

## Development of novel enzyme assay procedures to determine demethylation of kraft lignin by wood-decay Fungi: Modifying lignin as substitute for phenol in formaldehyde-based polymers

Balaji Venkatesagowda<sup>1</sup>, Iryna Bastan-Kandybovich, Aneli Barbosa and Robert Dekker  
Biorefining Research Institute, Lakehead University, Canada

Lignin is a highly methylated natural aromatic biopolymer found in the cell walls of plants. Biological demethylation by wood-decay fungi removes O-methyl groups from lignin increasing its phenolic content. The objective was to demonstrate a new enzyme associated with lignin demethylation (lignin demethylase, LDM) that cleaves O-methyl groups from benzyl rings comprising lignin making the latter more phenolic in structure to serve as phenol substitutes in formaldehyde-based polymers. Assays for LDM activity were developed that specifically measured (i) methanol liberated as a consequence of demethylation, followed by (ii) an increase in pyrocatecholic structure content of modified lignin. Wood-decay fungi isolated from the Boreal Forest (31 isolates growing solely on Kraft lignin, KL) were screened for enzymes that modify lignin. An assay for LDM consisted of coupling an enzymatic oxidation step using alcohol oxidase to convert methanol released to formaldehyde and hydrogen peroxide, and then employing specific reagents to yield colored complexes measured spectrophotometrically. Five biochemical methods were developed to determine LDM using KL and lignin-like model compounds (LMC; guaiacol, veratryl, syringyl alcohols). Formaldehyde was measured using (i) pentan-2,4-dione/ammonium acetate. (ii) Hydrogen peroxide was assayed against ABTS in the presence of horseradish peroxidase. The increase in pyrocatecholic content of modified LMC's and KL as consequence of demethylation, was determined spectrophotometrically by either (iii) complexation with Ti(III)-nitrilotriacetic acid, or (iv) reaction with 4-aminoantipyrine. Biologically modified KL structures were determined by FT-IR. SIFT-MS(v), a highly sensitive procedure that measures volatile organic compounds, was also used to measure methanol, and demonstrated unequivocally that methanol was released from LMC's and KL examined. The potential of ligninolytic enzymes to modify KL and LMCs was performed on 6 ligninolytic fungal isolates, and the results obtained are discussed. Supported by NSERC-CRD.

### Biography

Balaji Venkatesagowda completed his PhD at the University of Madras (India) and joined the Biorefining Research Institute in 2010. His specialty is biodiesel production from seed oils using fungal lipases. Lately, his research focuses on biological modification of lignin for polymer applications.

kvsbalaj@gmail.com