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Supplementary Material

The Effect of Acetylation on Iron Uptake and Diffusion in Water Saturated Wood Cell Walls and Implications for Decay

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S1. Iron and oxalate concentrations in lumen water during brown rot decay

As the findings appear to have a very strong dependence on oxalate concentration, we provide here a table of Fe and oxalate concentrations found in water squeezed from wood blocks during brown rot degradation. Given that the presence of oxalate has such a strong effect on the results obtained, it is interesting to note that the oxalate concentration used in this work was on the low end of this range.

Table 1. Iron and oxalate concentrations in lumen water of wood blocks during brown rot degradation.

	Fungus	Time in Culture	Fe (μM)	Oxalate (μM)
This Work			100	1,000
Suzuki 2006 [1]	*Gloeophyllum traebeum*	1–5 wks	11–12	500–1,100
Wei 2010 [2]	*Postia placenta*	1–3 weeks	36–39	1,600–21,700
Korripally 2013 [3]	*Serpula lacrymans*	8–14 days	460–470	8,700–12,500

S2. Iron species in the solution

S2.1 Oxalate Solution

Because the focus of this work is the transport of iron species, it is instructive to explore the chelation state of the iron under the two solution conditions, oxalate and acetate buffer. Because of its importance in both biology and geochemistry, iron in water solution has long been the subject of studies. In addition, the complexity of its behavior in water has led to many experimental difficulties. Under highly acidic conditions, where hydrolysis is suppressed, the dominant iron species has all six of its octahedral chelation sites occupied by associations with the oxygen lone pair electrons from six water molecules. As the pH rises, i.e., hydronium ion concentration falls, hydrolysis of water leads to the formation of hydroxides. These hydroxides can then condense and form a wide range of multinuclear hydroxide and oxo complexes, some of which are insoluble [4]. This hydrolysis chemistry can be suppressed by the introduction of strong ligands, as shown by the difference in oxo products when oxalate is present (Figure S1), and absent (Figure S2).

To accomplish the task of estimating the concentration of the various iron species in solution a collection of stability constants is required. While the National Institute of Standards and Technology (NIST) has ended support for its NIST Standard Reference Database 46: NIST Critically Selected

Stability Constants of Metal Complexes Database, we have been able to develop a Microsoft Access version of the database from the archived data files [5]. The Access file is available from the authors. All equilibrium constants used in the subsequent calculations are obtained from this Access database. In cases where data spanned a range of ionic strengths, a quadratic fit was used to extrapolate to the desired ionic strength. In other cases where data are limited the single value with the closest ionic strength was chosen. All constants were for 25 °C.

Given that the solutions in this work were dilute, ionic strength < 0.2 M, one can assume that activity coefficients are near 1 and the activities of each of the species is equal to its concentration. Determination of speciation of various components then involves a series of equilibrium expressions and corresponding balance equations. Algebraic manipulation of these equations results in polynomial equations, with solutions being the relevant concentrations.

S2.1. Oxalate Solution

S2.1.1 Concentrations

Oxalic acid (Oxt): 1,000 μM with sufficient NaOH to bring the pH to 4.0
Iron (Fet): 100 μM as FeCl3 (for this derivation we assume that chloride is not significant)

S2.1.2 Equilibrium expressions

K1 = [FeOx]/[Fe][Ox] (log(K1) = 7.493 at I = 0.007)
K2 = [FeOx2]/[Fe][Ox]2 (log(K2) = 13.81 at I = 1)
K3 = [FeOx3]/[Fe][Ox]3 (log(K3) = 18.6 at I = 1)
Ka1 = [H2Ox]/[HOx][H] (log(Ka1) = 1.163 at I = 0.007)
Ka2 = [HOx]/[Ox][H] (log(Ka2) = 3.839 at I = 0.007)
KOH1 = [FeOH]/[Fe][OH] (log(KOH1) = 11.36 at I = 0.007)
KOH2 = [Fe(OH)2]/[Fe][OH]2 (log(KOH2) = 22.16 at I = 0.007)
Kw = [H][OH] (log(Kw) = -13.783 at I = 0.007)

S2.1.3 Balance Equations

Oxt = [FeOx] + 2[FeOx2] + 3[FeOx3] + [HOx] + [H2Ox] + [Ox]
Fet = [FeOx] + [FeOx2] + [FeOx3] + [FeOH] + [Fe(OH)2] + [Fe]

S2.1.4 Substitution and rearrangement

K3(1+Ka2[H]+Ka1Ka2[H]2)[Ox]4 +
(K2(1+Ka2[H]+Ka1Ka2[H]2) + K3(3Fet-Oxt))[Ox]3 +
(K1(1+Ka2[H]+Ka1Ka2[H] 2) + K2(2Fet-Oxt))[Ox]2 +
((1+Ka2[H]+Ka1Ka2[H] 2) (KOH2*Kw*Kw/[H]2 + KOH1*Kw/[H] + 1) + K1(Fet-Oxt))[Ox] -
Oxt(KOH2KwKw/[H] 2 + KOH1*Kw/[H] + 1) = 0

This polynomial is quartic in free oxalate concentration [Ox], for which, the largest positive real root is the correct desired solution. Once the concentration of [Ox] is known all the other concentrations can be calculated by substitution. A Python code to solve and plot the results was developed and is attached at the end of this document.

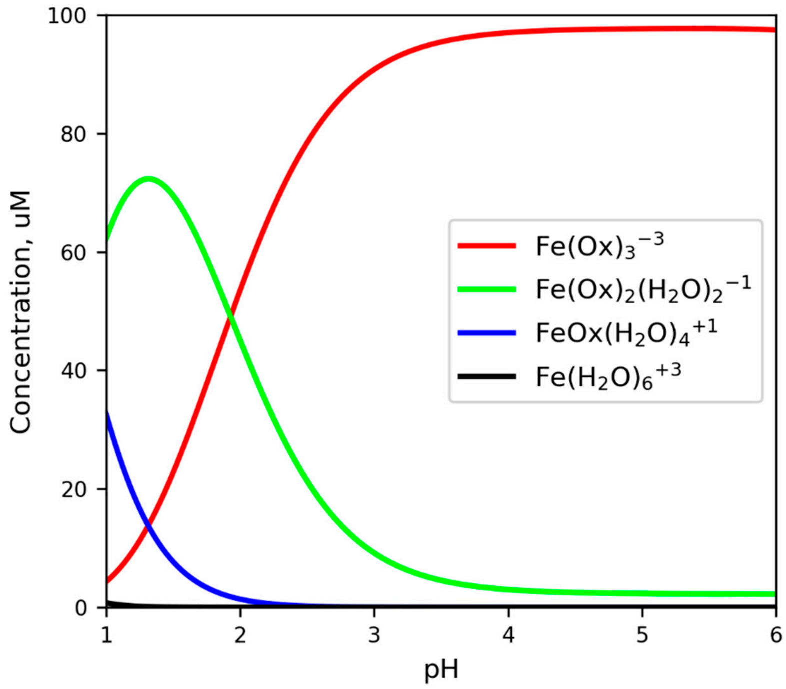

Figure S1. Iron species concentration for a solution of 100 μM iron(III) chloride in 1000 μM oxalate buffer at various pH values.

S2.2 Acetate Solution

S2.2.1 Concentrations

Acetic acid (Act): 100,000 μM with sufficient NaOH to bring the pH to 4.0
Iron (Fet): 200 μM as FeCl3
Chloride (Clt): 3Fet = 600 μM

S2.2.2 Equilibrium expressions

KOH1 = [FeOH]/[Fe][OH] (log(KOH1) = 11.27 at I = 0.2M)
KOH2 = [Fe(OH)2]/[Fe][OH]2 (log(KOH2) = 21.93 at I = 0.2M)
KOH3 = [Fe(OH)3]/[Fe][OH]3 (log(KOH3) = 30.20 at I = 0.2M)
KCl1 = [FeCl]/[Fe][Cl] (log(KCl) = 0.776 at I = 0.2M)
KAc = [FeAc]/[Fe][Ac] (log(KAc) = 3.30 at I = 0.2M)
Ka = [HAc]/[Ac][H] (log(Ka) = 4.60 at I = 0.2M)
Kw = [H][OH] (log(Kw) = -13.78 at I = 0.2M)

S2.2.3 Balance Equations

Act = [FeAc] + [HAc] + [Ac]
Fet = [FeOH] + [Fe(OH)2] + [Fe(OH)3] + [FeCl] + [FeAc] + [Fe]
Clt = [FeCl] + [Cl]

S2.2.4 Substitution and rearrangement

(KOH3 Kw Kw Kw/[H]3 + KOH2 Kw Kw/[H]2 + KOH1 Kw/[H] + 1)K1Cl K1Ac[Fe]3 +
((KOH3 Kw Kw Kw/[H]3 + KOH2 Kw Kw/[H]2 + KOH1 Kw/[H] + 1)(Ka[H]+1.0)(K1Cl+K1Ac) + K1Cl K1Ac(1+Clt+Act-Fet))[Fe]2 +

((KOH3 Kw Kw Kw/[H]3 + KOH2 Kw Kw/[H]2 + KOH1Kw/[H] + 1 +Clt-Fet))(Ka[H]+1) + K1Ac*(Act-Fet))[Fe] - Fet *(Ka*[H]+1.0)

This cubic equation in [Fe] was again solved and the results plotted using Python.

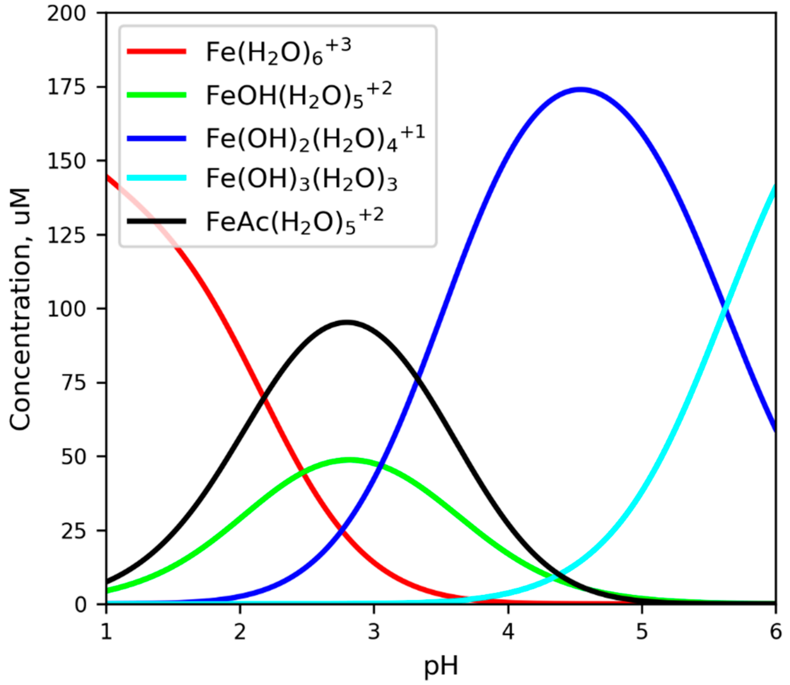

Figure S2. Iron species concentration for a solution of 200 μM iron(III) chloride in 100 mM acetate buffer at various pH values.

Our experiments were conducted in a solution adjusted to pH 4.0, with sodium hydroxide. Note the combined concentration of acetate and acetic acid was 100 mM, which is 500 times the iron concentration. Inspection of Figure S2 shows that, even with a large excess of acetate, it is only a minor component of the ligand sphere. While many iron-centered species can form under these conditions, the model predicts that the hydrolysis of water leads to the majority of the solution species being Fe(OH)2 (H2O)4 at pH 4. This leaves open coordination sites on the iron available for reaction with hydroxyl groups on hemicellulose, cellulose, and lignin. It should be noted that the Ksp of iron oxide under these conditions indicates that precipitation will occur, fortunately that rate of the oxide formation should be slow under these conditions, and likely not significant over the 48 hours of these experiments. This lack of precipitate was confirmed by the very clear (not orange) color of the 18 L of soak solution even when viewed at the end of the acetate experiment, ~25 cm deep in a clean white bucket.

S3. Python Code for Calculating and Plotting Iron Speciation

S3.1 Oxalate Buffer

```
#this is a plot of Oxalate iron speciation, using Pythion version 3.8.3
#Carl Houtman 5/6/20 carl.houtman@usda.gov

import numpy as np
import matplotlib.pylab as plt
import sys

numberPoints = 400
```

```
# input concentrations
Ot = 1000.0/1.0e6                           # uM Oxalate
Fet = 100.0/1.0e6                           # uM Iron
pH = np.linspace(1.0, 6.0, numberPoints)    # pH range
H = np.power(10.0, -pH)                     # convert to [H+]

# log equilibrium constants all from NIST 46
pKa1 = 1.163          #H + HOx --> H2Ox
pKa2 = 3.839          #H + Ox --> HOx
pK1 = 7.493           #Fe + Ox --> FeOx
pK2 = 13.81           #Fe + 2Ox --> FeOx2
pK3 = 18.6            #Fe + 3Ox --> FeOx3
pKOH1 = 11.360        #Fe + OH --> FeOH
pKOH2 = 22.162        #Fe + 2OH --> FeOH2
pKw = -13.783         #H + OH --> H2O

# convert from Log10
Ka1 = np.power(10.0, pKa1)
Ka2 = np.power(10.0, pKa2)
K1 = np.power(10.0, pK1)
K2 = np.power(10.0, pK2)
K3 = np.power(10.0, pK3)
KOH1 = np.power(10.0, pKOH1)
KOH2 = np.power(10.0, pKOH2)
Kw = np.power(10.0, pKw)

# coefficients of the polynomial solution for the concentration of oxalate
C4 = K3*(Ka1*Ka2*H*H + Ka2*H + 1.0)
C3 = K2*(Ka1*Ka2*H*H + Ka2*H + 1.0) + K3*(3.0*Fet - Ot)
C2 = K1*(Ka1*Ka2*H*H + Ka2*H + 1.0) + K2*(2.0*Fet - Ot)
C1 = (Ka1*Ka2*H*H + Ka2*H + 1.0)*(KOH1*Kw/H + KOH2*Kw*Kw/(H*H) + 1.0) +
K1*(1.0*Fet - Ot)
C0 = - Ot*(KOH1*Kw/H +
KOH2*Kw*Kw/(H*H) + 1.0)

# solve the polynomial
Ox = np.zeros(numberPoints, dtype = np.double)
for i in range(numberPoints):
    Ox[i] = np.max(np.real(np.roots([C4[i], C3[i], C2[i], C1[i], C0[i]])))     # the np.max picks the
only real positive root

# do the calculations
Fe = Fet/(1.0 + K1*Ox + K2*Ox*Ox + K3*Ox*Ox*Ox + KOH1*Kw/H + KOH2*Kw*Kw/(H*H))
H2Ox = Ka1*Ka2*Ox*H*H
HOx = Ka2*Ox*H
FeOx = K1*Fe*Ox
FeOx2 = K2*Fe*Ox*Ox
FeOx3 = K3*Fe*Ox*Ox*Ox
FeOH = KOH1*Kw*Fe/H
FeOH2 = KOH2*Kw*Kw*Fe/(H*H)
```

```
# set our colors
color1 = [256./256.,0./256.,0./256.]
color2 = [0./256.,256./256.,0./256.]
color3 = [0./256.,0./256.,256./256.]
black = [0,0,0]
gray = [0.35,0.35,0.35]

# start the frame
figure1=plt.figure(figsize=(4.5,4.0), dpi=300) # size in inches
#plt.tight_layout()
ax1 = plt.subplot(111)

# add the plots
ax1.plot(pH,FeOx3*1e6,'-', color=color1, linewidth=2.0, label='$\mathregular{Fe(Ox)_3}^{-3}$')
ax1.plot(pH,FeOx2*1e6,'-', color=color2, linewidth=2.0, label='$\mathregular{Fe(Ox)_2(H_2O)_2}^{-1}$')
ax1.plot(pH,FeOx*1e6,'-', color=color3, linewidth=2.0, label='$\mathregular{FeOx(H_2O)_4}^{+1}$')
#ax1.plot(pH,FeOH*1e6,'--', color=color3, linewidth=2.0, label='FeOH^{+2}')
#ax1.plot(pH,FeOH2*1e6,'-', color=color3, linewidth=2.0, label='FeOH2^{+1}')
ax1.plot(pH,Fe*1e6,'-', color=black, linewidth=2.0, label='$\mathregular{Fe(H_2O)_6}^{+3}$')
ax1.legend(loc='center right', fontsize=10)

#set the axis
ax1.set_ylim([0, Fet*1e6])
ax1.set_ylabel('Concentration, ($\mathrm{\mu}$M)', fontsize=10)
ax1.set_xlim([np.min(pH),np.max(pH)])
ax1.set_xlabel('pH', fontsize=10)
ax1.tick_params(axis='both', which='major', labelsize=8)

#save the figure
plt.savefig('iron_oxalate.png')

# show the result
plt.show()
```

S3.2 AcetateBuffer

```
# show the result
plt.show()
#this is a plot of acetate/hydroxide iron speciation, using Pythion version 3.8.3
#Carl Houtman 5/6/20 carl.houtman@usda.gov

import numpy as np
import matplotlib.pylab as plt
import sys

numberPoints = 300

# input concentrations
Act = 100000.0/1.0e6        # uM Acetate
Fet = 200.0/1.0e6           # uM Iron
Clt = 3.0*Fet               # uM Chloride
```

```
pH = np.linspace(1.0, 6.0, numberPoints)    # pH range
H = np.power(10.0, -pH)                      # convert to [H+]
I = Act+Fet*3.0*3.0+Clt+H                    # ionic strength

# log equilibrium constants all from NIST 46 (fits to ionic strength are from an excel file Oxalate_iron.xlsx)
pK1OH = 11.3621-0.4929*I+0.036971*I*I        # Fe + OH --> FeOH
pK2OH = 22.1658-1.2536*I+0.4305*I*I          # Fe + 2OH --> FEOH2
pK3OH = 30.2                                 # Fe + 3OH --> FEOH3
pK1Cl = 0.8066-0.1617*I+0.0564*I*I           # Fe + Cl --> FeCl
pK1Ac = 3.5670-1.3878*I+0.4265*I*I           # Fe + Ac --> FeAc
pKa = 4.6047-0.0583*I+0.0491*I*I             # H + Ac --> HAc
pKw = -(13.7832-0.01818*I+0.036971*I*I)      # H2O --> H + OH

# convert from Log10
K1OH = np.power(10.0, pK1OH)
K2OH = np.power(10.0, pK2OH)
K3OH = np.power(10.0, pK3OH)
K1Cl = np.power(10.0, pK1Cl)
K1Ac = np.power(10.0, pK1Ac)
Ka = np.power(10.0, pKa)
Kw = np.power(10.0, pKw)

# coefficients of the polynomial solution for the concentration of oxalate
C3 = (K1OH*Kw/H + K2OH*Kw*Kw/(H*H) + K3OH*Kw*Kw*Kw/(H*H*H))*K1Cl*K1Ac
C2 = (K1OH*Kw/H + K2OH*Kw*Kw/(H*H) + K3OH*Kw*Kw*Kw/(H*H*H) + 1.0)*(Ka*H+1.0)*(K1Cl+K1Ac) + K1Cl*K1Ac*(1.0+Clt+Act-Fet)
C1 = (K1OH*Kw/H + K2OH*Kw*Kw/(H*H) + K3OH*Kw*Kw*Kw/(H*H*H) + 1.0 + Clt - Fet)*(Ka*H+1.0) + K1Ac*(Act-Fet)
C0 = -Fet*(Ka*H+1.0)

# solve the polynomial
Fe = np.zeros(numberPoints, dtype=np.double)
for i in range(numberPoints):
    Fe[i] = np.max(np.roots([C3[i], C2[i], C1[i], C0[i]]))    # the np.max picks the only positive root

# do the calculations
Ac = Act/(1.0 + K1Ac*Fe + Ka*H)
Cl = Clt*K1Cl*Fe/(1.0 + K1Cl*Fe)
FeOH = K1OH*Kw*Fe/H
FeOH2 = K2OH*Kw*Kw*Fe/(H*H)
FeOH3 = K3OH*Kw*Kw*Kw*Fe/(H*H*H)
FeCl = K1Cl*Fe*Cl
FeAc = K1Ac*Fe*Ac
HAc = Ka*Ac*H

# set our colors
color1 = [256./256.,0./256.,0./256.]
color2 = [0./256.,256./256.,0./256.]
color3 = [0./256.,0./256.,256./256.]
color4 = [0./256.,256./256.,256./256.]
```

```
black = [0,0,0]

# start the frame
figure1=plt.figure(figsize=(4.5,4.0), dpi=300) # size in inches
#plt.tight_layout()
ax1 = plt.subplot(111)

# add the plots
ax1.plot(pH,Fe*1e6,'-', color=color1, linewidth=2.0, label='$\mathregular{Fe(H_2O)_6}^{+3}$')
ax1.plot(pH,FeOH*1e6,'-', color=color2, linewidth=2.0, label='$\mathregular{FeOH(H_2O)_5}^{+2}$')
ax1.plot(pH,FeOH2*1e6,'-', color=color3, linewidth=2.0, label='$\mathregular{Fe(OH)_2(H_2O)_4}^{+1}$')
ax1.plot(pH,FeOH3*1e6,'-', color=color4, linewidth=2.0, label='$\mathregular{Fe(OH)_3(H_2O)_3}$')
ax1.plot(pH,FeAc*1e6,'-', color=black, linewidth=2.0, label='$\mathregular{FeAc(H_2O)_5}^{+2}$')ax1.legend(loc='best', fontsize=10)

#set the axis
ax1.set_ylim([0, Fet*1e6])
ax1.set_ylabel('Concentration, ($\mathrm{\mu}$M)', fontsize=10)
ax1.set_xlim([np.min(pH),np.max(pH)])
ax1.set_xlabel('pH', fontsize=10)
ax1.tick_params(axis='both', which='major', labelsize=8)

#save the figure
plt.savefig('iron_acetate.png')

# show the result
plt.show()
```

References

1. Suzuki, M.R.; Hunt, C.G.; Houtman, C.J.; Dalebroux, Z.D.; Hammel, K.E. Fungal hydroquinones contribute to brown rot of wood. *Environmental Microbiology* **2006**, *8*, 2214-2223, doi:https://doi.org/10.1111/j.1462-2920.2006.01160.x.
2. Wei, D.S.; Houtman, C.J.; Kapich, A.N.; Hunt, C.G.; Cullen, D.; Hammel, K.E. Laccase and Its Role in Production of Extracellular Reactive Oxygen Species during Wood Decay by the Brown Rot Basidiomycete Postia placenta. *Applied and Environmental Microbiology* **2010**, *76*, 2091-2097, doi:10.1128/aem.02929-09.
3. Korripally, P.; Timokhin, V.I.; Houtman, C.J.; Mozuch, M.D.; Hammel, K.E. Evidence from Serpula lacrymans that 2, 5-dimethoxyhydroquinone is a lignocellulolytic agent of divergent brown rot basidiomycetes. *Appl. Environ. Microbiol.* **2013**, *79*, 2377-2383, doi:10.1128/AEM.03880-12.
4. Byrne, R.; Luo, Y.-R.; Young, R. Iron hydrolysis and solubility revisited: observations and comments on iron hydrolysis characterizations. *Marine Chemistry* **2000**, *70*, 23-35.
5. Smith, R.; Martell, A.; Motekaitis, R. Critically selected stability constants of metal complexes. In *NIST standard reference database*, National Institute of Standards: 2004; Vol. 46, p. 8.