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SMECTITE-CATALYZED DEHYDRATION OF GLUCOSE

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Abstract—The objective of this study was to determine whether smectites abiotically catalyze transformation of glucose under conditions relevant to soil organic matter (SOM) formation. Four smectites saturated with Na, Ca, Fe and Al were incubated under abiotic conditions with glucose solutions for 21 days at 37ºC. After the incubations, soluble organic C recoveries ranged from 95 to 109.3%, relative to the amount of C added as glucose; however, glucose recoveries in the solutions ranged from 18.3 to 98.3%. The results indicate that a significant amount of the added glucose was abiotically transformed to soluble organic compounds other than glucose during the incubations. In general, glucose recoveries decreased with the acidic character of smectites: SWa-1 > Panther > Otay. Also, within clays, glucose recoveries decreased as the exchangeable cation became more acidic: Na > Ca > Al. Higher glucose recoveries were obtained for 'Fe-rich' smectites relative to 'Fe-poor' smectites, suggesting that Fe-oxyhydroxy coatings on smectite surfaces inhibit the transformation of glucose. High-pressure liquid chromatography analysis of the incubation solutions revealed small peaks for 5-(hydroxymethyl)-2-furaldehyde along with peaks for other unknown compounds. The results suggest that under conditions similar to those found in soils, smectites catalyze glucose dehydration to form furfural compounds. Polymerization of furfural compounds may be a major pathway leading to the formation of new humic materials in soils.

Key Words—Abiotic Transformation, Carbohydrates, Catalysis, Furfural Compounds, Glucose Dehydration, Polymerization, Smectite, Soil Organic Matter Formation.

INTRODUCTION

Soil organic matter (SOM) performs many important functions in soils: it significantly improves stability of soil structure, and thus increases aeration, water-holding capacity and permeability of soils. It also indirectly increases nutrient availability to plants via mineralization of organic forms of N, P, S and other nutrients. From an environmental point of view, SOM reduces transport of organic and inorganic contaminants to surface and groundwater by binding those contaminants to its network. Furthermore, the sequestration of C in SOM is one means of reducing the rate of increase of greenhouse gases in the atmosphere. Therefore, new soil management practices that enhance the formation of SOM are needed. The development of such practices will be furthered by a better understanding of the fundamental chemical processes leading to the formation of new humic materials.

The lignin hypothesis is the most widely accepted theory for the formation of new humic materials in soils (Koivula and Hanninen, 2001). According to this theory, polyphenols and other aromatic degradation products of lignin are abiotically or enzymatically copolymerized with other molecular products of biodegradation to form new humic substances. Aromatic moieties derived from lignin are believed to form the aromatic backbone of humic materials. Although much laboratory-scale research has supported the lignin hypothesis (Haider and Martin, 1976), conclusive evidence of the importance of this mechanism is elusive. Furthermore, there is a simple observation that is problematic for the lignin hypothesis. Woody plants are enriched with lignin relative to grasses, yet prairie soils typically have greater humus content than forest soils.

The Maillard reaction has also been considered for many years as a potential pathway for the formation of humic materials (Ikan *et al.*, 1986; Stevenson, 1982). In the Maillard reaction, reducing sugars and amino acids are condensed to form various melanoidins, brown nitrogenous compounds. The first step in this reaction is the dehydration of the reducing sugars to form various furfural compounds. Under acidic conditions, enolization is the rate-limiting step and β elimination leads to the formation of furfural compounds (Pigman and Anet, 1972). Under alkaline conditions, β elimination is the rate-limiting step in the transformation of glucose to saccharinic acids (Pigman and Anet, 1972). Furfural compounds contain a furan ring and thus their copolymerization with other compounds could contribute to the aromatic backbone of humic materials (Ikan *et al.*, 1986; Rubinsztain *et al.*, 1986). The primary challenge limiting wider acceptance of the Maillard reaction hypothesis has

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been the difficulty of explaining how reducing sugars can be dehydrated in soil environments, which are aqueous systems.

Carbohydrates are by far the most abundant biopolymer added to soils each year in plant and animal residues (Koivula and Hanninen, 2001). However, carbohydrates do not persist in soils because they are a primary source of energy for soil microorganisms. Soil carbohydrates account for only ~10% of the SOM (Cheshire, 1979), and much of that is probably in the form of undecomposed plant residues and recently synthesized microbial products. In soils, extracellular enzymes degrade carbohydrates rapidly releasing various oligosaccharides to the soil solution. Although most of these compounds will be consumed by microbes, some will reach the surfaces of soil clays.

Soil clay minerals, predominantly smectites, are very important in SOM stabilization and humification because of their chemical properties. Surface acidity is one of the most important properties of smectites (Johnston and Tombácz, 2002; Mortland and Raman, 1968). Because of surface acidity, smectites can catalyze numerous organic reactions (Balogh and Laszlo, 1993). Furthermore, there is evidence that pillared montmorillonites facilitate four acid-catalyzed reactions involving sugars: isomerization of glucose to fructose; dehydration of glucose to 5-(hydroxymethyl)-2-furfural (HMF); cleavage of HMF to formic acid and levulinic acid; and formation of solid and water-soluble humic-like substances (Lourvanij and Rorrer, 1994; Lourvanij and Rorrer, 1997). The relevance of the reactions between glucose and smectites to the formation of SOM has not been established.

The objective of this study was to determine whether different types of smectites and their associated saturating metal cations abiotically catalyze transformation of glucose under conditions relevant to the formation of humic materials in soils.

MATERIALS AND METHODS

Chemicals

The American Chemical Society reagent-grade D-glucose, 5-(hydroxymethyl)-2-furaldehyde, 2-furaldehyde and 5-methyl-2-furaldehyde (Sigma-Aldrich Corp., St. Louis, Missouri) were used without further purification and DABS-hydrazine, synthesized from DABS-chloride[4-(Dimethylamino)azobenzene-4-sulfonyl chloride] (96% purity, Sigma-Aldrich Group, St. Louis, Missouri), was used for the pre-column derivatization of glucose. Milli-Q water (Milli-Q system, Millipore, Bedford, Massachusetts) with a resistivity of 18.2 MΩ-cm was used to prepare all solutions.

Samples

Four different reference smectites with a range of surface properties were used. Panther Creek beidellite (Panther) was obtained from the A.D. Scott collection, Iowa State University, Ames, Iowa. Otay white montmorillonite (Otay) was collected at an exposure near San Diego, California. IMV saponite (Saponite) was obtained from IMV Corporation, a division of Floridin Company (Amargosa Valley, Nevada). Ferruginous smectite SWa-1 (SWa-1) was obtained from the Source Clays Repository of The Clay Minerals Society (Purdue University, Indiana). For each sample, the clay fraction (<2 μm) was separated from the bulk ore by sedimentation. The clays were Na-, Ca- and Al-saturated by washing the <2 μm clays twice with 1 M solutions and twice with 0.1 M solutions of the appropriate salts (NaCl, $CaCl_2$ and $Al(NO_3)_3$). Excess salt was removed by dialyzing the M^{n+}-saturated clays (where M^{n+} is the saturating cation) using Spectra/Por® 3500 molecular weight cut off (MWCO) porous membrane tubing (Spectrum Laboratories Inc., Rancho Dominguez, California) against Milli-Q water until the conductivity of the dialysate was <0.6 μS/cm. The M^{n+}-saturated clay samples were freeze dried and saved for later use.

Portions of the Na-clays were coated with polymeric $Fe(OH)_3$ (Fusi *et al.*, 1989). Briefly, polymeric $Fe(OH)_3$ was prepared by dialyzing 0.1 M $Fe(NO_3)_3$ against deionized water for 6 h, using a 3500 MWCO molecular porous membrane tubing. Then, 25 mL of the polymeric $Fe(OH)_3$ were added to a Na-saturated clay suspension (5 g clay + 150 mL water) to prepare the $Fe(OH)_3$-coated clays (Fe-coated clays), mixed for 1 h, washed and centrifuged until the conductivity of the supernatant liquid was <0.6 μS/cm. The Fe-coated clay samples were freeze dried and saved for later use.

Incubations

About 0.25 or 0.50 g of M^{n+}-saturated clays were placed in 15 mL amber vials and loosely capped using PTFE/silicone septa (Supelco, Bellefonte, Pennsylvania). The capped vials were sterilized in an autoclave for 15 min at 120ºC. Glucose solutions were prepared fresh for each experiment, filter-sterilized with 150 mL Nalgene® MF75 Series sterile disposable tissue culture filter units (Nalge Nunc International, Rochester, New York), and added to the cooled vials containing the sterilized clay under sterile conditions to avoid microbial contamination. The solutions were dispensed using a Repeater® Plus pipet with autoclave-sterilized 50 mL Eppendorf Combitip Plus tips (Eppendorf AG, Hamburg, Germany). The final concentration of glucose was ~1.0 mmol g^{-1} clay and the total solution volume was 5 mL. The vials containing the M^{n+}-saturated clay + glucose systems were capped tightly and a strip of wax paper was wrapped around the caps to ensure the integrity of the systems. The vials were vortex-mixed and incubated in the dark for 21 days in a temperature-controlled incubator at 37±0.5ºC. Three replicates were run for each treatment.

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Analysis

After incubation, the evolved CO_2 was measured with a CI-301 CO_2 infrared (IR) gas analyzer (CID, Inc., Vancouver, Washington). The CO_2 concentrations in the vial's headspace were determined by circulating He gas through a closed loop connected to the IR analyzer. Standard curves were constructed for CO_2 by injecting a range of known concentrations of CO_2 into the IR gas analyzer. Certified standard CO_2 (10% in He) was obtained from Scott Speciality Gases (Troy, Michigan). After the CO_2 analysis, 5 mL of a filter-sterilized 100 mM $CaCl_2$ solution were added to the vials under sterile conditions, mixed and centrifuged for 10 min at $4500 \times g$. The supernatant liquid was analyzed for glucose and soluble organic C, and its pH was measured.

Glucose analysis was performed by reverse phase-high pressure liquid chromatography (RP-HPLC) using pre-column derivatization with DABS-hydrazine (Muramoto *et al.*, 1987) to form a chromophore because molecules with single bonds (such as glucose) do not absorb UV/Vis radiation at the range of the detector used in this study. The DABS-hydrazine was synthesized in our laboratory following the procedure of Muramoto and co-workers (1987). A DABS-hydrazine solution (0.1%, w/v) was prepared by dissolving 10 mg of DABS-hydrazine in 10 mL of 100% ethanol; 250 µL of aqueous trichloroacetic acid (10%) were added, the solution was mixed, and then stored at 4ºC. For the glucose analysis, a 25 µL aliquot of supernatant liquid from one of the incubated clay + glucose samples was placed in a 2 mL clear vial and dried under a stream of N_2 gas in a sand bath at 40ºC. Then, 400 µL of DABS-hydrazine solution were added to the clay sample; the vials were capped and reacted for 30 min at 70ºC. The solution was allowed to cool at room temperature and immediately analyzed by RP-HPLC using a HP 1050 quaternary pump and an 1100 diode array detector (Hewlett-Packard, Wilmington, Delaware). A reverse-phase ODS C_{18} column (4.6 by 150 mm, 5 µm particle size) (Supelco, Bellefonte, Pennsylvania) was used. The injection was performed using a 10 µL loop and absorbance was measured at 470 nm. The mobile phases consisted of acetonitrile (A) and a mixture of 40% acetonitrile/60% Milli-Q water adjusted to pH 3.0 with HPLC-grade H_3PO_3 (B). A flow rate of 0.8 mL min^{-1} was used with the following gradient profile: 0 min 100% B; 6.0 min 100% B; 10 min 10% B; 15 min 10% B, with a re-equilibration time of 5 min. The retention time for glucose was 5.6 min. Calibration was performed using external standards. Peaks for the un-reacted DABS-Cl and DABS-hydrazine were observed at 3.0 and 14 min, respectively. The detection limit for this method is 2 pmol (Muramoto *et al.*, 1987).

Furfural compounds were identified by RP-HPLC (Ferrer *et al.*, 2000) using the same HPLC system as in glucose analysis. An aliquot from the supernatant liquid of the samples was filtered through a 0.02 µm pore-size filter and injected using a 10 µL injection loop. The mobile phases consisted of 5% acetonitrile in water (A) and acetonitrile (B). A flow rate of 0.85 mL min^{-1} was used with the following gradient profile: 0 min 100% A; 8.0 min 100% A; 10 min 50% A; 14 min 50% A, with a re-equilibration time of 5 min. The absorbance was measured at 284 nm. The retention times for 5-(hydroxymethyl)-2-furaldehyde, 2-furaldehyde, and 5-methyl-2-furfaldehyde were 5.6, 6.8, and 11.8 min, respectively.

Total C and N were determined in untreated clay samples by thermal combustion using a Carlo Erba NA1500 NSC elemental analyzer (Haake Buchler Instruments, Paterson, New Jersey). After incubation, total organic carbon in the supernatant liquids was determined using a modified dichromate oxidation method (Yeomans and Bremner, 1988). An aliquot of 2.0 mL of supernatant liquid, 5.0 mL of 1 N $K_2Cr_2O_7$ and 7.5 mL of concentrated H_2SO_4 were added to a 125 mL Erlenmeyer flask with a TS Joint. The flask was placed on a hot plate and connected to a Liebig condenser for 15 min. The temperature was controlled in order to have a stable boiling of the solution. The flasks were allowed to cool to room temperature, 0.3 mL of indicator solution (0.1 g of *N*-phenylanthranilic acid and 0.1 g Na_2CO_3 dissolved in 100 mL of Milli-Q water) was added, and the samples were titrated with Mohr's salt solution. The end-point is evident by a bright violet color. The Mohr's salt solution consisted of 156.8 g of ferrous ammonium sulfate $[Fe(NH_4)_2(SO_4)_2.6H_2O]$ dissolved in 100 mL concentrated sulfuric acid and then taken to 2.0 L volume with Milli-Q water and kept in an amber bottle. This solution was standardized daily because it undergoes slow oxidation during storage. Two boiled controls and two unboiled controls were also prepared.

X-ray diffraction (XRD) patterns for the clay minerals were obtained using a Scintag XDS-2000 powder diffractometer (Thermo ARL, Ecublens, Switzerland) equipped with an air-cooled Kevex Psi Peltier silicon detector, and CuKα radiation. About 100 mg of M^{n+}-saturated clay were slurried in 95% ethanol, oriented on glass slides by the paste method and dried above a saturated solution of $Mg(NO_3)_2$ (54% R.H.). Data were collected from 2 to 32º2θ. Elemental analysis of the clay samples was performed by inductively coupled plasma-atomic emission spectroscopy using the suspension nebulization technique (Laird *et al.*, 1991).

One-way analysis of variance (ANOVA) and pairwise multiple comparison procedures using the Student-Newman-Keuls Method ($P < 0.05$) were performed using SigmaStat software (SPSS Science, Chicago, Illinois).

RESULTS AND DISCUSSION

Characterization of clay minerals

The four clay minerals used in this study were chosen because of their chemical differences. Results for the elemental analysis for all clays are shown in Table 1. The

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Table 1. Elemental analysis of the untreated clay minerals used in this study.

Clay mineral	C	N	Interlayer charge	CEC[¶]	– Tet[‡] –		——— Oct[§] ———			Tet charge	Fe^{3+} coating clays
	%		e fu^{-1}	mmol g^{-1}	Si^{4+}	Al^{3+}	Al^{3+}	Fe^{3+}	Mg^{2+}	%	mg g^{-1}
					cations per formula unit[#]						
SWa-1	0.21	0.02	0.46	1.25	3.70	0.30	0.41	1.44	0.14	64	145
Panther	0.11	0.01	0.38	1.35	3.86	0.14	1.43	0.35	0.21	37	124
Saponite	0.39	0.01	0.58	1.36	3.77	0.23	0.88	0.09	1.37	39	221
Otay	n.d.[†]	nd	0.53	1.38	3.99	0.01	1.32	0.06	0.67	2	169

[†] n.d.: not detected
[‡] Tet: Tetrahedral sheet
[§] Oct: Octahedral sheet
[¶] Cation exchange capacity
[#] Formula unit based on $M^{n+}_{x+y}-(Si_{4-x}Al_x)(Al_{2-y-z}Mg_yFe^{3+}_z)O_{10}(OH)_2$

XRD patterns (traces not shown) for the Ca-saturated samples equilibrated at 54% R.H., showed large 001 smectite peaks with *d* spacings ranging from 1.37 to 1.56 nm. In addition to the smectite peaks, the Saponite XRD pattern had two small peaks with *d* spacings of 1.02 and 0.33 nm, indicating the presence of admixtures of illite and quartz, respectively. The total C and N content of the untreated samples ranged from 0 to 0.39% and from 0 to 0.02%, respectively (Table 1). The interlayer charge (from 0.38 to 0.58 e fu^{-1}), determined from the sum of Ca^{2+}, K^+ and Na^+ cations, is within the range for smectites (Table 1). The smectites are classified as beidellite (Panther), montmorillonite (Otay) and saponite (Saponite). There is a debate whether SWa-1 is a nontronite or not (Frost and Kloprogge, 2000); however, it is clearly a high-Fe smectite.

C in solution

The samples were initially sterilized, and sterile techniques were used throughout the incubations. Furthermore, analysis of the trapped gasses at the end of the incubations indicated that CO_2-C was <0.2% of the total C added for all of the samples. Thus, all reactions occurring during the incubations are believed to be strictly abiotic.

The total soluble organic C recovered at the end of the incubations, as determined by the dichromate oxidation method, ranged from 95.0 to 109.6% relative to the amount of C added initially as glucose (Table 2). Generally, total soluble organic C recoveries were significantly different for the cation saturation treatments (Fe > Ca = Na > Al) but not for the different smectites. The measured soluble organic C slightly exceeded the total amount of C added as glucose for most samples. This discrepancy is believed to be an artifact of using the dichromate oxidation method to measure soluble organic C, based on the measurement of unused dichromate after oxidation of the soluble organic C. There are two conditions that may cause an overestimate of the soluble organic C: (1) the presence of Fe^{2+}; and (2) the presence of Cl^- according to the following reactions:

$$Cr_2O_7^{2-} + 6Fe^{2+} + 14H^+ = 2Cr^{3+} + 7H_2O \quad (1)$$

$$K_2Cr_2O_7 + 4KCl + 6H_2SO_4 = 2CrO_{2+}Cl_2 + 6KHSO_4 + 3H_2O \quad (2)$$

Table 2. Fraction of added organic C recovered as C in solution for the clay + glucose systems[†].

Cation	Clay mineral			
	SWa-1	Panther	Saponite	Otay
	% C recovered			
Na	104.0 A[‡] a[§]	101.4 A b	101.3 A a	102.9 A ab
Ca	102.7 A a	102.0 A b	102.8 A a	105.3 B b
Al	109.6 C b	95.0 A a	99.0 B a	101.4 B a
Fe	108.2 A b	108.3 A c	105.6 A b	109.3 A c

[†] C in solution determined by a modified dichromate oxidation method (Yeomans and Bremner, 1988).
[‡] For each cation treatment, there was no significant difference between means with the same upper case letter by the Student-Newman-Keuls test ($P < 0.05$)
[§] For each clay mineral, there was no significant difference between means with the same lower case letter by the Student-Newman-Keuls test ($P < 0.05$).

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Most of the Fe in the system was in the ferric form, though a small amount of ferrous iron was probably present as a structural ion in the SWa-1 and Panther clays and some Fe^{2+} may have been present in the Fe-coated clays. The extent of the Cl^- interference with the dichromate oxidation method should be both small and the same for all samples because the same amount of $CaCl_2$ was added to all samples after incubation.

The amount of glucose-C recovered from the solutions at the end of the incubations varied from slightly to substantially less than the amount of glucose-C initially added to the systems (Table 3). Glucose recoveries in the various M^{n+}-clay + glucose systems ranged from 18.3 to 98.3% of the total glucose added. The type of clay significantly influenced glucose recovery for the Na-, Ca- and Al-clay systems (SWa-1 > Panther > Otay >=< Saponite). Depending on the saturating cation, glucose recovery for Saponite was variable and it was either greater than, equal to, or less than that for Otay. Glucose recoveries were also influenced by the saturating cations; for SWa-1, Na > Al = Fe > Ca; for Panther, Fe > Na > Ca = Al; for Saponite, Fe > Na = Ca = Al; and for Otay, Fe > Ca > Na > Al (Table 3). No significant differences were observed in glucose recoveries for the Fe-coated clays (Table 3). The DABS-hydrazine is oxidized by Fe^{3+} in aqueous solutions (Moliner and Street, 1989), though the high recoveries of glucose in the Fe-coated clays (78.2–91.9%) suggest that oxidation of DABS-hydrazine by Fe^{3+} was not a problem, probably because there was little or no Fe^{3+} in the solutions. Together, the results in Tables 2 and 3 indicate that a substantial amount of the glucose initially added to the systems was abiotically transformed into soluble organic compounds other than glucose during the incubations. There are three variables in the clay + glucose systems that could affect glucose recoveries: pH, type of clay, and saturating cation. The pH of the systems ranged from 3.0 to 6.3. Both forms of glucose (cyclic and open chain structures) react most rapidly with hydrazine to form hydrazones in the pH 4–5 range. Thus, if solution pH influenced glucose recovery, higher recoveries would be expected either under acidic (pH <4) or slightly acidic (pH >5) conditions. In this study, however, both low and high glucose recoveries were obtained under acidic conditions (Al-Otay, pH 3.3, 18.3% and Al-SWa, pH 3.0 and 83.9% glucose recovery). Also, both low and high glucose recoveries were obtained under slightly acidic conditions (Ca-Saponite, pH 5.8, 43.9% and Fe-Saponite, pH 5.8, 83.9% glucose recovery). Thus, in this study, clay type and saturating cation clearly had a much greater influence on glucose recoveries than pH.

Interestingly, Fe^{3+} content had a positive effect on glucose recoveries. Glucose recoveries increased as structural Fe^{3+} increased in Na-, Ca- and Al-clay systems (Table 3), and high recoveries of glucose were obtained for all of the Fe-coated clays. These results suggest that Fe^{3+}, probably as Fe oxyhydroxide surface coatings, inhibit the abiotic clay-catalyzed transformation of glucose, probably by preventing direct contact of glucose with the clay surface.

Controls consisting of fresh 100 mM glucose in 100 mM $Fe(NO_3)_3$, and $Al(NO_3)_3$ solutions (1:1 v/v) were analyzed. There was no peak for glucose in the chromatogram for the glucose + $Fe(NO_3)_3$ system, indicating that Fe^{3+} in solution had oxidized all of the DABS-hydrazine. By contrast, there was nearly complete recovery of glucose from the fresh glucose + $Al(NO_3)_3$ control. The opposite trend was observed for the clay + glucose systems; recoveries of glucose were highest for the Fe-clay systems and lowest for the Al-clay systems. This contrasting effect of Fe and Al in the aqueous controls and clay systems is further evidence that the smectites catalyzed the transformation of glucose in the systems studied.

Strong acids are known to catalyze dehydration of reducing sugars to form furfural compounds (Feather and Harris, 1973), which are highly reactive intermediate compounds that are polymerized in more advanced stages of the Maillard reaction (Ferrer *et al.*, 2000). Under acidic conditions, glucose dehydrates to form

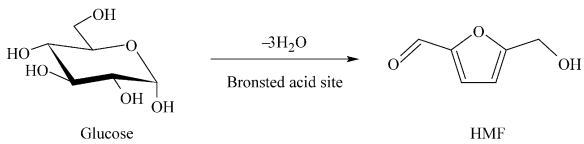
Table 3. Fraction of total added C recovered as solution glucose-C for the clay + glucose systems[†].

Cation	Clay mineral			
	SWa-1	Panther	Saponite	Otay
	% C recovered			
Na	98.3 C[‡] c[§]	81.2 B b	39.6 A a	43.9 A b
Ca	65.3 D a	56.9 C a	43.9 A a	50.7 B c
Al	83.9 D b	59.3 C a	20.7 B a	18.3 A a
Fe	82.0 A b	91.9 A c	83.9 A b	78.2 A d

[†] C-glucose recovery for controls (aqueous solution of glucose) was 88.0%.
[‡] Within each cation, means followed by the same upper-case letter are not significantly different by the Student-Newman-Keuls test (P<0.05).
[§] Within each clay mineral, means followed by the same lower-case letter are not significantly different by the Student-Newman-Keuls test (P <0.05).

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5-(hydroxymethyl)-2-furaldehyde (HMF) (Lourvanij and Rorrer, 1994; Lourvanij and Rorrer, 1997) as follows:



The HMF has three reactive groups: the hydroxymethyl group, the aldehyde group, and the furan ring. The hydroxymethyl group behaves as a primary alcohol; thus, ester formation and oxidation of this group may occur. The aldehyde group is subject to oxidation to a carboxyl, reduction to an alcohol, and nucleophilic addition reactions. The most important reaction involving the furan ring is hydrogenation, which causes ring cleavage. Aqueous HMF was completely transformed to unknown compounds after 186 days at room temperature (Lehnen *et al.*, 2001). In addition to furfural compound formation, maltol product formation may occur from the dehydration of carbohydrates, though in the pyrolysis of various carbohydrate systems, maltol was detected only in maltose and lactose systems (Patton, 1950).

An HPLC analysis of the solutions from several of the clay + glucose systems revealed small peaks for HMF and 2-furaldehyde (retention time of 5.55 and 6.77 min, respectively) and an unidentified shoulder on the HMF peak (Figure 1). Aqueous controls consisting of glucose in distilled water and glucose in 100 mM HCl, NaCl, $CaCl_2$, $Fe(NO_3)_3$ and $Al(NO_3)_3$ were incubated for 21 days at 37ºC. The chromatogram for the HCl control exhibited a substantial HMF peak (Figure 1). Chromatograms for only two of the metal-salt systems exhibited peaks for HMF ($Al(NO_3)_3$ > $Fe(NO_3)_3$). The $Al^{3+}$and Fe^{3+} ions are stronger Lewis acids than Na^+ and Ca^{2+} ions, thus the acid-catalyzed dehydration is facilitated by those metals in aqueous systems. Based on the knowledge above, we suggest that clay surfaces may catalyze the dehydration of glucose in our systems. The surface of smectites may contain both Lewis and Bronsted acidic sites. The former are usually coordinated unsaturated Al^{3+} ions and the latter arise from weakly acidic SiOH groups exposed at the structure edges or the basal surface defects, and the strongly acidic bridged-hydroxyl groups, and polarized water molecules adsorbed on the mineral surface, principally those water associated with the exchangeable cations (Solomon and Hawthorne, 1983). Moreover, Bronsted acidity of smectites is affected by the layer charge location: if isomorphous substitution occurs in the octahedral sheet, then the negative charge is delocalized over at least 10 basal oxygens of the tetrahedral sheet. Conversely, if the isomorphous substitution occurs in the tetrahedral sheet, then the negative charge is localized on the three basal oxygens directly bound to the constituent ion of the tetrahedral sheet. Accordingly, the surface of montmorillonite and hectorite are stronger Bronsted acids than the surface of the saponite and vermiculite (Farmer and Russell, 1971; Solomon and Hawthorne, 1983; Yariv, 1992). Clay minerals acid-catalyzed elimination reactions and the dehydration reactions of alcohols are some of the most documented reactions (Balogh and Laszlo, 1993). Furthermore, acid-catalyzed aldol condensates are very easily dehydrated, forming carbon−carbon bonds (Morrison and Boyd, 1987). Although only small amounts of HMF were detected for the clay + glucose systems, the results do demonstrate that clay surfaces are capable of catalyzing dehydration of glucose to form HMF. In hindsight, the furfural compounds are probably too unstable to have persisted in the solutions for the clay + glucose systems. The unidentified peaks in the chromatographs are probably polymerization products of HMF.

Carbohydrates are by far the most abundant form of biomass introduced into soils each year. As soil microorganisms degrade carbohydrates, primarily oligosaccharides but also some monosaccharides will be released to the soil solution. Although most of these compounds will be consumed by microbes, some will reach the surfaces of soil clays. The results of this study provide evidence that monosaccharides are transformed under abiotic conditions

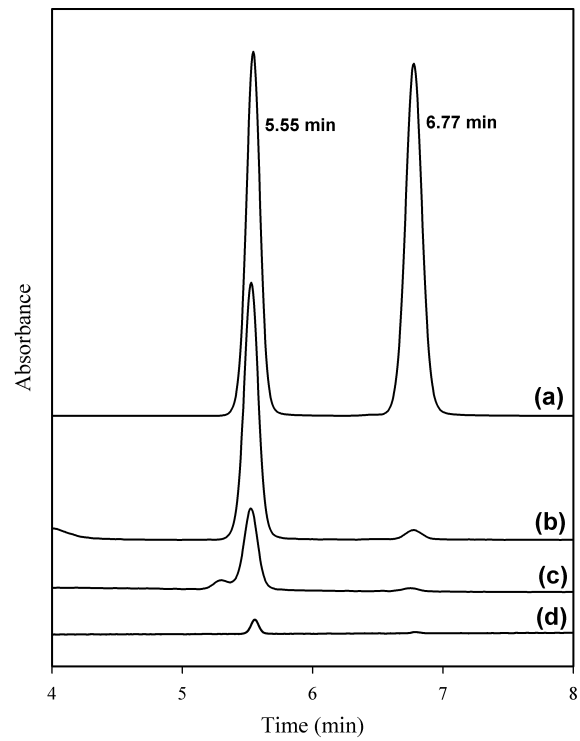


Figure 1. Chromatograms showing furfural compounds: (a) fresh HMF and 2-furaldehyde, 0.5 ppm each with retention times of 5.55 and 6.77 min, respectively; (b) HCl-glucose incubated for 64 h at 70ºC; and solutions from (c) Al-Otay + glucose and (d) glucose systems incubated for 21 days at 37ºC. The relative absorbance scale is the same for all chromatograms.

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by smectites into other soluble organic compounds. Under mildly acidic conditions, the clay surface-catalyzed dehydration of monosaccharides to form furfural compounds is probably the first step in this process. The furfural compounds are highly reactive, and thus are likely to be polymerized or co-polymerized with other organic compounds. Reactions of oligosaccharides with clay minerals were not addressed in this study. However, if oligosaccharides are subject to similar clay surface-catalyzed transformations, then this may be an important pathway for the formation of new humic-like substances and the incorporation of new C into SOM.

CONCLUSIONS

Smectites behave as Lewis and Bronsted acids and can catalyze several organic reactions such as addition and elimination reactions. The results of this study provide evidence that smectites are capable of abiotically catalyzing the dehydration of glucose, under conditions similar to those found in soils, to form furfural compounds, which are very reactive and contributors in the Maillard reaction. Thus, polymerization of furfural compounds may be a major pathway leading to the formation of new humic materials in soils.

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