

## AN OXIDANT TO REPLACE NITROBENZENE IN LIGNIN ANALYSIS

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Four metal organic frameworks (MOFs) are being evaluated as possible catalysts for alkaline lignin oxidation. One aspect of the screening process is the oxidation of in-situ hardwood lignin by a high mole ratio of Cu, Fe, or Cu + Fe contained in the MOFs. The MOF's were prepared in a microwave-assisted synthesis. One of the MOFs, a benzenetricarboxylic acid complex of Cu (II) and Fe (III), converted the phenylpropane (C<sub>9</sub>) units in in-situ poplar lignin to approximately 50% monomers. The ratio of syringyl (S) to guaiacyl (G) monomers was similar to the S:G ratio obtained from nitrobenzene oxidation (NBO). The S:G ratio from NBO was then compared to that from MOF oxidation (MOFO) for two other poplars. The S:G ratios for the three poplars by MOFO were 1.20, 1.51 and 1.67, respectively while the corresponding NBO values were 1.25, 1.45 and 1.68. These initial results indicate that MOFO could be developed into a credible replacement for NBO, because it affords a higher yield of monomers when a large MOF dose is used. Some preliminary results are also presented for MOF acting as a catalyst (100 ppm Cu (II) plus 81 ppm Fe (III) in NaOH) for oxygenation of the hardwoods. The products from O<sub>2</sub> + MOF are identical to MOF only, but the ratios amongst them are different.

*Keywords:* Metal organic frameworks; Metal oxides; S:G ratio; Hardwoods; Lignin oxidation.

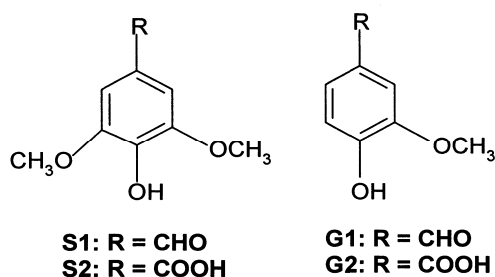
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## INTRODUCTION

Four metal organic frameworks (MOFs) are being evaluated as possible catalysts in our laboratories. The reaction of interest is lignin oxidation by oxygen in hot alkali to produce the four monomers shown in Fig. 1. One aspect of the screening process is straight oxidation of in-situ lignin in milled hardwood with a high mole ratio of Cu (II), Fe (III), or Cu (II) + Fe (III) to phenylpropane (C<sub>9</sub>) units. The Cu (II) or Fe (III) would be obtained from CuO, Fe<sub>2</sub>O<sub>3</sub>, or the MOFs.

One of the MOFs gave a higher total yield of the products in Fig. 1 than was obtained with nitrobenzene oxidation (NBO), and the ratio of (S1 + S2)/(G1 + G2) appeared to be similar. A new project was initiated to see if MOFO could replace NBO in determining the syringyl to guaiacyl (S:G) ratio of hardwood and other lignins. Nitrobenzene is a liquid with low volatility at room temperature but is a confirmed

animal carcinogen (Lewis 2008). The only volatile organic that is involved in the synthesis of MOF's is N, N-diethylformamide, a non-carcinogen (Fisher Scientific 2008). Although the health risk associated with the use of nitrobenzene in the laboratory is minimal, it is a good practice to investigate replacements that may be safer. We are now reporting on a comparison between NBO and MOFO in determining the S:G ratio of three poplars. Some preliminary results are also presented on generation of the four S and G products by oxygenation of hardwood catalyzed by a low concentration of the MOF in hot NaOH.



**Figure 1.** Products from metal oxide, nitrobenzene, and MOF oxidation of hardwood lignin.

## MATERIALS AND METHODS

### Poplar Wood Meals

The three extractive-free poplar wood meals were previously described in an earlier paper, where their S:G ratios were determined by NBO (Bose et al. 2009). An extractive-free sugar maple (*Acer saccharum*) wood meal was also investigated. The lignin content of poplar D 109 was 25.4%, while it was 17.6% for D 105; 20.4% for Crandon, and 25.0% for sugar maple.

### Synthesis of Cu<sup>2+</sup>/Fe<sup>3+</sup> Metal-Organic Framework of 1,3,5-Benzenetricarboxylic Acid

In a typical synthesis of the Cu<sup>2+</sup> - Fe<sup>3+</sup> metal-organic framework ferric nitrate nonahydrate (2.62 g or 6.5 mmoles of Fe(NO<sub>3</sub>)<sub>3</sub> · 9 H<sub>2</sub>O) was dissolved in 42.4 g of the solvent, N,N-diethylformamide (DEF), in a 100 mL round bottom flask. The mixture was stirred until a clear, orange solution was obtained. To this solution was added 1.83g (8.7 mmoles) of the linker, 1,3,5-benzenetricarboxylic acid, and the mixture was stirred until all of the solid acid was dissolved. Cu(NO<sub>3</sub>)<sub>2</sub> · 2½ H<sub>2</sub>O (1.52g or 6.5 mmoles) was then added to the DEF solution of Fe (III) and carboxylic acid. The solution was stirred until all of the copper compound was dissolved and an optically dense, homogenous, brown solution was obtained. The flask was placed in the microwave synthesizer (Discover Model, CEM Corporation) that was fitted with a water-cooled condenser. The condenser was connected to an ice-cooled collector to retain solvent that might by-pass the condenser. The instrument was programmed to heat the sample to 90°C and to hold the sample at 90°C for 10 min. The maximum power input to the reaction mixture was

200W. The rate of application of microwave energy to the reaction mixture was automatically attenuated during the synthesis so that the power was sufficient, during the heating cycle, to rapidly attain the reaction temperature (90°C), then reduced to a lower level, sufficient only to maintain the mixture at the reaction temperature. Following the reaction, it was observed that the flask was filled with a dark, olive-green solid product consisting principally of masses of small wet granules surrounded by a lesser quantity of a thick microcrystalline-MOF/DEF suspension. Typically, 90-92% of all the DEF could be accounted for as occluded in the wet product. The product was stored under a constant flow of air, at room temperature, for two days, resulting in the removal of all solvent. To assess the porosity and stability of the dry solid, a sample of the solid was reheated at the synthesis temperature for 10 min. in DEF. The solid was observed to maintain its structural integrity; the mass of the DEF absorbed during the procedure was approximately twice that of the dried solid. This preliminary work suggests that these solids may be mesoporous, possessing both a permanent porosity and a high surface area. These properties would be important in the action of these solids as catalysts for the oxidative processes that we report here. The first preparation of this MOF contained 6.9% Cu and 5.5% Fe, as measured by ICP-AES analysis. A second preparation was made at a later date and it contained 6.2% Cu and 5.0% Fe.

### **Metal Oxide, MOF or O<sub>2</sub> + MOF Oxidation of Wood Meals**

The nearly identical procedures for nitrobenzene, MOF, CuO, and Fe<sub>2</sub>O<sub>3</sub> oxidation of hardwood lignin to the products in Fig. 1 are documented in the literature (Chen 1992; Bose et al. 2009). A sample of extracted wood meal (equivalent to 50mg of lignin) was added to a small stainless steel autoclave along with 40 ml of 2M NaOH and a known mass of MOF, CuO, or Fe<sub>2</sub>O<sub>3</sub>. The autoclave has a valve for gas addition and an O<sub>2</sub> partial pressure of 0.30 MPa was used for O<sub>2</sub> + MOF. The autoclave was tightly sealed with a screw cap fitted with a Teflon gasket and placed in a 170°C oil bath for 2.0h with occasional mixing. At the end of the reaction the autoclave was removed and then cooled with ice cold water. The reaction mixture was filtered on Whatman 1 filter paper and washed with 2M NaOH solution. The filtrate was then extracted with 3 x 50 ml of CHCl<sub>3</sub>. The pH of the aqueous phase was then adjusted to approximately 2-3 with concentrated HCl and extracted again with diethyl ether (3 x 50 ml). The internal standard, 2,6-dimethoxyphenol, was added to the ether extract, which was then dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness at reduced pressure. The residue was dissolved in 2 ml of dichloromethane. A small portion (0.1 ml) was taken in a vial and 0.1 ml of BSTFA with a drop of pyridine was added to it for silylation prior to GC analysis using a DB-17 or SE-30 capillary column.

## **RESULTS AND DISCUSSION**

### **Calculation of S:G Ratio from Yields of S and G Products**

During alkaline lignin oxidation most, if not all, of the yield of S1, S2, G1, and G2 (Fig. 1) is believed to be derived from the uncondensed fraction of the lignin (Bose et al. 2009). An uncondensed C<sub>9</sub> unit is defined as one that does not contain a C-C bond at

any ring position except for C-1 (sidechain) nor connected to another C<sub>9</sub> unit by a diaryl ether linkage. In the earlier investigation (Bose et al. 2009), hardwood lignins were analyzed by permanganate oxidation and NBO, both under alkaline conditions. Permanganate oxidation was used to quantify condensed and uncondensed C<sub>9</sub> units in wood meals from two poplars plus a 1:1:1 mixture of sugar maple (*Acer saccharum*), paper birch (*Betula papyrifera*), and cottonwood (*Populus deltoides* Bartr.). It was determined that 66.6% of the guaiacyl C<sub>9</sub> units in the hardwoods were uncondensed, while the value was 96% for the syringyl fraction. The 96% value for the S fraction was nearly identical for the three hardwoods, while the 66.6% value for the G fraction was an average of 65.4%, 65.4%, and 69.0% (Bose et al. 2009). If the yield of (S1 + S2) was to be divided by 0.96 and the yield of (G1 + G2) divided by 0.666, then the relationship in equation [1] would be obtained. Further research is needed on this 0.694 correction factor in equation [1]. However, it is likely that an average value for most hardwoods would be in the range of 0.70. In their comprehensive examination of the lignin in *Eucalyptus globulus*, Evtuguin et al. (2001) found that the uncondensed fraction of the G units was 61.0% while the value was 84.4% for the S fraction. Those two numbers would give a correction factor of 0.722.

$$\text{S:G Ratio} = 0.694 (\text{S1} + \text{S2})/(\text{G1} + \text{G2}) \quad [1]$$

The 0.694 correction factor in equation [1] enhances the credibility of NBO and other oxidation techniques by providing a more realistic value of the S:G ratio. The lower corrected S:G value would correlate better with the methoxyl content of the lignin. The lignin of aspen (*Populus tremuloides*) has been widely investigated, and literature data will be used to demonstrate the significance of the 0.694 correction factor. Typical values of uncorrected S:G ratios determined by NBO for aspen are approximately 2.5 (Kavanagh and Pepper 1955; Pepper et al. 1967). The lowest value we could find was 2.21 (Funaoka and Lai 1993). Aspen lignin is known to contain ~5% (C<sub>9</sub> basis) of p-hydroxyphenylpropane units (Pearl and Beyer 1960; Larson 1967). If the remaining 95 C<sub>9</sub> units are divided into a S:G ratio of 2.21, then the lignin should have a methoxyl content of 1.60 OCH<sub>3</sub>/C<sub>9</sub>. This number is much greater than some of the higher methoxyl contents (1.44 – 1.47 OCH<sub>3</sub>/C<sub>9</sub>) that are reported for aspen lignin (Musha and Goring 1975; Chua and Wayman 1979; Obst and Landucci 1986). When the 0.694 correction factor is applied to the S:G ratio above, a new value of 1.53 (2.21x 0.694) is obtained, and this would correspond to 1.52 OCH<sub>3</sub>/C<sub>9</sub> unit. This is much closer to the 1.44 – 1.47 OCH<sub>3</sub>/C<sub>9</sub> discussed above.

### S:G Ratios by Metal Oxide and MOF Oxidations

The yields of the four products (Fig. 1) from CuO, CuO + Fe<sub>2</sub>O<sub>3</sub>, and MOF oxidation are documented for the D 109 poplar in Table 1. The values are in mmoles of products per 100 mmoles of C<sub>9</sub> units. A wide range of literature data was examined and it appears as if the structural formula for various native hardwood lignins is fairly constant, except for the number of methoxyl groups. The average formula is close to C<sub>9</sub>H<sub>8.7</sub>O<sub>2.9</sub>(OCH<sub>3</sub>)<sub>x</sub> with x varying from 1.30 to 1.57 (Gellerstedt et al. 1988, Fengel and Wegener 1989; Dence and Lin 1992). If x = 1.50 is assumed, then a MW of 210 is obtained. It

should also be noted that varying  $x$  from 1.30 to 1.57 only changes the  $C_9$  MW by 8.4 units. A constant  $C_9$  MW of 210 is used throughout this paper.

A S:G ratio of 1.25 was previously obtained for this poplar by NBO (Bose et al. 2009). When the poplar was oxidized by metal oxides and MOF, wood meal corresponding to 50 mg of lignin was used (lignin content given in Materials & Methods). It can be seen that 100mg of Cu (II) from CuO gave a higher yield of products as compared to 50mg of Cu (II) + 50mg of Fe (III) from  $Fe_2O_3$ . When 100 mg of Fe (III) was added to 100 mg of Cu (II), the total product yield increased from 35.7% to only 37.1%. When the MOF (138 mg Cu + 110 mg Fe) was used, the total product yield was ~33% higher than when CuO +  $Fe_2O_3$  (100 + 100) were used. The first MOF preparation was used, and it contained 6.9% Cu and 5.5% Fe (Materials & Methods). There was a clear trend of a lower S:G ratio as the total product yield increased. The S:G ratio from MOF was of great interest because it was obtained from a total product yield of 49 monomers/100  $C_9$  units and the 1.20 value obtained was close to the 1.25 value obtained from a total product yield of 42% by NBO (Bose et al. 2009). The ratio of G1 to G2 and S1 to S2 were 2.0 and 14.2, respectively for MOFO. The corresponding values for NBO were 15.7 and 14.9. These results are not tabulated. In most trials MOFO afforded a much higher yield of vanillic acid (G2) as compared to NBO.

**Table 1.** Oxidation of D 109 Poplar Wood Meal with CuO, CuO +  $Fe_2O_3$ , and MOF (wood meal equivalent to 50 mg of lignin)

Oxidant	S1	G1	S2	G2	Total Yield <sup>1</sup>	S:G Ratio <sup>2</sup>
Cu (100 mg Cu) <sup>3</sup>	21.1	8.2	2.3	4.1	35.7	1.32
Cu + Fe (50 + 50) <sup>4</sup>	20.2	7.5	2.0	3.8	33.5	1.36
Cu + Fe (100 + 100)	21.6	8.5	2.5	4.5	37.1	1.28
MOF (138 + 110)	28.8 (29.3) <sup>5</sup>	11.9 (12.0)	2.0 (2.1)	5.9 (6.1)	48.6 (49.5)	1.20 (1.20)

<sup>1</sup> mmoles products/100 mmoles of  $C_9$  units;  $C_9$  MW estimated at 210 (see text)

<sup>2</sup> Calculated from equation [1]; a value of 1.25 was obtained by NBO

<sup>3</sup> 100 mg of Cu from CuO

<sup>4</sup> 50 mg Cu from CuO + 50 mg Fe from  $Fe_2O_3$

<sup>5</sup> Oxidation performed in duplicate

Typical chromatograms for the reaction products from CuO and MOF oxidation are shown in Fig. 2. The peaks at 26.5 min. and 28.1 min are characteristic of C-5 condensed dimers. Their concentrations were quite variable, but always low. This was also the case for NBO.

A second batch of the MOF was prepared (6.2% Cu and 5.0% Fe), and it was used to oxidize two other poplars. These poplars were extensively analyzed by NBO, and we are very confident about the S:G ratios obtained by that method (Bose et al. 2009). The first is a poplar identified as D 105 with an S:G ratio of 1.68, and the second is a poplar called Crandon with an S:G ratio of 1.45 (Bose et al. 2009). The D 105 was investigated first, and an MOF dose of 112mg Cu + 90mg Fe afforded a total product yield of 56.2 monomers/100  $C_9$  units and a S:G ratio of 1.64. The same dosage was used at a later date, and a total yield of 48.2 monomers/100  $C_9$  and S:G of 1.69 were obtained (Table 2). The total product yield was 46% for D 105 when NBO was used (Bose et al. 2009). The next experiment was with the Crandon wood meal, and only 56mg of Cu +



MOF alone. Also, product yield increased with an increase in the S:G ratio from these trials. These results would suggest that in oxygen delignification of hardwood chemical pulps, a furnish whose lignin is rich in syringyl units, should afford a higher rate of overall lignin (S+G) solubilization.

**Table 2.** Oxidation of D 105 (D) and Crandon (C) Wood Meal (50 mg of lignin) with MOF

Oxidant	S1	G1	S2	G2	Total Yield	S:G Ratio <sup>1</sup>
MOF (112 + 90) <sup>2</sup> (D)	34.8	14.7	4.7	2.0	56.2	1.64 (1.68)
MOF (112 + 90) <sup>3</sup> (D)	31.1	10.8	3.1	3.2	48.2	1.69 (1.68)
MOF (56 + 45) (C)	22.1	6.9	3.9	5.0	37.9	1.51 (1.45)

<sup>1</sup> S:G ratio by NBO in parentheses; similar calculation methodologies to Table 1

<sup>2</sup> mg of Cu + mg of Fe in parentheses

<sup>3</sup> Repeat experiment at a later date

**Table 3.** Catalytic Oxygenation of hardwoods to produce the monomers in Fig. 1

Hardwood	S1	G1	S2	G2	Total Yield	S:G Ratio <sup>1</sup>
D-105	39.5	17.1	4.44	7.51	68.6	1.24
	39.7	17.1	4.45	7.72	68.9	1.23
	39.5	17.1	4.49	7.55	68.7	1.24
Crandon	26.3	12.3	3.32	4.84	46.7	1.20
D-109	23.6	13.8	2.51	4.00	43.9	1.02
Sugar Maple	18.1	11.0	1.64	3.93	34.7	0.93

<sup>1</sup> similar calculation methodologies to Table 1

## CONCLUSIONS

Results are presented showing that when a MOF was used to oxidize in-situ hardwood lignins in 2M NaOH, the calculated S:G ratio was similar to that obtained by NBO. The comparison of S:G ratios for three poplars (NBO value given first) were 1.25 vs. 1.20; 1.68 vs. 1.67; and 1.45 vs. 1.51. The MOF is relatively simple to synthesize and all the required reagents are commercially available. Preliminary results are also presented for oxygenation catalyzed by a low concentration of the MOF in the 2M NaOH. This method appears promising as a means of producing benzaldehydes and benzoic acids from hardwood lignin.

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