

## Birnessite catalysis of the Maillard reaction: Its significance in natural humification

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Although mineral colloids are known to play a significant role in transforming organic matter in soils and sediments, there still are many gaps in our understanding of the mechanisms of organic-mineral interactions. In this study, we investigated the role of a major oxide-mineral birnessite (a form of Mn(IV) oxide) in catalyzing the condensation reaction between sugars and amino acids, the Maillard reaction, for forming humic substances. The Maillard reaction is perceived to be a major pathway in natural humification. Using a suite of spectroscopic methods (including ESR, XANES, EXAFS and  $^{13}\text{C}$  NMR), our results show that Mn(IV) oxide markedly accelerates the Maillard reaction between glucose and glycine at ranges of temperatures and pH typical of natural environments. These results demonstrate the importance of manganese oxide catalysis in the Maillard reaction, and its significance in the natural abiotic formation of humic substances.

### 1. Introduction

In soils and sediments, humification is pivotal in transforming organic molecules originating from organized structures typical of organisms (e.g., carbohydrates and proteins) to randomly polymerized, heterogeneous humic macromolecules characteristic of geological systems. The Maillard reaction [Maillard 1913] is thought to be a major pathway in humification because of significant similarities between humic substances and melanoidins formed through this pathway involving sugar-amino acid condensations [Ikan *et al.*, 1996]. Evershed *et al.* [1997] detected the presence of characteristic products of the Maillard reaction (alkyl pyrazines) in archaeological plant remains up to 1500 years old from Egypt. Despite the importance of the Maillard reaction, the mechanisms and rates of polycondensations of sugars and amino compounds in natural systems remain obscure [Ikan *et al.*, 1996]. Soil mineral colloids are significant in turnover of organic matter [Shindo and Huang, 1982; Torn *et al.*, 1997]. Manganese oxides are common mineral constituents of soils and sediments, and birnessite [ $\delta$ -Mn(IV) oxide] is one of the most widespread [McKenzie, 1989]. The objective of the present study was to investigate the role of birnessite in the Maillard reaction forming macromolecules typical of humic substances from the polycondensation

of sugars and amino acids. The model reactants, glucose and glycine, used are important constituents of carbohydrates and proteins, respectively, and are released from plant and animal materials by microbial decomposition [McKeague *et al.*, 1986].

### 2. Experimental Methods

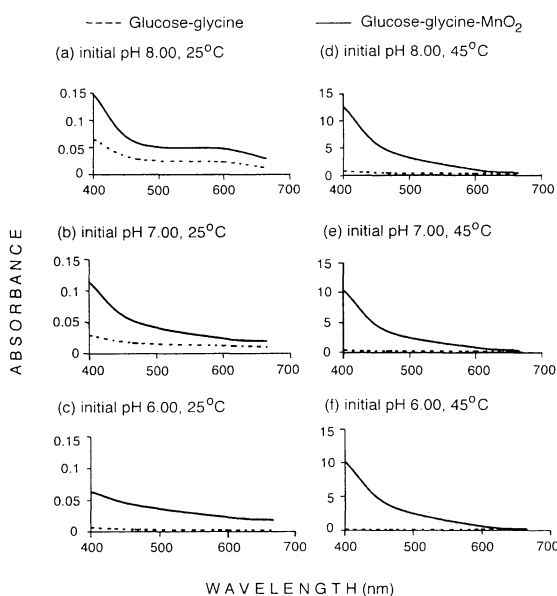
Birnessite was synthesized according to McKenzie's method [1971]. Glucose (ACS reagent grade) and glycine (Sigma Ultra grade purity) were obtained from Sigma Chemical Co. (St. Louis, MO). Sealed flasks, each containing 2.5 g suspended birnessite in a 100 mL solution of either an equimolar mixture of glucose and glycine, or glucose or glycine alone (0.05 mole each), were incubated in an oscillating water bath under ambient light conditions. The pH of the system ranged from 6 to 8. The reaction mixture was incubated for 15 d at 45°C. This is the approximate temperature of the soil's surface on a day when the air temperature is 25°C [Baver, 1956; Jury *et al.*, 1991], and thus frequently occurs in tropical and subtropical areas, and even in temperate areas during the summer. For comparison, we also incubated at 25°C with incubation time extended to 30 d to compensate for the reduced reaction rate at the lower temperature. We maintained sterile conditions by autoclaving all apparatuses used, the water and the birnessite. In addition, we added an antibacterial agent, thimerosal (0.02% w/v, final volume), to the reaction systems. We verified the absence of microbial growth by culturing aliquots from the reaction mixtures on Trypticase Soy Agar (TSA) plates [Dandurand and Knudsen, 1997].

We used visible absorption spectroscopy to monitor the formation of humic substances by measuring changes in absorbance of the supernatant of the reactions systems in the visible range from 400 to 665 nm. The Mn content of the supernatants was determined at 279.5 nm by atomic absorption (AA) spectroscopy. Electron paramagnetic resonance spectrometry was performed using a Bruker B-ER418S spectrometer (Bruker, Germany). We employed x-ray absorption spectroscopy (both XANES, x-ray absorption near-edge structure, and EXAFS, extended x-ray absorption fine structure) to follow the changes in the speciation of Mn in birnessite. Both Mn K-edge XANES and EXAFS spectra were measured at the X16C beamline at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory. The EXAFS data were analyzed by non-linear least square fitting, using theoretical EXAFS signal calculated with FEFF program [Zabinsky *et al.*, 1995].

Humic substances were isolated according to Swift's procedure [1996] with one modification in the acid used. The fulvic acid fraction obtained

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**Figure 1.** Absorbance vs. wavelength plots in the Maillard reaction between glucose and glycine as influenced by birnessite catalysis. (a), (b) and (c): 30 d reaction period. (d), (e) and (f): 15 d reaction period.

from the supernatant of the glucose-glycine-birnessite system was treated only with HCl and not HCl/HF because silica was not present in the system studied. The fulvic acid fraction was analyzed by  $^{13}\text{C}$  CPMAS NMR spectroscopy on a Bruker Avance 360 DRX spectrometer (Bruker Analytik, Rheinstetten/Karlsruhe Germany). Optimal spectral conditions were obtained with a spinning rate of 12 kHz, a contact time of 3.0 ms, and a relaxation delay of 2.0 s. Under these conditions, no rotor effects were observed. Chemical shifts are expressed relative to tetramethylsilane (TMS = 0 ppm).

### 3. Results and Discussion

Adding birnessite to the glucose-glycine system significantly increased the extent of browning compared to a control solution over the pH range 6–8 (Figure 1); the trend was similar both at 25°C and 45°C. Large amounts of Mn(II) accumulated in the supernatant of the glucose-glycine-birnessite system and the glucose-birnessite systems (both at 25°C and 45°C) (Table 1). The presence of Mn(II) was confirmed by electron paramagnetic resonance (EPR) spectroscopy with typical hyperfine

coupling constant  $A = 92.65$  G, characteristic of  $\text{Mn}^{2+}$  six-line splitting, and  $g = 2.0062$  for the system at 25°C (spectra not shown). In the glucose-glycine-birnessite system at 45°C, the pH rose to 7.89 from 7.00 (Table 1). In contrast, there was no significant change in pH in the control system (glucose-glycine), also starting at 7.00. A similar increase in pH was observed at 25°C in the glucose-glycine-birnessite system (7.39 from 7.00) compared to the control system (glucose-glycine) (7.06 from 7.00). The combined pH + pE value of the glucose-glycine-birnessite system at 45°C was lower (10.3) than that of the glucose-glycine system (13.8). Since the pH + pE value is a function of  $\text{O}_2$  concentration, it is a measure of the redox status of a system [Lindsay, 1979]. At 25°C, essentially no variation was seen in the sum of pH + pE values.

Figure 2 gives the Mn K-edge XANES spectra indicating changes in manganese speciation in the different experimental systems: (1) glucose-glycine-birnessite, (2) glucose-birnessite, and (3) glycine-birnessite, all at pH 7.00. Several features in the spectra in the energy region between 6545 and 6565 eV (Figure 2) suggest distinct changes in the oxidation state of Mn in the different samples. We gained more detailed information by deconvoluting the spectra into several gaussian peak functions and an arctangent step function (Figure 2B shows a typical deconvolution). Our fitting showed three major gaussian peaks representing Mn(II), Mn(III), and Mn(IV) states. The precision of the estimates of the different Mn species from XANES deconvolution is in the range of 10 to 20%. The energies corresponding to these different oxidation states, indicated by the letters (a), (b) and (c), are in good agreement with those obtained from several reference Mn-containing compounds [Manceau et al., 1992].

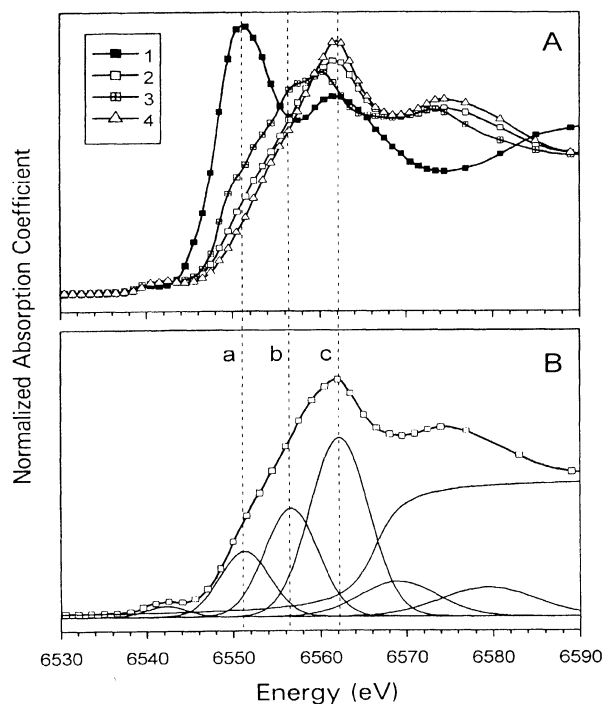
Although atomic absorption (AA) spectroscopic data (Table 1) suggest that a substantial amount of the Mn(II) entered the liquid phase in the glucose-birnessite system, the XANES spectrum of the solid phase of the glucose-birnessite system showed only a slight increase in the amount of reduced Mn(II) compared with the pure birnessite (Figure 2). The amount of Mn(III) remained almost unchanged in the glucose-birnessite system. In contrast to the glucose-birnessite system, only a small amount of Mn(II) entered into solution in the glycine-birnessite system (Table 1). However, the XANES spectrum showed that the relative amounts of both Mn(II) and Mn(III) increased substantially in the solid phase compared to pure birnessite. Thus, it appears that unlike the glucose-birnessite system, where most of the reduced Mn entered the liquid phase, most of the Mn(II) generated in the glycine-birnessite system remained on the birnessite. The reasons for this difference in partitioning of the Mn(II) between the solution and the solid phases in the different systems are not clear, although complexation with different types of ligands may be a factor. The increase of Mn(III) probably suggests that the reduction of Mn(IV) to Mn(II) proceeded through this intermediate stage. Our results show a dramatic increase in the amount of Mn(II), both in the solid and liquid

**Table 1.** Effect of birnessite on glucose and glycine solutions at an initial pH of 7.00

Treatment <sup>a</sup>	Absorbance		pH	Mn ( $\mu\text{g mL}^{-1}$ )	pH + pE
	400 nm	600 nm			
<b>45°C</b>					
Glucose + glycine + birnessite	10.4 ± 0.3	0.74 ± 0.01	7.89 ± 0.08	8286 ± 108	10.3 ± 0.3
Glucose + glycine	0.34 ± 0.02	0.093 ± 0.005	7.03 ± 0.05	0	13.8 ± 0.2
Glucose + birnessite	0.043 ± 0.002	0.027 ± 0.001	7.47 ± 0.08	2797 ± 75	12.0 ± 0.2
Glucose	0.038 ± 0.002	0.022 ± 0.001	6.69 ± 0.04	0	13.2 ± 0.2
Glycine + birnessite	0.021 ± 0.001	Colourless <sup>b</sup>	7.46 ± 0.04	20.9 ± 0.8	13.9 ± 0.1
Glycine	0.020 ± 0.001	Colourless <sup>b</sup>	6.95 ± 0.05	0	13.2 ± 0.2
<b>25°C</b>					
Glucose + glycine + birnessite	0.114 ± 0.007	0.023 ± 0.001	7.39 ± 0.11	2341 ± 56	13.8 ± 0.2
Glucose + glycine	0.025 ± 0.001	0.011 ± 0.001	7.06 ± 0.05	0	14.0 ± 0.3
Glucose + birnessite	Colourless <sup>b</sup>		6.85 ± 0.09	1001 ± 17	13.0 ± 0.3
Glucose	Colourless <sup>b</sup>		6.65 ± 0.07	0	13.4 ± 0.2

<sup>a</sup> The reaction periods were 15 and 30 d, respectively, at 45 and 25°C.

<sup>b</sup> The supernatant was colourless with an absorbance < 0.01 at the wavelengths studied

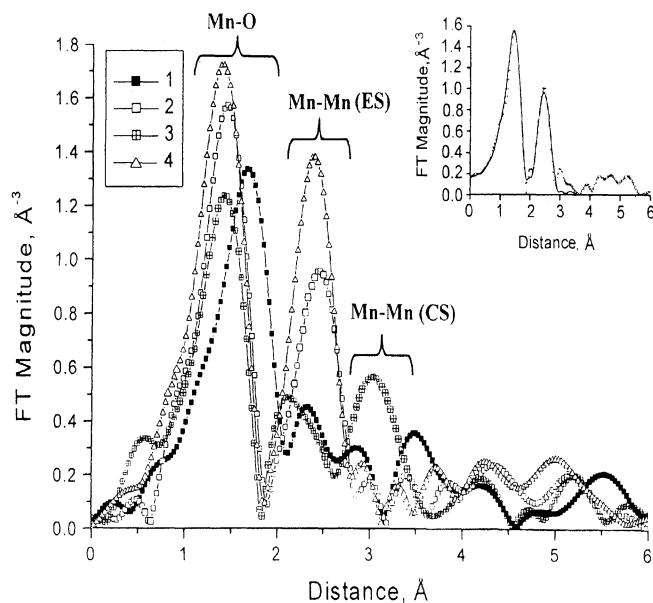


**Figure 2.** (A) Manganese K-edge XANES spectra of the solid phase products of birnessite reaction with glucose and/or glycine in the Maillard reaction. 1: glucose-glycine-birnessite, 2: glucose-birnessite, 3: glycine-birnessite, all at an initial pH 7.00, following reaction at 45°C for 15 d, and 4: pure birnessite. (B) Typical deconvolution of an XANES spectrum (sample 2) into several gaussian peak functions and an arctangent step function which reflects continuum transition of the photoelectron. As described earlier, the three major peaks were taken to represent different oxidation states of manganese (2+ (a), 3+ (b) and 4+ (c)). The two minor peaks at the end were required to obtain a complete fitting of the spectrum, and probably represent unspecified transitions. The relative percent contributions of the +2, +3, and +4 states are for 1: Mn(II) 50%, Mn(III) 10%, Mn(IV) 40%; 2: Mn(II) 20%, Mn(III) 30%, Mn(IV) 50%; 3: Mn(II) 20%, Mn(III) 45%, Mn(IV) 35%; and 4: Mn(II) 10%, Mn(III) 30%, Mn(IV) 60%.

phases, only in the glucose-glycine-birnessite system (Table 1, Figure 2), which also shows maximum browning due to the Maillard reaction. Thus, it is clear that the glucose-glycine-birnessite system involved a redox reaction, with the reduction of Mn(IV) to Mn(II), which then entered the supernatant, and the concomitant oxidation of glucose, possibly forming  $\alpha$ -dicarbonyl compounds which then couple with glycine. Therefore, we suggest that birnessite accelerates the Maillard reaction (abiotic browning) by directly mediating an oxidative coupling between a reducing sugar and an amino acid.

The pH rises as the reaction proceeds (Table 1), because of the consumption of protons in the reduction of birnessite [ $\text{MnO}_2 + 4\text{H}^+ + 2\text{e}^- \leftrightarrow \text{Mn}^{2+} + 2\text{H}_2\text{O}$ ]. The polycondensation reaction between glucose and glycine may proceed on the surface of birnessite generating colored Maillard reaction products. Hedges [1978] noted that for a range of amino acids in the Maillard reaction, the rate of polycondensate formation increased with increasing pH. Therefore, the increase in pH caused by the reduction of the birnessite may also have an added effect in facilitating the polycondensation reaction.

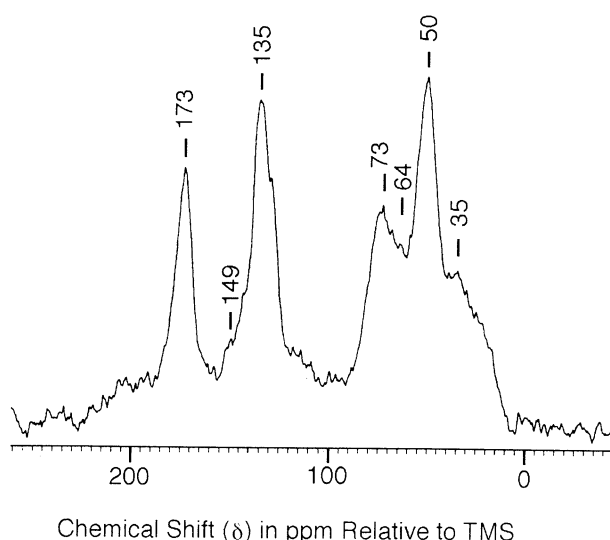
Our EXAFS results provide further support for changes in Mn speciation associated with the Maillard reaction. Figure 3 shows the Fourier-transformed EXAFS spectra for all the samples. The peaks are shifted toward lower distances by ca. 0.5 Å relative to true interatomic distances because of the uncorrected photoelectron phase shift. For pure



**Figure 3.** Fourier transform magnitudes (uncorrected for the phase shift functions) of the  $k^2$ -weighted EXAFS spectra of the solid phase products of birnessite reaction with glucose and/or glycine in the Maillard reaction. 1: glucose-glycine-birnessite, 2: glucose-birnessite, 3: glycine-birnessite, all at an initial pH 7.00, following reaction at 45°C for 15 d, and 4: pure birnessite. The characteristic distances (uncorrected for the phase shift functions) for the Mn-O and two Mn-Mn pairs (originating from either edge-sharing (ES) or corner-sharing (CS)  $\text{MnO}_6$  octahedra) are indicated by brackets. The inset shows the representative fit (solid) to the data for Sample 2 (glucose-birnessite).

birnessite (Sample 4), the range of the Mn-O distance was 1.90-1.94 Å (phase shift adjusted by 0.5 Å) which agrees well with that typical for predominantly Mn(IV) [Wehrli et al., 1995]. The strong peak at ca. 2.4 Å (uncorrected value) corresponds to  $\text{Mn}^{4+}$ - $\text{Mn}^{4+}$  pairs at ca. 2.9 Å originating from the edge-sharing  $\text{MnO}_6$  octahedra in samples 2 and 4. The coordination number and interatomic distances for these pairs were consistent with six edge-sharing octahedral coordination of each Mn site [Silvester et al., 1997]. In the glucose-birnessite system (sample 2), there is apparent domination of Mn(IV) as in birnessite (sample 4) which is in agreement with the XANES results. However, the number of  $\text{Mn}^{4+}$ - $\text{Mn}^{4+}$  pairs per Mn atom is fewer than those in birnessite, while the Mn-O and Mn-Mn distances are the same as in birnessite, within the uncertainties. The disorder in both bond lengths was greater compared with sample 4. In the glycine-birnessite system (sample 3), the contribution of the  $\text{Mn}^{3+}$  state is the strongest, consistent with the XANES data. The presence of  $\text{Mn}^{4+}$ - $\text{Mn}^{3+}$  pairs from the corner-sharing octahedral is notable in Sample 3 data as a peak at 3 Å. The Mn-O distance of  $2.19 \pm 0.01$  Å in glucose-glycine-birnessite (Sample 1) agrees well with that typical for predominantly Mn(II) oxidation state, i.e., 2.208 Å in  $\text{Mn(OH)}_2$  and 2.222 Å in MnO [Christensen, 1965]. The indication that Mn-O distance is shorter (ca. 0.03 Å) than that in reference Mn(II) compounds suggests admixture with Mn(IV) state, consistent with our XANES results. In summary, our EXAFS results accord with the XANES data, indicating birnessite is reduced with the concomitant formation of humified products in the Maillard reaction.

We also verified the formation of humic substances in the glucose-glycine-birnessite system by measuring the  $^{13}\text{C}$  CPMAS NMR spectrum of the isolated fulvic acid (FA) (Figure 4). The literature reveals that humic substances from different sources exhibit similar chemical shifts but their relative intensity may differ substantially [Malcolm and MacCarthy, 1991]. As Figure 4 shows, in the region of 35 ppm, signals arise from



**Figure 4.**  $^{13}\text{C}$  CPMAS NMR spectrum of fulvic acid extracted from the supernatant of the glucose-glycine-bimessite system at an initial pH of 7.00 following reaction at  $45^\circ\text{C}$  for 15 d.

predominantly methylene carbons [Schnitzer, 2000]. A strong peak with a maximum at 50 ppm is attributed to methoxyl carbon [Schnitzer, 2000]. The signals at 64 and 73 ppm are ascribed to carbohydrate structures. The strong resonance signal in the aromatic region with a peak maximum at 135 ppm is attributed either to alkylbenzenes and other aromatic rings [Hatcher et al., 1980; Schnitzer, 2000], or to alkenic moieties conjugated with furanoid or aromatic rings [Benzing-Purdie et al., 1985]. The signal at 149 ppm arises from phenolic or aromatic amine carbon [Hatcher et al., 1980]. Heterocyclic or heteroaromatic products known to be produced by the dehydration of carbohydrates [Ikan et al., 1986] apparently account for the origin of the NMR signals in the aromatic region (Figure 4). The intense peak with maximum at 173 ppm is due to carboxyl- and possibly amide-carbon. Thus, it is clear that the chemical shifts of the FA resemble those of natural humic substances such as soil and stream fulvic acids [Malcolm, 1989; Schnitzer, 2000].

In conclusion, our data clearly show that bimessite greatly accelerates the Maillard reaction between glucose and glycine forming polycondensation products resembling humic substances under ambient conditions. The results indicate that catalysis by mineral colloids such as bimessite merits close attention in the abiotic formation of humic substances through the Maillard reaction in soils and sediments.

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