Organic Matter Transformation Catalysed by Clays: Model Reactions for Carbon Sequestration in Soils

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Abstract

Stabilisation of carbon in humic substances, by a variety of coupling reactions, is an important route to carbon sequestration, and is linked to soil enzymes and abiotic components of soils, such as clay minerals. Oxidative coupling reactions of model compounds: vanillin, 2-naphthol, anthrone, 2,6-dimethylphenol, 2,4-dimethylphenol and 2-hydroxy-6-naphthalene-sulfonic acid (chosen to represent phenolic fragments of soil organic matter) mediated by abiotic catalysts, was investigated. Iron (III) loaded clays exhibited good catalytic activity yielding 1,1'-binaphthalene-2,2'-diol and bianthronyl. Soluble iron (III) salts also effect oxidative coupling of certain substrates in aqueous environments, but the process is not catalytic, indicating that the clay components of soils may be important in a variety of similar carbon-carbon bond forming reactions and thus in stabilisation of organic matter, aiding carbon sequestration in soils.

Key Words

Carbon sequestration, clay catalysts, humic substances, iron (III), oxidative coupling

Introduction

Carbon sequestration in soils depends on the capacity to store organic matter for long time periods. Conversion of native vegetation to agricultural uses, such as cropping and grazing, often leads to the depletion of soil organic carbon (Ingram and Fernandes 2001). Furthermore, it has been established that the global soil resource contains far more carbon than the combined stocks of the atmosphere and terrestrial vegetation (Swift 2001). As such a large area of land has been utilised for agriculture, there exists a great potential for stabilisation and replenishment of carbon in organic matter depleted agricultural soils.

The potential for sequestration is linked to the extent of accumulation and protection of stable organic structures, such as humic substances and mineral-associated organic matter (Swift 2001). Humic substances are chemically stable, whereas mineral-associated 'protected' organic matter is temporarily stable due to its relationship with mineral matrices. Protection of organic molecules refers to conditions where organic matter decomposition is reduced when associated with mineral components of soils, compared with free molecules (Baldock and Skjemstad 2000). The capacity for soil to protect organic structures relies on the mineral composition of the soil, and predominantly involves weak non-covalent interactions between mineral surfaces and organic molecules (Theng 1974). Protection can also occur within hydrophobic domains of humic substances (Piccolo 2002). The chemical properties that help to stabilise humic structures include covalent bonding within, and non-covalent interactions between, structural units. The heterogeneity and molecular size of humic substances are also important, as microbial degradation is slowed when organic molecules are too large to pass through cell membranes, and when exoenzymes are only suitable for hydrolysing repeating units (Swift 2001). Processes that increase the proportion of covalent bonds in humic systems are relevant to improved soil fertility and carbon retention/sequestration.

Oxidative coupling of phenols is a significant reaction in the formation of humic structures in the soil (Bollag, Dec *et al.* 1998). The formation of carbon-carbon bonds between aromatic moieties increases molecular weight and may lead to polymerisation (Bollag, Dec *et al.* 1998). Cross coupling of different organic compounds results in increased heterogeneity of humic molecules resulting in increased resistance to enzymatic hydrolysis (Bollag, Minard *et al.* 1983). These reactions can be catalysed enzymatically by peroxidases, tyrosinases and laccases, which commonly contain heme iron, di-nuclear copper and multi-nuclear copper respectively at the active site (Claus 2004; Conesa, Punt *et al.* 2002; Garcia-Borron and Solano 2002). Additionally, oxidative coupling can be catalysed by abiotic components of the soil, such as layered silicates, primary minerals, and metal oxides (Bollag, Dec *et al.*

1998). Studies have focussed on polymerisation of phenols considered to be important lignin fragments, such as pyrogallol, resorcinol, catechol and hydroquinone (Shindo and Huang 1985). The choice of such easily polymerised substrates reduces the capacity to adequately control reaction progress, and makes identification and quantification of coupling products difficult. Typically, indirect analytical methods, such as observation of coloured products or oxygen uptake and release (Pal, Bollag *et al.* 1994) have been employed to detect mineral catalysed covalent bond formation in organic substrates. The study of model compounds, which yield discrete isolable products, allows quantification of catalytic effects and characterisation of products.

Clay minerals have been widely employed in synthetic procedures as effective catalysts for covalent bond formation between a range of organic substrates (Balogh and Laszlo 1993). In addition, modification of clay mineral surfaces and interlayers with hydrated metal ions enhances the reactivity of clays in certain reaction types (Cornelis and Laszlo 1985). Smectite clay minerals naturally contain hydrated metal ions in interlayer spaces to balance negative surface charge resulting from isomorphous substitution (Velde 1992). However, by purposely saturating the surface sites with homo-ionic cations, the catalytic properties of the clay can be enhanced compared with the mixed cation environment seen in natural clays (Laszlo and Mathy 1987). This allows comparison of different hydrated metal ions in order to determine which are most effective in promoting covalent bond formation.

In this study, model compounds (**1-6**) specifically representing simple phenolic fragments of soil organic matter, were used to investigate oxidative coupling in the presence of iron (III) salts, copper (II) and iron (III)-loaded clays. Substrates were chosen with different ring substituents to provide varying degrees of activation towards coupling. 2-Naphthol (**1**) readily forms 1,1'-binaphthalene-2,2'-diol (**7**) under a range of conditions and is used as a probe for reactivity.

Methods
Materials
2-Naphthol (**1**), anthrone (**2**), vanillin (**3**), 2,6-dimethylphenol (**4**), 2,4-dimethylphenol (**5**), 2-hydroxy-6-naphthalene-sulfonic acid sodium salt (**6**), K10 montmorillonite clay, iron (III) trichloride hexahydrate and anhydrous iron (III) trichloride, were purchased from Sigma-Aldrich chemical company (except for vanillin obtained from Ajax Chemicals Ltd and iron trichloride hexahydrate obtained from BDH). All reagents were used as purchased from the supplier without any further purification.
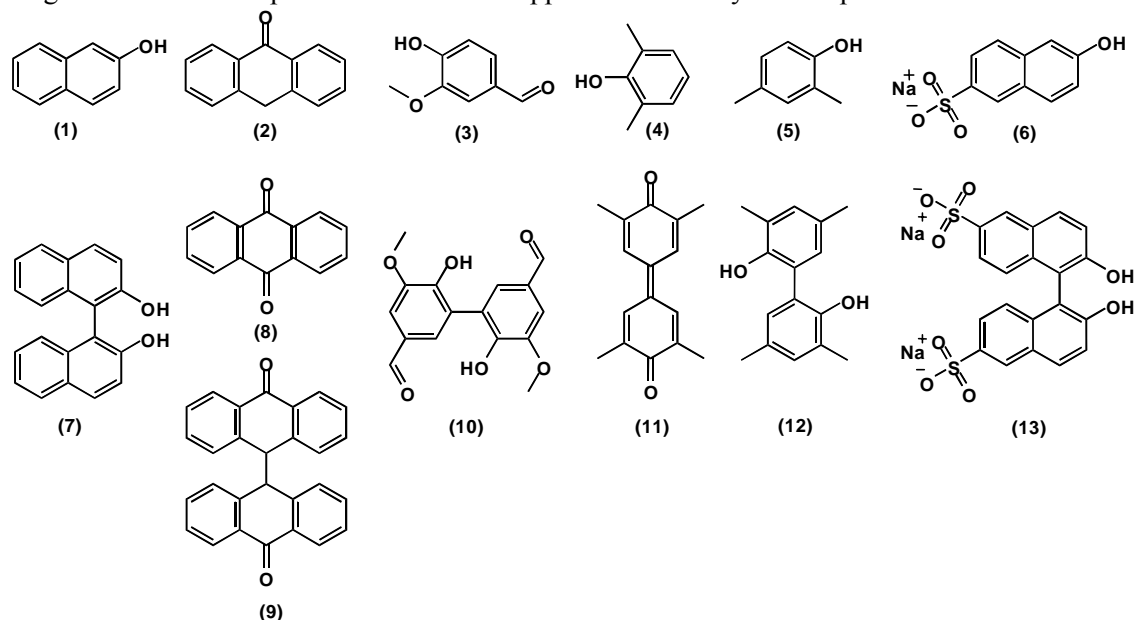
Analysis
[1]H Nuclear Magnetic Resonance (NMR) and ^{13}C NMR spectra were recorded on 200MHz, 300MHz or 400MHz Bruker spectrometers in CDCl$_3$, DMSO-d$_6$ or D$_2$O with tetramethylsilane (TMS) as an internal standard ($\delta = 0$ ppm), except in the case of D$_2$O (H$_2$O $\delta = 4.79$ ppm). Conversions were derived from comparison of integrated areas of well-resolved signals in ^{1}H NMR spectra (±1% error was determined for this method). Infrared (IR) spectra were recorded on a Perkin Elmer Series 1600 FTIR spectrometer as

a KBr disc or Nujol mull. Atomic absorption spectroscopy (AAS) was carried out using a Perkin Elmer Atomic Absorption Spectroscope Model 3110. Melting points were recorded on a Gallenkamp variable heat melting point apparatus at a heating rate of 1 °C/min. Thin layer chromatography (TLC) was carried out using silica gel 60 F-254 (Merck) TLC plates, with visualisation under 254 nm and/or 365 nm radiation. Semi-quantitative TLC was used to determine reaction conversions in some instances and an estimate of error (±5%) was determined for this method, corresponding to the visual limits of detection of any changes in sample intensity under 254 nm radiation.

Clay loading and determination of metal content
Samples of *ca* 400 g clay was added to 2 L of 1 *M* HCl and stirred overnight. The suspension was filtered and washed with deionised water until chloride ions could no longer be detected *via* a silver nitrate test. Dried clay was crushed in a mortar and pestle, and added to *ca* 50 g $FeCl_3$ in 500 mL water. The suspensions were stirred at 35°C for three hours, then at 20°C overnight. All clays were rinsed until no chloride ions could be detected. Dried clay was ball-milled in a stainless-steel pot and sieved into size fractions of <180 µm, 180-250 µm and 250-355 µm. The 180-250 µm size fraction was analysed for metal content and used in all reactions. Copper and iron were measured at 222.6 nm and 372 nm respectively, both with a high and wide slit. Three accurately weighed *ca* 300 mg loaded clay samples were allowed to stir in 10 mL of 1 *M* HCl for 1 hour. The clays were filtered and further treated with another 10 mL of 1 *M* HCl with stirring for a further hour. The combined supernatants obtained from each stripping were separately made up to 50 mL with 1 *M* HCl in a volumetric flask. Clay loading was determined by AAS against a series of 8 standards for copper and iron, and loading values are given (Table 1).

Table 1. Metal content on clay (g metal/g clay)

Clay type	g Cu^{2+} / g clay	g Fe^{3+} / g clay
Montmorillonite	0.0065 ± 0.0001	0.0120 ± 0.0006
Vermiculite	0.0180 ± 0.0001	0.0277 ± 0.0010
Bentonite	0.0140 ± 0.0001	0.0192 ± 0.0008

Long-term incubation of modified clays with 2-naphthol, solvent-free at ambient temperature
Accurately weighed samples of *ca* 0.25 g 2-naphthol (**1**) dissolved in diethyl ether (10 mL) were added to *ca* 4 g loaded clay, and the slurry stirred for 24 hours at 20°C. An amount of clay, corresponding to 0.5 mol equivalents of the hydrated metal ion content to moles of substrate, was used. The solvent was then removed *in vacuo* and intimately associated mixture transferred to a vial (20 mL) with a stirring bead. The stoppered vial was stored in the dark at ambient temperature. The contents were stirred daily in air, except Saturday and Sunday, using a magnetic stirrer (5 mins). No coupled product (**7**) was detected (TLC) immediately after solvent removal. For analysis, samples of approximately 0.05 g were removed after 1, 4, 10, 24, 38 and 64 days and slurried in ether (~5 mL) for 10 min. The clay was removed by filtration, the ether filtrate recovered and solvent removed *in vacuo* prior to analysis by ^{1}H NMR.

Example procedure for clay-catalysed reactions in toluene
Anthrone (**2**) (0.6855 g, 3.529 mmol) and iron (III)-loaded K10 montmorillonite (0.8112 g, 0.174 mmol Fe^{3+}) were slurried in toluene (35 mL) at reflux (111°C) for 24 h. After cooling, solids were removed by filtration through celite and washed with a further aliquot of toluene. The organic solution was dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give a yellow solid (0.6342 g, 93% mass recovery). ^{1}H NMR ($CDCl_3$, TMS, 200 MHz) of recrystallised fractions revealed complete conversion of anthrone to 77% anthraquinone (**8**) m.p. 279-280°C (lit m.p. 282-283°C (Bhawal, Khanapure *et al.* 1991)) and 23% bianthronyl (**9**) m.p. 257-260°C (lit m.p. 256-258°C (Dabestani, Ellis *et al.* 1995)).

Procedure for reoxidation / rehydration of clay catalyst
Iron (III)-loaded clay remaining from the clay-catalysed oxidative coupling of 2-naphthol (following the general procedure for clay-catalysed reactions in toluene) was divided into two batches. One batch of the clay thus recovered (0.46 g) was stirred in 30% H_2O_2 (20 mL) for 48 hours. The second batch of clay (0.44 g) was stirred in H_2O (20 mL) for 48 hours. The separate clay samples were dried at 120°C overnight. Reoxidised and rehydrated clays were both submitted to the same reaction conditions for clay-catalysed reactions in toluene for 6 hours, giving 66% conversion to the product (**7**) for the reoxidised clay and 17% conversion for the rehydrated clay.

Example procedure for solvent-free reactions with clays
Anthrone **(2)** (0.2054 g, 1.058 mmol) and iron (III)-loaded K10 montmorillonite (0.2466 g, 0.053 mmol Fe^{3+}) were ground together in an agate mortar and pestle, placed in a sealed Pyrex test-tube in an aluminium dry block heater and kept at 50°C for 24 hours. An amount of clay, corresponding to 0.05 mol equivalents of the hydrated metal ion content to moles of substrate, was used. The organic component was extracted with dichloromethane, filtered to remove clay, washed with an aliquot of dichloromethane, dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give a yellow solid (0.1846 g, 90% mass recovery). ^{1}H NMR ($CDCl_3$, TMS, 200 MHz) revealed conversion of anthrone to 14% anthraquinone **(8)** and 4% bianthronyl **(9)**.

Example procedure for solvent-free reactions with iron salts
2-Naphthol **(1)** (1.0092 g, 6.999 mmol) and iron trichloride hexahydrate (3.7841 g, 13.990 mmol) were ground together in an agate mortar and pestle, placed in a sealed Pyrex test-tube in an aluminium dry block heater and kept at 50°C for 2 hours. The reaction was quenched with 1 *M* HCl, washed with an aliquot of distilled water and the organic component extracted with dichloromethane. The solution was dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give a white solid (0.9991 g, 99% mass recovery). ^{1}H NMR ($CDCl_3$, TMS, 300 MHz) revealed complete conversion to 1,1'-binaphthalene-2,2'-diol **(7)**: confirmed by IR (Nujol), m.p. 214-216ºC (lit 214-216°C (Ding, Wang *et al.* 1996)) and TLC (95:5 DCM/EtOAc) R_f= 0.58.

Example procedure for aqueous-slurry reactions
2,4-Dimethylphenol **(5)** (0.195 mL, 1.612 mmol) was added to a solution of iron trichloride hexahydrate (0.8885 g, 3.287 mmol) in distilled water (30mL) and slurried at 50°C for 2 hours. The organic component was extracted with dichloromethane, dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give a light-yellow solid (0.1500 g, 75% organic mass recovery). ^{1}H NMR ($CDCl_3$, TMS, 400 MHz) revealed 75% 3,3',5,5'-tetramethyl-biphenyl-2,2'-diol **(12)** and 25% 2,4-dimethylphenol **(5)**

Results and Discussion
Oxidative coupling of six substrates, representing phenolic fragments of soil organic matter, was achieved under a range of conditions (Table 2). 2-Naphthol **(1)** coupled to form 1,1'-binaphthalene-2,2'-diol **(7)**, anthrone **(2)** oxidised to form anthraquinone **(8)** and coupled to form bianthronyl **(9)**, vanillin **(3)** coupled to form divanillin **(10)**, 2,6-dimethylphenol **(4)** coupled and oxidised to form 3,3',5,5'-tetramethyl-diphenoquinone **(11)**, 2,4-dimethylphenol **(5)** coupled to form 3,3',5,5'-tetramethyl-biphenyl-2,2'-diol **(12)**, and 2-hydroxy-6-naphthalene-sulfonic acid sodium salt **(6)** coupled to form 2,2'-dihydroxy-1,1'-binaphthalenyl-6,6'-disulfonic acid sodium salt **(13)**.

Oxidative coupling of model compounds proceeded slowly under solvent-free conditions with iron (III)-loaded K10 montmorillonite clay (Table 2). An amount of clay, corresponding to 0.05 mol equivalents of the hydrated metal ion content to moles of substrate, was used in these experiments. After 48 hours at 50°C, 63% conversion of 2-naphthol to **(7)** and 18% conversion of anthrone was observed. Most of the anthrone formed **(8)** rather than the coupled product **(9)**). The remaining substrates **(3-6)** showed no appreciable conversion to coupled products under elevated temperatures over a 24-hour time period.

Soil processes operate at low temperatures over long time periods, therefore mild reaction conditions were explored. After only 48 hours at 20°C with iron (III)-loaded K10 montmorillonite, 8% conversion of 2-naphthol to **(7)** was observed. Long-term solvent-free reactions with the model compound 2-naphthol were performed at ambient temperatures, using a range of clays loaded with copper (II) and iron (III) cations (Figure 1). Copper (II)-loaded clays yielded the greatest quantities of coupled product **(7)**, in the order bentonite> montmorillonite> vermiculite. Iron (III)-loaded montmorillonite yielded more coupled product **(7)** than iron (III)-loaded bentonite and vermiculite. The level of conversion exceeded the level of metal ion loading, implying catalytic behaviour due to aerial reoxidation of metal ions. Further reaction appeared possible in all systems except copper (II)-bentonite.

Table 2. Conditions, conversions and recovery of organic substrates in selected reactions.

Conditions	2-Naphthol (1)	Anthrone (2)	Vanillin (3)	2,6-Dimethyl phenol (4)	2,4-Dimethyl phenol (5)	2-Hydroxy-6-naphthalene sulfonic acid (6)
Neat Fe^{3+}-K10 *(5% Fe^{3+})*	50ºC 48hr [a,b,c] 13% conv., 35% recov. 87%(1) 13%(7)	50ºC 24hr 18% conv., 90% recov. 82%(2) 14%(8) 4%(9)	50ºC 24hr 0% conv., 99% recov. 100%(3) 0%(10)	50ºC 24hr 0% conv., 99% recov. 100%(4) 0%(11)	50ºC 24hr 0% conv., 99% recov. 100%(5) 0%(12)	50ºC 24hr 0% conv., 97% recov. 100%(6) 0%(13)
Toluene Fe^{3+}-K10 *(5% Fe^{3+})*	111ºC 7hr 67% conv., 79% recov. 33%(1) 67%(7)	111ºC 24hr 100% conv., 93% recov 0%(2) 77%(8) 23%(9)	111ºC 6hr 0% conv., 96% recov. 100%(3) 0%(10)	111ºC 6hr [a] 0% conv., 50% recov. 100%(4) 0%(11)		
Neat $FeCl_3 \cdot 6H_2O$ *(2 mol eq)*	50ºC 2hr 100% conv., 94% recov. 0%(1) 100%(7)	50ºC 2hr 93% conv., 80% recov. 7%(2) 63%(8) 30%(9)	50ºC 2hr 0% conv., 99% recov. 100%(3) 0%(10)	50ºC 6hr 0% conv., 93% recov. 100%(4) 0%(11)	50ºC 2hr 69% conv., 92% recov. 31%(5) 69%(12)	50ºC 2hr 0% conv., 99% recov. 100%(6) 0%(13)
Neat $FeCl_3$ *(1.1 mol eq)*	50ºC 2hr [d] 72% conv., 78% recov. 28%(1) 72%(7)	20ºC N_2 24hr [e] 89% conv., 80% recov. 11%(2) 16%(8) 73%(9)	20ºC N_2 24hr 0% conv., 86% recov. 100%(3) 0%(10)			
Water $FeCl_3 \cdot 6H_2O$ *(2 mol eq)*	50ºC 2hr 100% conv., 99% recov. 0%(1) 100%(7)	50ºC N_2 4hr 12% conv., 87% recov. 88%(2) 9%(8) 3%(9)	50ºC 10hr [f] 100% conv., 31% recov. 0%(3) 100%(10)	50ºC N_2 4hr 100% conv., 63% recov. 0%(4) 100%(11)	50ºC N_2 2½hr 75% conv., 75% recov. 25%(5) 75%(12)	50ºC N_2 2½hr 100% conv., 97% recov. 0%(6) 100%(13)

[a] Low recovery possibly due to adsorption to clay surface,
[b] Conversion refers to the observed distribution of numbered compounds,
[c] mass recovery refers to the total percentage of organic material recovered,
[d] 2 mol equivalents of iron,
[e] air excluded with N_2,
[f] low recovery due to solubility differences between product and starting material, following literature method (Yamamoto, Hoshino *et al.* 1999).
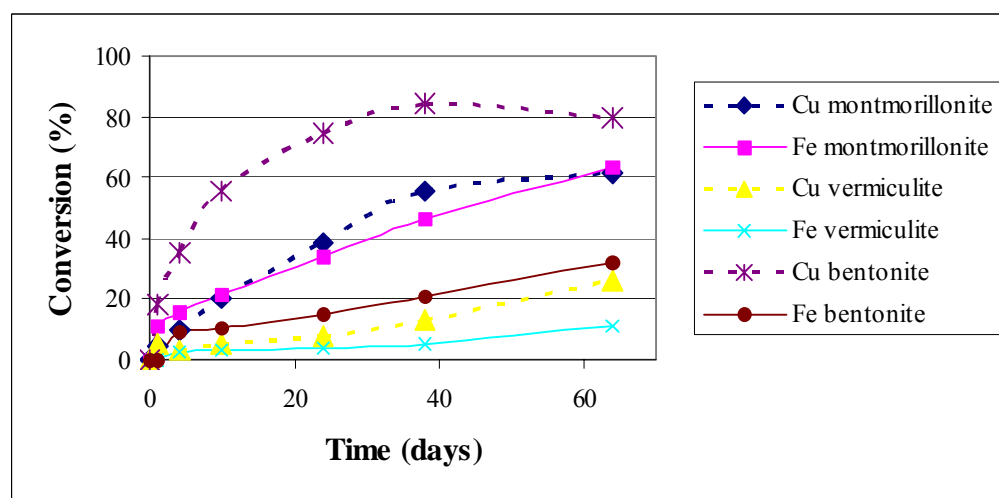

Figure 1. Long-term incubation of surface-modified clays (50% M^{n+}) with 2-naphthol, solvent-free at ambient temperature (20ºC).

Moisture is usually present in a natural soil environment, hence a series of experiments were conducted with dissolved iron (III) salts. Hydrated metal salts and hydrated metal cations in solution are known to promote oxidative coupling reactions (Ding, Wang *et al.* 1996), and may promote covalent bond formation where they exist in soils. Iron (III) salts are highly water soluble, forming the hexa-aquo iron cation $[Fe(H_2O)_6]^{3+}$ in water. The substrates chosen for this study, with the exception of 2-hydroxy-6-naphthalene-sulfonic acid sodium salt **(6)** are poorly water-soluble, the reactions were thus conducted as a slurry. All substrates exhibited conversion to coupled products in the presence of dissolved iron (III) chloride (Table 2), though the rates differ, with readily coupled 2-naphthol yielding **(7)** almost quantitatively, while the less activated substrate vanillin, required extended reaction time to effect coupling to divanillin **(10)**. In some cases further oxidation occurred post coupling with 2,6-dimethylphenol forming the coupled and oxidised 3,3',5,5'-tetramethyl-diphenoquinone **(11)** in the

presence of four equivalents of iron (III). 2,4-Dimethylphenol coupled to form **(12)** in aqueous iron, with high conversion and mass recovery and 2-hydroxy-6-naphthalene-sulfonic acid sodium salt formed the highly fluorescent product **(13)** under these conditions. Anthrone did not show much reactivity in this system, with little overall conversion. Anthrone is the least water-soluble of the selected substrates (* log P (anthrone) = 3.576±0.237 *cf* log P (2-naphthol) = 2.713±0.189) and thus this truly biphasic system may be less reactive. (* Calculated using Advanced Chemistry Development (ACD/Labs) Software Solaris V4.67 © 1994-2004 ACD/Labs. Obtained from SciFinder[®] Scholar 2004)

Aside from the effect of solubility on substrate diffusion, a number of other factors may have limited the reactivity in these systems. Intimate association of substrates with iron (III) salts or clay-supported cations is required, and this can be inhibited by negatively charged clay surfaces preventing intercalation of substrate molecules. Also, adsorption of substrates or products to clay surfaces may decrease their recovery. Reactions carried out in water yielded high conversions to the coupled products where the substrates were slightly water-soluble. The lack of water in clays under solvent-free conditions may decrease their ability to promote coupling, possibly due to reduced interlayer spacing.

To test the effect of water on substrate diffusion and association with iron (III) cations, reactions were carried out using both hydrated and anhydrous metal salts under solvent-free conditions. Oxidative coupling reactions of 2-naphthol were conducted using anhydrous iron (III) trichloride and iron (III) trichloride hexahydrate as the reaction promoters. The metal salts were used in 0.1 mol equivalents [1:10] relative to 2-naphthol to determine if the salts act as catalysts, and 2 mol equivalents [2:1] to ascertain whether complete conversion is possible. Reactions were performed at ambient temperature (20°C) and 50°C (Figure 2).
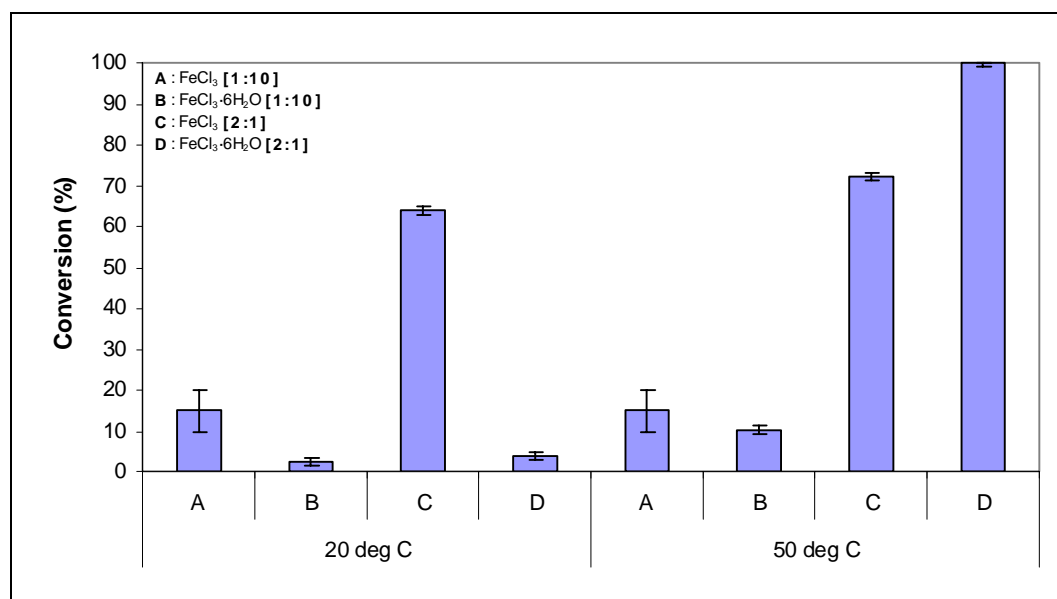

Figure 2. Solvent-free oxidative coupling of 2-naphthol using anhydrous iron (III) trichloride and iron (III) trichloride hexahydrate.

Oxidative coupling of 2-naphthol requires one equivalent of iron for each mole of substrate (Toda, Tanaka *et al.* 1989), and thus reactions performed using catalytic amounts of each iron (III) salt did not proceed beyond 10% conversion to **(7)**, indicating that reoxidation of iron (II) salts did not occur under these conditions. When an excess of each iron salt was used, a large difference in reactivity was evident. At ambient temperature (20°C), anhydrous iron (III) trichloride yielded nearly as high conversion to coupled product as at 50°C, albeit over a longer time period. Conversely, a significant difference in reaction progress was seen when using iron (III) trichloride hexahydrate at ambient (20°C) and elevated (50°C) temperatures. Iron (III) trichloride hexahydrate was observed to liquefy above 37°C, and is reported to contain octahedral $Fe(H_2O)_4Cl_2^+$ and tetrahedral $FeCl_4^-$ dissolved in a small volume of water, in equal concentrations (Murata and Irish 1988). Therefore reaction proceeded in a liquid medium, which was effectively a highly concentrated solution of iron (III) salts. This may explain why the reaction proceeded well at temperatures above 37°C, and poorly below this temperature.

Iron (III) trichloride hexahydrate-mediated coupling under solvent-free conditions (50°C, 2 h) did not proceed equally well for all substrates tested (Table 2). 2-Naphthol, anthrone and 2,4-dimethylphenol showed good conversion to coupled products, and starting material was recovered unaltered for substrates **(3)**, **(4)** and **(6)** even though these all exhibited some oxidative coupling product in aqueous solution (Table 2). Close observation of the reactant mixtures indicated a single liquid phase for mixtures of 2-naphthol and iron (III) trichloride hexahydrate at 50°C. However, vanillin, while turning black upon contact with iron (III) trichloride hexahydrate, did not result in a liquid mixture, implying that the existence of a melt phase may be required to effect reaction between the oxidant and substrate (Rothenberg, Downie *et al.* 2001).

Accumulation of soil organic matter can occur *via* protection within hydrophobic domains of humic substances, preferentially occurring within associations of fine mineral particles and organic matter (Piccolo 2002). Accordingly, oxidative coupling with loaded clays was attempted using a low-polarity solvent as the reaction medium (Table 2). An amount of clay, corresponding to 0.05 mol equivalents of the hydrated metal ion content to moles of substrate, was used in these experiments. Reactions in toluene were typically carried out at higher temperatures over shorter time periods of approximately 6 hours, without exclusion of air. Complete conversion of anthrone occurred, although only 23% of this was to the coupled product **(9)** and 77% to the oxidised form **(8)**. Moderate conversion of 2-naphthol to the coupled product **(7)** was observed. Also, reoxidation of the iron supported on the clay catalyst was important. This was demonstrated by renewal of activity in oxidative coupling reactions of 2-naphthol after reoxidation with 30% aqueous hydrogen peroxide, which enhanced reactivity, whereas rehydration alone did not (Figure 2).
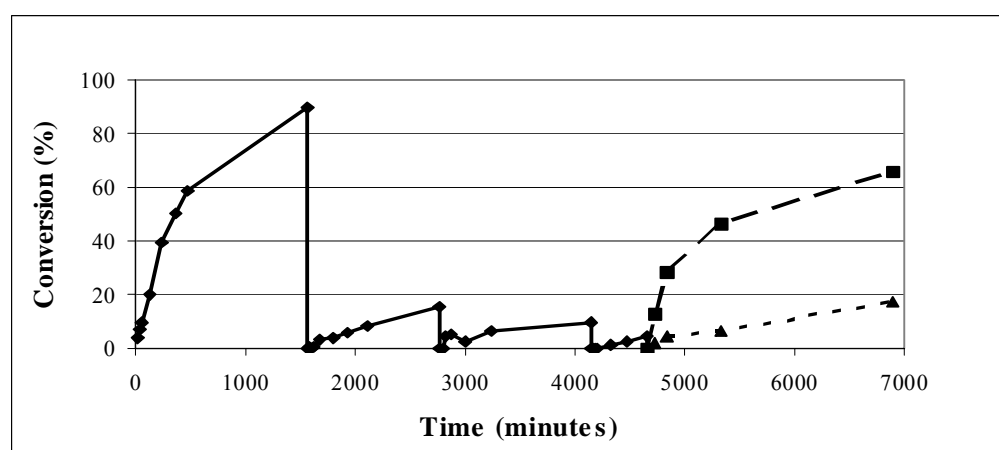

Figure 2. Oxidative coupling using iron (III)-loaded montmorillonite with further additions of starting material, reoxidation with H_2O_2 (- - -) and rehydration ($\cdots$).

In summary, aqueous iron (III) chloride solution was the most effective set of conditions for oxidative coupling of the selected substrates. Iron (III) and copper (II)-loaded clays showed catalytic activity in oxidative coupling of 2-naphthol, indicating a possible source of aryl-aryl oxidatively coupled products in soils where metal cations and smectite clays exist together. Reoxidation of the iron loaded on the clay was important for further reaction to occur. Similarly, reoxidation of metals in soils is vital for these to continue to act as electron acceptors, and thus promote covalent bond formation. Effective reaction was limited by factors that control contact between the substrate and iron (III) cations, such as substrate solubility, diffusion of substrate molecules and iron (III) cations, and the repulsive effect of negatively charged clay surfaces.

Conclusion

Model compounds, representing phenolic fragments of soil organic matter, were converted to larger molecular weight coupled compounds by the formation of new carbon-carbon bonds in oxidative coupling reactions. Dissolved iron (III) salts were effective reaction mediators but did not, in the timeframes tested, act catalytically while iron (III) ions, intimately associated with smectite clays, acted as catalysts, effecting coupling under mild conditions. The catalytic effect of such abiotic soil

components may be important in a variety of similar carbon-carbon bond forming reactions and thus in stabilisation of organic matter, aiding carbon sequestration in soils.

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