Nitrate Remeidation by Iron Redox Reactions in Soils

Basic Information

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<td>Principal Investigators:</td>
<td>Joseph Stucki, Zachary B. Day, Fabian G. Fernandez</td>
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Publications

There are no publications.
PROBLEM AND RESEARCH OBJECTIVE

Nitrate is an important inorganic nitrogen species, key to the healthy growth of all vegetation either through direct uptake or indirectly through processes involving a range of bacteria within the soil and root systems. Logically, this makes nitrate a target of most fertilizer regimens, with tons applied annually to fields in most all agricultural regions. Combined with irrigation, rain, natural water tables and nitrate’s high solubility, its widespread contamination of water sources in human populated areas is not surprising.

A litany of negative human health effects are attributed to these high levels of nitrate, including an increased risk of a number of cancers, chronic diseases and birth defects (Camargo and Alonso, 2006). Nitrate also contributes extensively to the acidification of, eutrophication of, and hypoxia in bodies of water in nearly every coastal region with significant human population. Hypoxia is especially of major concern in the Gulf of Mexico, particularly near the Mississippi River delta (Camargo and Alonso, 2006; Hypoxia Action Plan, 2007). Beyond the direct health and environmental stresses caused by nitrate, economic side effects can be felt in the strains on health care these issues pose and the loss of income in areas reliant on clean waters for fishing and tourism.
Additionally, clean up and immobilization efforts at sites dealing with contamination by oxidation-reduction (redox) active heavy metals (e.g. cobalt, technetium, and uranium) are hampered by the high levels of nitrate that are also present. Nitrate, sitting higher in the reduction series, must be dealt with first before any method of sequestration relying on redox reactions within the organic, inorganic, or both portions of the soil is attempted. To this end, understanding the interactions between nitrate with the redox active, iron containing portions of soils is necessary.

Most studies of nitrate and the nitrogen cycle within soils have focused primarily on bacterial processes. Some studies, however, have investigated abiotic nitrate reduction which have focused primarily on two aspects. The first is on a class of compounds known as green rusts (layered Fe(II)Fe(III) hydroxide, GR) which have been shown in the laboratory to be an excellent reductant of nitrate. While thought to exist in soils, they have yet to be characterized in situ due to their highly oxygen sensitive nature (Hansen et al., 1994, 1996, 1998, 2001). Studies by numerous groups have also shown zero-valent iron to be an excellent reductant of nitrate (Miehr et al., 2004; Alowitz et al., 2002; Sianta et al., 1996; Till et al., 1998; Huang et al., 1998; Devlin et al., 2000; Westerhoff, 2003; Sohn et al., 2006).

A third, but little studied avenue of nitrate abiotic nitrate reduction was proposed by Ernstsen (Ernstsen, 1996; Ernstsen et al, 1998) who observed a sharp, dramatic decrease in nitrate levels within Danish soil profiles. Denitrifying bacteria were absent from these soils, demonstrating the existence of some type of abiotic pathway leading to nitrate reduction. She proposed that Fe(II) within the structure of the soil clay minerals that was responsible for this reduction. The structural Fe(II) was produced by iron-reducing bacteria within the soil. Because Ernstsen’s hypothesis concerning redox active soil clay minerals interacting with nitrate has
received no further study but significant merit for a more detailed investigation, the present study was undertaken to determine whether such a phenomenon can be demonstrated in the laboratory.

METHODOLOGY

Sample Preparation and Reduction

All experiments were performed using the Source Clays Repository recognized ferruginous smectite, SWa-1—hereafter referred to as “SWa-1” and “clay” within the methods section for ease of reading. All water used is first purified by a Barnstead Fisher NanoPure System Model D4741, fed by a DI water source.

Each experiment described was performed in triplicate, with each reaction in a separate 50 mL polycarbonate centrifuge tube. The centrifuge tubes were sealed with an air-tight septum cap and all manipulation were performed under inert-atmosphere conditions, using needles to access the sample suspensions through the septum before or after centrifugation (Stucki et al, 1984).

Each sample tube received a fresh preparation of citrate-bicarbonate (C-B) buffer from stock solutions for each trial. The buffer components were 5 mL 0.18 M sodium citrate (Fisher Scientific, Waltham, Massachusetts) in water, 10 mL 0.36 M sodium bicarbonate (Fisher Scientific, Waltham, Massachusetts) in water, and an additional 15 mL of water. Final buffer concentrations were 0.03 M sodium citrate and 0.12 M sodium bicarbonate in a 30 mL total volume. Into each tube was then weighed a 50 mg sample of SWa-1. Tubes were capped and mechanically vortexed for 20 min, suspending their contents.
Tube contents were chemically reduced using sodium dithionite (Mallinckrodt Baker, Lopatcong Township, New Jersey) as described by Stucki et al (1984). A 200 mg portion of sodium dithionite was weighed and added to the C-B clay suspension in each reaction tube, after which the tube was immediately septum sealed and placed in a 70 °C water bath. Two needles were inserted through the septum cap. One needle brought nitrogen gas, which previously passed through an oxygen trap, into the tube; the second needle vented excess gas, purging gaseous reaction products (e.g. H₂S) from the system. Because the mass of reductant added to each reaction was constant, the extent of reduction was controlled by reaction time within the water bath. Reactions were quenched by submersion of the tubes into liquid nitrogen. Experimental runs were defined by these reduction times as follows: 10 min, 30 min, 60 min, 240 min and an unaltered control group that was not reduced.

After quenching the reaction in liquid nitrogen, tubes were centrifuged at 19000 rpm (18000 x g) for 20 min in a Sorvall RC 50 Plus centrifuge. Tube contents were washed three times with deoxygenated 5 mM NaCl solution in water. The clay mixtures were resuspended by vortexing between washes. A fourth, final wash was performed with 18 MOhm-cm pure water before analyses for iron oxidation state and/or before reaction with nitrate.

Iron Analysis

The washed clay with little remaining supernatant was analyzed for Fe(II) and total Fe content using a 1,10-phenanthroline and UV light method, modified slightly from Komadel and Stucki (1988). Method modification required digestion of samples and standards in polycarbonate centrifuge tubes instead of polypropylene tubes to avoid sample transfer resulting in loss and reoxidation. Analysis batches averaged between three and six washed clay samples,
representing one or two different reduction time trials, as well as four standards to verify the accuracy of the analysis.

Each standard was prepared in a polycarbonate centrifuge tube identical to the reduction reaction tubes. Tubes were labeled according to their approximate iron concentrations after final dilution as 0, 1, 3, and 5 ppm. The mass of each empty tube was recorded and into each approximately 7 mg of ferrous ammonium sulfate (Allied Chemical and Dye, New York, New York) was weighed per integer of final desired concentration in ppm. The weight of the tube and the standard were recorded so exact concentration could be determined calculated. After adding the standard to the tube, all white lights in the room where the analysis was being performed were turned off in favor of red lights. This prevented ferric iron in samples, which also forms a complex with 1,10-phenanthroline, from being photo-chemically reduced before Fe(II) concentrations had been determined.

Digestion and complexing reagents were added in fast succession to each standard and sample to prevent as much reoxidation of the reduced clay samples in the presence of air as possible; reduced clay samples were only uncapped just before addition of digestion reagents. A 12.0 mL aliquot of 3.6 N sulfuric acid produced from concentrated sulfuric acid (Mallinckrodt Baker, Lopatcong Township, New Jersey), a 2.0 mL aliquot of 10% by weight 1,10-phenanthroline (Sigma-Aldrich, St. Louis, Missouri) in 95% ethanol, and 1.0 mL of 49% hydrofluoric acid in water (Acros Organics, Geel, Belgium) were added to all standards and samples. The centrifuge tubes were placed in a boiling water bath for 30 min to digest their contents, followed afterwards by a 15 minute cooling period. Once cooled, 10.0 mL of 5% by weight boric acid (Acros Organics, Geel, Belgium) in water was added to each centrifuge tube.
Centrifuge tube contents were quantitatively transferred to 100 mL polypropylene tubes, which had previously been weighed while empty. Each of these “iron analysis tubes” was filled to within an inch of its tops with water and its final mass recorded. Mass was converted to volume assuming a density of 1.000 g/ml. A piece of parafilm was placed securely across the top of each tube to prevent spilling and the tube was then inverted several times to induce mixing. After mixing, a Brinkmann Dosimat dilutor was used to draw a 2.0 mL aliquot from the tube and to expel it into a 50-mL Erlenmeyer flask along with 20.0 mL of 1% by weight sodium citrate solution in water. Two such flasks were produced from each iron analysis tube for duplicate absorbance measurement.

Final solutions were analyzed on a Varian Cary 5 UV-Vis spectrophotometer equipped with a Routine Sampling Accessory (RSA) Internal Sipper using the Concentration software application provided by Varian. The spectrometer was allowed to warm up for a minimum of 30 min prior to zeroing and sample analysis. The absorbance of the tris-(1,10-phenanthroline)Fe(II)$^{2+}$ complex was measured at 510 nm, with two replicate measurements taken from each flask. During Fe(II) analysis the room was illuminated with only subdued red light, and the sipper line was rinsed twice with water between samples to prevent cross contamination. After absorbance values were collected for Fe(II), all flasks were placed in an enclosure with two high intensity mercury vapor lamps for 2 h to reduce the ferric iron complex with 1,10-phenanthroline (Stucki and Anderson, 1981). The total Fe absorbance values were then measured at 510 nm.

*Nitrate Addition*
A second group of reduced, washed clay samples from each reduction-time set were identically prepared to be reacted with nitrate. An 88 μM solution of sodium nitrate (EM Science, Gibbstown, New Jersey) was prepared in water. For each sample a 20.0 mL aliquot of nitrate was prepared in a capped centrifuge tube. Each was deoxygenated by flowing nitrogen into the solution for a minimum of 30 min through a needle which penetrated the septum cap, venting excess gas through a second needle. Nitrogen flow was continued while the aliquot was drawn from the centrifuge tube by a gas tight syringe and injected into a tube containing the washed, reduced clay, also equipped with nitrogen supply and vent needles.

After injection, the clay was suspended in the nitrate solution by mechanical vortex and then tubes were placed on a shaker plate for 18 h. Samples were centrifuged and affixed with a nitrogen needle and vent needle in the headspace, so as not to disturb the supernatant liquid or the collected solid at the bottom. The supernatant was carefully removed by gas tight syringe and placed in a new tube which was then frozen for shipping to be analyzed for NO$_x$ and nitrite as described below. The remaining clay was analyzed by the 1,10-phenanthroline method for iron content to compare with samples not reacted with nitrate.

A control group for the 10-min, 30-min, 60-min, and 240-min reduction time trials was created as well. These samples were reduced and treated exactly as those that were treated with nitrate up to the point of nitrate addition. Instead of a dilute nitrate solution, these samples were treated with 20 mL each of deoxygenated water for 18 h. As no nitrate was present, supernatants were not analyzed for nitrogen. This control experiment was performed to account for any reoxidation of the clay structure not attributed to nitrate.

*Nitrogen Speciation Analysis*
Nitrite and nitrate were analyzed together using a modification of the method described by Braman and Hendrix (1989) to determine NO$_x$ concentrations using a Thermo Model 42i Chemiluminescence Analyzer. The sample holder on the instrument was filled with 100 mL of acidified (1-2 M HCl) 0.10 M V(III) solution, bubbled with helium. Instrument response was calibrated by 100 μL injections of NO$_x$ standards ranging in concentration from 1 to 50 μM. Evolved NO was carried by helium flow to the analyzer and the peak area recorded. Sample injection volume was varied between 50 and 200 μL.

Nitrite alone was measured using a modified Garside (1982) method. The method was modified such that it was identical to the combined nitrite and nitrate method described above with the exception of the reagent solution in the sample holder. The nitrite only mixture was a 1:3:6 ratio of 3% w/v sodium iodide in water, glacial acetic acid, and pure water. Calibration and sample analysis were carried out as above.

Results

Iron Analysis

Following each iron analysis trial, absorbance values for the standards were plotted against their known iron concentrations to create calibration curves. Curves were used to verify both that the method returned a linear response within the concentration range and that the specific trial set was responding properly. Calibration curves constructed for both Fe(II) and total Fe for a single trial run were uniformly linear with correlation coefficients within the same range as those in the figures, excepting one trial with one outlying standard.

Accuracy and precision were also correlated across the entire experimental range using the Beer-Lambert Law.
\[ A = \varepsilon \cdot c \cdot l \]  

where \( A \) is the absorption value, \( \varepsilon \) is the absorptivity coefficient in M\(^{-1}\)cm\(^{-1}\), \( c \) is the molar concentration and \( l \) is the path length (1 cm) of the cell used. Using the known concentrations of the standards analyzed in each trial in conjunction with the absorption values obtained, the absorptivity of all standards was calculated. The absorptivity values were averaged, excluding the single outlier, resulting in absorptivity coefficients of 931 M\(^{-1}\)cm\(^{-1}\) ± 5% for Fe(II) and 1044 M\(^{-1}\)cm\(^{-1}\) ± 4% for total Fe. The slightly higher value of the total Fe absorptivity coefficient versus that of Fe(II) is in agreement with the findings of Komadel and Stucki (1988).

Using the absorptivity coefficients derived from the standards, the Beer-Lambert law was again used to calculate the concentrations of Fe(II) and total Fe present in the sample solutions. The concentrations were normalized by dividing by the mass of clay used in each trial, and then these values are averaged across each time set and a ratio between Fe(II) and total Fe was derived (Table 1).

The “unaltered” samples—those that were unreacted with dithionite—showed only a trace amount of Fe(II) which is negligible compared to the total Fe content. The amount of Fe(II) present in the unaltered samples is the same both before and after reaction with a dilute nitrate solution. The remaining sample sets in the before nitrate reaction group have consistent total Fe concentrations. The total Fe values after reaction with nitrate are likewise consistent within themselves, but show a slight decrease, likely due to dissolution in the unbuffered nitrate solution.

After only 10 min of reduction in the presence of dithionite, the Fe(II) content increased dramatically accounting for roughly a quarter of the total Fe in the structure. Following reaction with nitrate, the Fe(II) content of the 10-min trials, however, failed to drop significantly.
Interestingly, the extent of reduction in the 30-minute 60-min samples was similar, with both falling in the 50-60% range. The values after introduction to nitrate were likewise similar to each other, but a large amount of reoxidation was evident in both cases. After 420 min of reduction, nearly all ferric iron in the clay structure was converted to Fe(II). Comparing all the values obtained for all time trials revealed that a large amount of reduction occurred in approximately the first 30 min of reaction and the rate of the reaction significantly decreased after that point. The Fe(II) to total Fe ratios in 10-, 30- and 60-min control trials, in which reduced clay was exposed only to water, were not significantly different from those of the clay directly after reduction; so most of the reoxidation occurring was attributed to the redox reaction with nitrate.

The 240-min samples exhibited the most reoxidation in the presence of nitrate. Note, however, that the 240-min samples were not reoxidized to the same extent as the 30-min and 60-min samples, which in turn were not as reoxidized as the 10-min samples. This suggests that the structural Fe(II) is not all available to be reacted with nitrate. This may in large part be due to the random reduction of Fe sites at the basal surface. The nitrate anion is unlikely to react at the basal surfaces due to coulombic repulsion. Reaction then only occurs at the comparatively small surface area of the edge sites (approximately 0.1% of clay surface area), which contain a small percentage of the total Fe(II) within the structure, as illustrated by Ribeiro et al. (2009). If so, some form of electron transfer to the edge sites is necessary for the complete reoxidation of the clay structure. The 240-min samples were the only group that exhibited significant reoxidation in water, with the structural Fe(II) to total Fe ratio some 20% lower than in the clay after being reduced.

Nitrogen Speciation
The nitrogen speciation for the reduced clay samples (Table 2) revealed that the general trend is for NO$_x$ values to decrease from their original 88 μM concentration as the extent of reduction in the clay increases. An unexpected slight decrease in NO$_x$ concentration within the unaltered samples, comparable to that in the 10-min samples, suggests a slight error in the method of approximately ± 1.5 μM. No nitrite appears in the solutions of the unaltered samples, confirming that no nitrate was likely lost from the unaltered samples. Nitrite concentrations among all reduced samples were extremely low and comparable with one another. Nitrite, therefore, was not a major reduction product in these reactions: far more NO$_x$ was lost relative to how much of the remaining NO$_x$ was nitrite. Furthermore, nitrite concentrations failed to increase with increasing extent of reduction or with total nitrate reduced (Table 3). Nitrate remaining was calculated as nitrite values subtracted from NO$_x$ and total nitrate reduced is the calculated nitrate concentration subtracted from the initial concentration in the nitrate solution used of 88 μM multiplied by the volume of the aliquot introduced to the sample (20 mL).

Looking at the nitrogen speciation and iron analysis together as a whole, chemically reduced clay clearly was very much capable of nitrate reduction, as hypothesized. By plotting the amount of Fe(II) initially available vs. the amount of nitrate reduced (Figure 1), a clear trend between the extent of reduction within the clay structure and the amount of reduced nitrate is visible. The points do not fall all exactly upon a line, because, as noted previously, the state of reduction within the structure was such that variable amounts of Fe(II) are available at the edge given the same overall amount of reduction of the structure.

PRINCIPAL FINDINGS AND SIGNIFICANCE
The data presented show that nitrate is abiotically reduced by chemically reduced clays, which concomitantly are oxidized in the process, thus removing the nitrate from the system. A clear relationship was drawn between the amount of Fe(II) present in the clay structure and the amount of nitrate that is removed from a dilute solution. The reactive sites evidently are located on the edge surfaces of the clay layers, which present much less reactive surface area than the basal surfaces. Not explored in these experiments was the effect of different clays upon the reactions. SWa-1 was selected for this pilot study because it is known to have one of the highest rates of iron substitution in the octahedral sheet. It stands to reason that clays containing less octahedral iron, containing tetrahedral iron, and those with a different layer structure (1:1, 2:1:1) will behave differently. Since a smectite such as SWa-1 is not representative of the minerals found at all sites requiring nitrate remediation, it must be ascertained whether or not other phylosillicates have similar reactivity. As well, the kinetics of these reactions are not understood. The methods used in the experiments described here could easily be altered to assess the amount of reoxidation and nitrate removal at intervals other than 18 h.

While the findings of these experiments were all based upon chemical reduction, it seems logical that bacteria reduced clays found in natural settings would be capable of the same reaction. This is especially true in light of the fact that bacteria reduced clays have been shown to have their Fe(II) concentrated at edge sites, which is conducive to the proposed mechanism for reduction of nitrate by the reduced clay minerals. Further experiments using various clays in addition to SWa-1, as detailed above, may confirm or disconfirm this hypothesis.

There is still a great deal more to be explored concerning reduced mineral interactions with nitrate. With any luck, the literature will eventually be as detailed for this set of reactions as for nitrate’s interactions with green rust and with zero-valent iron.
NOTABLE ACHIEVEMENTS

This study has increased scientific understanding, providing evidence that redox active, iron-bearing clay phases within soils are capable of, and may play a key role in, nitrate reduction. The 1,10-phenanthroline iron analysis method was also slightly improved to allow analysis of wet and oxygen sensitive samples without initial transfer and loss to new analysis tubes.

STUDENT SUPPORTED WITH FUNDING

Zachary B. Day, Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, Master of Science, May 2010.

PUBLICATIONS AND PRESENTATIONS


Westerhoff, P. (2003) Reduction of nitrate, bromate and chlorate by zero valent iron (Fe0).
Journal of Environmental Engineering, 129, 10-16.

**TABLES**

**Table 1.** Iron Analysis Results

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<th>Reduction Time (min)</th>
<th>0*</th>
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<th>30</th>
<th>60</th>
<th>240</th>
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<td><strong>Before Nitrate Treatment</strong></td>
<td>mmol Fe(II) / g Clay</td>
<td>0.01</td>
<td>0.42</td>
<td>0.98</td>
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<td>mmol Fe / g Clay Fe(II)/Fe</td>
<td>2.68</td>
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<td>1.63</td>
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<td>Fe(II)/Fe</td>
<td>0.00</td>
<td>0.25</td>
<td>0.60</td>
<td>0.52</td>
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<tr>
<td><strong>After Nitrate Treatment</strong></td>
<td>mmol Fe(II) / g Clay</td>
<td>0.01</td>
<td>0.33</td>
<td>0.47</td>
<td>0.61</td>
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<td></td>
<td>mmol Fe / g Clay Fe(II)/Fe</td>
<td>1.49</td>
<td>1.53</td>
<td>1.43</td>
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<td>Fe(II)/Fe</td>
<td>0.01</td>
<td>0.21</td>
<td>0.33</td>
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<td><strong>After Water Treatment</strong></td>
<td>mmol Fe(II) / g Clay</td>
<td>n.d.</td>
<td>0.43</td>
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<td>mmol Fe / g Clay Fe(II)/Fe</td>
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<td>Fe(II)/Fe</td>
<td>n.d.</td>
<td>0.28</td>
<td>0.55</td>
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*Unaltered Sample

**Table 2.** Nitrogen Speciation Results

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<th>Sample</th>
<th>[NO\textsubscript{x}\textsuperscript{-}] (\textmu M)</th>
<th>[NO\textsubscript{2}\textsuperscript{-}] (\textmu M)</th>
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<tr>
<td>Unaltered</td>
<td>86.7</td>
<td>0.0</td>
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<td>10-min</td>
<td>87.0</td>
<td>0.1</td>
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<td>30-min</td>
<td>84.7</td>
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<td>60-min</td>
<td>83.4</td>
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<td>240-min</td>
<td>83.0</td>
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Table 3. Nitrate Reduced

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<th>Reduction Time</th>
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<th>[NO$_2^-$] Present (mM)</th>
<th>NO$_3^-$ Reduced (mmol)</th>
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<tr>
<td>Unaltered</td>
<td>0.0867</td>
<td>0.0000</td>
<td>2.6 x 10$^{-5}$</td>
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<tr>
<td>10 min</td>
<td>0.0870</td>
<td>0.0002</td>
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<tr>
<td>30 min</td>
<td>0.0848</td>
<td>0.0001</td>
<td>6.6 x 10$^{-5}$</td>
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<tr>
<td>60 min</td>
<td>0.0834</td>
<td>0.0002</td>
<td>9.6 x 10$^{-5}$</td>
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<tr>
<td>240 min</td>
<td>0.0830</td>
<td>0.0001</td>
<td>1.02 x 10$^{-4}$</td>
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FIGURES

![Figure 1](image.png)

**Figure 1.** Plot showing the effect of the extent of reduction of a clay mineral on the amount of nitrate reduced.