communications 12, 527, 2010.

[9] J. Colmenares, R. Luque, J. Campelo, F. Colmenares, Z. Karpinskiand A. Romero, Materials, 2, 2228, 2009.

[10] O. Goldschmid, Analytical Chemistry, 26, 9, 1421, 1954.

[11] A. Machado,A. Furuyama,S. Falone, R. Ruggiero, D. Perez and A. Castellan, Chemosphere, 40, 115,2000.

[12] M. Ugurlu and M. Karaoglu, Chemical Engineering Journal, 166 (3) 859, 2011.

[13] J. Villasenor, N. Duran and H. Mansilla, Environmental Technology, 23, 955, 2002.

[14] S. Kansal, M. Singh and D. Sud, Journal of Hazardous Materials, 153 (1-2), 412, 2008.

[15] J. Zakzeski, P. Bruijnincx, A. Jongerius and B.Weckhuysen, Chem.Rev, 2, 2009.

[16] K. Niu, P. Chen, X. Zhang and W. Tan, Journal of Chemical Technology and Biotechnology, 84 (8)1240, 2009.

[17] L. Bandounas, N. Weirckx, UJ.de Winde and H. Ruijssenaars, BMC Biotechnology, 11, 94,2011.

[18] K. DeAngelis, M.Allgaier,Y.Chavarria, J. Fortney, P. Hugenholtz, B. Simmons, K. Sublette, W.Silver and T. Hazen, PLoS ONE 6, 4, 2011.

[19] T. Dittmar and R. Lara, Geochimica et Cosmochimica Acta, 65(9)1417, 2001.

[20] R. Benner, A. Maccubbin and R. Hodson, Applied and Environmental Microbiology, 7(5) 998, 1984.