

Enhanced Degradation of TNT and RDX by Bio-reduced Iron Bearing Soil Minerals

Changhyun Cho, Sungjun Bae and Woojin Lee*

Department of Civil and Environmental Engineering, Korea Advanced Institute of Science and Technology,
291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea

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Abstract. We demonstrated that reductive degradation of 2,4,6-Trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (Royal Demolition Explosive, RDX) can be enhanced by bio-reduced iron-bearing soil minerals (IBSMs) using *Shewanella putrefaciens* CN32 (CN32). The degradation kinetic rate constant of TNT by bio-reduced magnetite was the highest (0.0039 h^{-1}), followed by green rust (0.0022 h^{-1}), goethite (0.0017 h^{-1}), lepidocrocite (0.0016 h^{-1}), and hematite (0.0006 h^{-1}). The highest rate constant was obtained by bio-reduced lepidocrocite (0.1811 h^{-1}) during RDX degradation, followed by magnetite (0.1700 h^{-1}), green rust (0.0757 h^{-1}), hematite (0.0495 h^{-1}), and goethite (0.0394 h^{-1}). Significant increase of Fe(II) was observed during the reductive degradation of TNT and RDX by bio-reduced IBSMs. X-ray diffraction and electron microscope analyses were conducted for identification of degradation mechanism of TNT and RDX in this study. 4-amino-dinitrotoluene were detected as products during TNT degradation, while Hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine, Hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine, and Hexahydro-1,3,5-trinitroso-1,3,5-triazine were observed during RDX degradation.

Keywords: 2,4,6-Trinitrotoluene (TNT); hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX); iron-bearing soil minerals; *Shewanella putrefaciens* CN32

1. Introduction

The cyclic nitramine explosives compounds are highly energetic chemicals that rapidly release large amounts of gaseous products and energy upon detonation (Lotufo *et al.* 2009). Because of their explosive properties, these chemicals are extensively used in the military, construction site, and mining industry. Due to intensive use of explosives as described above, the contamination of soil and groundwater by these has been continuously reported, especially in the proximity of munitions manufacturing plants (Hundal *et al.* 1997a, Boopathy and Manning 2000). Among them, 2,4,6-Trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (Royal Demolition Explosive, RDX) are the most widely used explosives all over the world. (Spain *et al.* 2000). TNT has been known to be carcinogenic and mutagenic and is acutely toxic to microbes, algae, fish, and other organisms (Spain *et al.* 2000). RDX, heterocyclic nitramine, is a persistent compound that can threaten human

*Corresponding author, Professor, E-mail: woojin_lee@kaist.ac.kr

health due to its toxicity (Yinon 1990).

Because of their toxicity, many efforts have been made to effectively degrade TNT and RDX to date. The reductive degradation of TNT by photocatalytic reaction (Schmelling *et al.* 1996), CO dehydrogenase (Huang *et al.* 2000), zero valent iron (Hundal *et al.* 1997b) have been reported. The reductive degradation of RDX by chemical reductants such as zero-valent iron (Singh *et al.* 1998, Naja *et al.* 2008), freshly precipitated iron minerals in aqueous Fe(II) solution (Boparai *et al.* 2010), and iron organic ligand complexes (Kim and Strathmann 2007) have shown successful degradation of RDX. Bioreduction of TNT and RDX using microorganism can be a great alternative for degradation of explosives in sediment or soil (Boopathy and Kulpa 1992, Adrian and Arnett 2004, Sherburne *et al.* 2005, Thompson *et al.* 2005, Meyers *et al.* 2007). It has been also reported that RDX degradation was greatly enhanced in anaerobic condition due to the initial nitro → nitroso degradation pathway (Adrian *et al.* 2003).

Degradation of various organic contaminants (e.g., chlorinated compounds and explosives) by iron-bearing soil minerals (IBSMs) has been reported in everywhere (Lee and Batchelor 2002a, b, Gregory *et al.* 2004, Larese-Casanovva and Scherer 2008, Oh *et al.* 2008). However, the IBSMs can be lost their reactivity after the reductive degradation of explosives due to the oxidation of Fe(II) to Fe(III) on their surfaces. The reduction potential of the IBSMs was decreased and the reductive degradation of the explosives was inhibited. Therefore, it is very important to recover the reactivity of the IBSMs for further reductive degradation reaction.

Bioreduction of IBSM by dissimilatory iron-reducing bacteria (DIRB) can be a great option to recover the reactivity of IBSMs. (Perez-Gonzalez *et al.* 2010, Maithreepala and Doong 2009, Fredrickson *et al.* 1998, O'Loughlin 2008, Zachara *et al.* 1998). However, there is lack of knowledge on the recovery of IBSM reactivities by DIRB for the reductive degradation of explosives to date. Thus, it is timely to investigate the possibility for the enhanced degradation of explosives by bio-reduced IBSMs.

The objectives of this study were to investigate the enhanced degradation of TNT and RDX by bio-reduced IBSMs, to find out the transformation products of TNT and RDX and propose the each reaction mechanism.

2. Materials and methods

2.1 Chemicals

Chemicals used in the experiment were TNT (Accustandard), RDX (Accustandard), sodium DL-lactate (Sigma), dipotassium phosphate (K_2HPO_4) (Junsei, Japan), monopotassium phosphate (KH_2PO_4) (Sigma), anthraquinone-2-sulfonic (AQS) (Sigma). Methanol (Merck) and acetonitrile (J.T.Baker) for preparation of TNT, RDX stock solution were all HPLC grade. 1,4-piperazinediehanesulfonic acid (PIPES) was used for buffered medium in all experiments. Deaerated deionized water (DDW, $18M\Omega\cdot cm$) was prepared by using ultra pure water from ELGA PURELAB Classic system and purging it by N_2 for 4 h and stored in an anaerobic chamber filled with 95% N_2 and 5% H_2 (Coy Laboratory Products Inc.). Unless stated otherwise, all experiments were prepared by using DDW purged by N_2 and conducted in an anaerobic chamber.

2.2 Preparation of IBSMs

Magnetite ($\text{Fe}^{\text{II,III}}_1\text{O}_4$) and green rust (GR- SO_4) were synthesized by following the procedure previously described (Bae and Lee 2010, Srinivasan *et al.* 1996). It was washed two times using DDW, freeze-dried, and stored in the anaerobic chamber. Lepidocrocite ($\gamma\text{-Fe}^{\text{III}}\text{OOH}$, Bayferrox 943), hematite ($\text{Fe}^{\text{III}}_2\text{O}_3$, Bayferrox 105M), and goethite ($\alpha\text{-Fe}^{\text{III}}\text{OOH}$, Bayferrox 3920) were purchased from LANXESS Corp. All IBSMs were screened with sieves and mineral particles under $150\ \mu\text{m}$ were collected for use. The IBSMs were identified by X-ray diffraction (XRD) to investigate their purity and identity. All IBSMs showed a good agreement with those in Joint Committee on Powder Diffraction Standards (JCPDS) diffraction data files (JADE 9, Materials Data, Inc.) (data not shown).

2.3 Bacteria culturing

Aerobically cultured CN32 cells in tryptic soy broth (TSB, 30 g/L) were prepared at 26°C under continuous shaking at 150 rpm. They were harvested at late growth phase and washed twice in 30 mM PIPES buffer (pH 7) to remove residual TSB by centrifuging at 12000 rpm for 5 min. Modified defined medium (MDM) was prepared using 30 mM PIPES buffer without NaHCO_3 to avoid the formation of siderite ($\text{Fe}(\text{II})\text{CO}_3$) (Bae and Lee 2012) and its pH was adjusted to 7.0 by adding 2 M NaOH. The prepared MDM was autoclaved, cooled at ambient temperature ($25 \pm 0.5^\circ\text{C}$).

2.4 Removal of IBSM reactivities and reductive degradations of TNT and RDX

Batch experiments for reductive degradation of TNT and RDX were conducted under anoxic condition using 250 mL of serum bottles sealed with aluminum cap and butyl rubber stopper. At first, we investigated abiotic degradation of TNT and RDX in magnetite, green rust, lepidocrocite, goethite, and hematite suspensions to remove their potentials for reductive degradation of TNT and RDX. MDM (200 mL) was transferred to the bottles containing 0.01 g of five different IBSMs (magnetite, green rust, lepidocrocite, goethite, and hematite). TNT (37.4 mM) and RDX (81 mM) stock solutions in methanol were prepared and its aliquot amount were (TNT 1.176 mL, RDX 110 μL) spiked to each bottle by a gastight syringe at the same time. The initial concentrations of TNT and RDX were 0.22 and 0.05 mM, respectively. The bottles were rapidly taken out of the anaerobic chamber and mounted on a tumbler at 7.5 rpm. After confirming that each IBSM could not degrade TNT and RDX, we centrifuged each bottle, washed once by DDW, and recollected the IBSMs by vacuum pump and $0.2\ \mu\text{m}$ sterilized PTFE membrane filter (Whatman). The similar procedure was used for sample preparation of the batch experiments to investigate the reductive degradation of TNT and RDX by bio-reduced IBSMs using CN32. MDM (180 mL) was transferred to the bottles containing 0.01 g of five different IBSMs (magnetite, green rust, lepidocrocite, goethite, hematite) and 5 mL of sodium lactate (30 mM, electron donor) and AQS (100 μM , electron transfer mediator) were added by a syringe with $0.2\ \mu\text{m}$ sterilized PTFE membrane filter. Washed cells (10 mL) were transferred to the bottles (3.33×10^7 cells/mL). Initial concentrations of TNT (0.22 mM) and RDX (0.05 mM) were injected into each bottle. The bottles were rapidly taken out of the anaerobic chamber and mounted on a shaking incubator at 150 rpm at 26°C . The reductive degradation kinetics of TNT and RDX were investigated by its aqueous concentration at each sampling time. Control (CN32 + MDM) was prepared to evaluate the loss of target compounds due to

microbial degradation of TNT and RDX.

2.5 Analytical procedures

TNT, RDX, and their transformation products such as 4-amino-dinitrotoluene (4-ADNT), Hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), Hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), and Hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) were analyzed using high-performance liquid chromatography (HPLC, Varian) with a variable wavelength photodiode array (PDA, 335 Varian) detector at 230 nm. The filtered samples were automatically injected into a RP C18 column (Shiseido, 250 mm × 4.6 mm) at ambient temperature. A mobile phase consisting of 40% phosphate buffer (pH 7.0) and 60% methanol was used at a flow rate of 1.2 mL/min (Bae 2006). TNT, RDX, and 4-ADNT were compared to certificated analytical standards in acetonitrile at known concentrations. Total iron was quantified by the Ferrozine assay (Stookey 1970). Each 1.5 mL aliquot sample from incubation bottles was diluted in 4.5 mL of 4 N HCl and 0.9 mL aliquot of the diluted solution was added to Ferrozine solution, and then quantified at 562 nm wavelength of UV/vis spectrophotometer (Agilent 8453).

XRD analysis was conducted for the identification of initial and secondary mineral phases using Rigaku automated diffractometer with Cu KN (D/MAX-2500). The bottles were centrifuged at 3000 rpm for 5 min at last sampling time. The precipitates were collected by filtration and dried in anaerobic chamber for 24 h. The samples were scanned between 5° to 80° 2 θ with scan speed of 4° min⁻¹. The XRD patterns of secondary minerals were analyzed with JCPDS diffraction data files.

The structure and morphology of IBSM particles were examined by TEM (Tecnai F20 model, Philips). Bottles were centrifuged at 3000 rpm for 5 min. Suspensions containing particles of IBSMs were replaced by ethanol in the anaerobic chamber and then dispersed by sonication for 4 min. A droplet of suspension was put on 300-mesh Cu TEM grids with a carbon film and dried in the anaerobic condition for 2 h.

3. Result and discussion

3.1 Abiotic degradation of TNT and RDX by IBSMs

Fig. 1 shows the reactivity removal of abiotic reduction potential of 5 IBSMs (green rust, magnetite, lepidocrocite, hematite and goethite) that can be used for TNT and RDX degradation. Green rust degraded 86.3% of TNT in first 1 h and approximately 100% of TNT in 44 h (Fig. 1(a)). To our knowledge, the degradation of TNT by green rust is firstly introduced by this study. Green rust also degraded 64.6% of RDX in 1 h and approximately 93% of RDX in 20 h exhibiting the highest reactivity to degrade contaminants among studied IBSMs (Fig. 1(b)). It has been reported that sulfate green rust (6 g/L) degraded 100% of RDX (40 μ M) in the presence of K₂SO₄ (50 mM) at pH 7 in 1 h (Larese-Casanovva and Scherer 2008). Our experimental results showed relatively slower kinetics compared to the previous research because 1.25 times higher concentrations of RDX and 12 times lower amount of green rust were used in our research than those of the previous research. The same amount of TNT and RDX were re-added after 44 and 20 h, respectively, to investigate the remaining reactivity of green rust. Fig. 1 shows that TNT and RDX were not further degraded by green rust after 60 h. This was due to the oxidation of Fe(II) on the surface of the green rust to

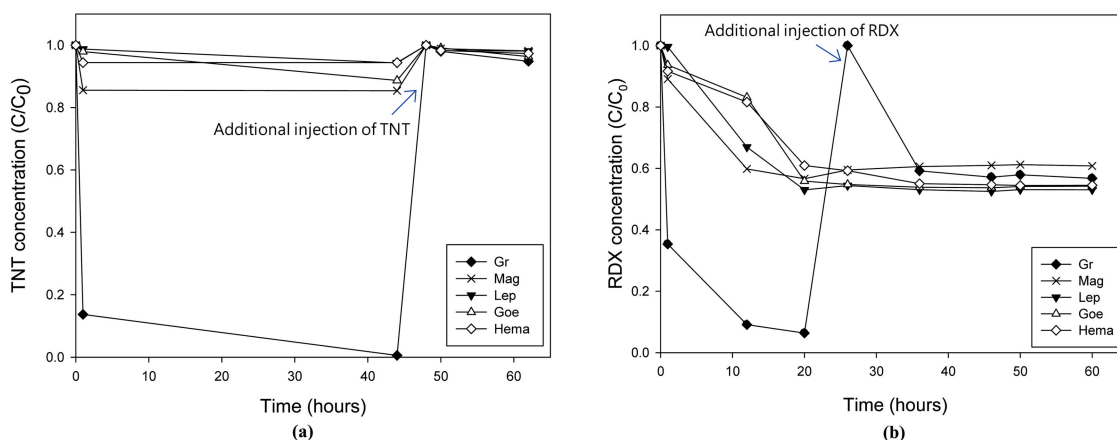


Fig. 1 Abiotic reductive degradations of (a) TNT and (b) RDX by IBSMs (green rust, magnetite, lepidocrocite, goethite, and hematite) (0.1 g/200 mL) in DDW. The initial concentrations of TNT and RDX were 0.22 and 0.05 mM, respectively

Fe(III) resulting in formation of magnetite by degrading TNT and RDX. XRD and TEM analysis in section 3.2 can explain the oxidation of green rust to magnetite. Magnetite degraded 17% of TNT in 44 h. It was relatively lower than green rust because magnetite composed of 1: 2 ratio of Fe(II) : Fe(III) ions (Lee and Batchelor 2002a). However, TNT was not degraded by magnetite after re-addition of TNT. The rest of other three IBSMs (lepidocrocite, hematite and goethite) did not show significant degradation of TNT. This was because they are Fe(II) deficient IBSMs that could not deliver electrons to TNT. Approximately 40% of RDX was removed in 58 h by magnetite, lepidocrocite, goethite and hematite (Fig. 1(b)), but expected by-products such as MNX, DNX and TNX were not detected during the reaction indicating that adsorption of RDX on the surface of IBSMs might be occurred.

3.2 Enhanced degradation of TNT by interaction of reactivity removed IBSMs and CN32

Fig. 2 shows the kinetics of TNT degradation by the interaction between reactivity removed IBSMs and CN32. The reactivity removed IBSMs regained their reactivity to degrade TNT by adding CN32. Especially, bio-reduced magnetite by CN32 showed the degradation of TNT (20.2%) in 58 h and the highest kinetic constant (0.0039 h^{-1}), followed green rust (0.0022 h^{-1}), goethite (0.0017 h^{-1}), lepidocrocite (0.0016 h^{-1}) and hematite (0.0006 h^{-1}) (Table 1). The control containing only CN32 and MDM showed low degradation of TNT (8.5%) and kinetic constant (0.0012 h^{-1}) in 58 h. This indicates that the degradation kinetic of TNT by each interaction was significantly different depending on types of IBSM.

3N HCl extractable Fe(II) concentrations of bio-reduced IBSMs were measured to investigate the effect of Fe(II) production on the degradation of TNT (Fig. 3). 3N HCl extractable Fe(II) in the IBSM suspensions with CN32 during the reductive degradation of TNT increased in 58 h. Magnetite and green rust, which showed high kinetic constants (0.0039 and 0.0022 h^{-1} , respectively), exhibited high Fe(II) productions (0.068 and 0.073 mM, respectively) among studied

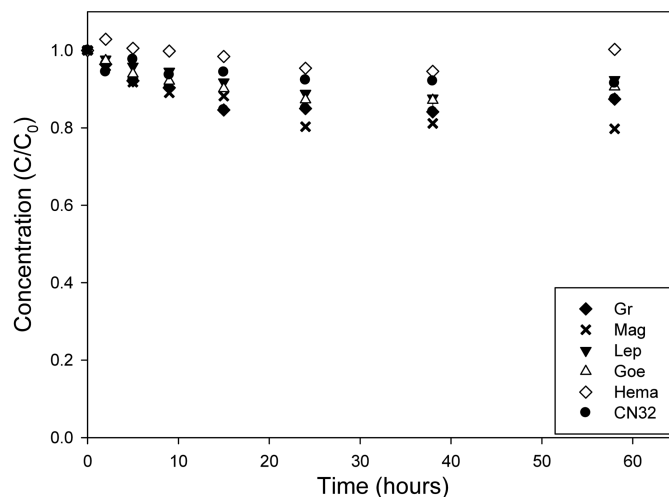


Fig. 2 Enhanced reductive degradation of TNT by bio-reduced IBSMs (green rust, magnetite, lepidocrocite, goethite, and hematite) with CN32 and only CN32 in MDM at pH 7 (PIPES). The initial concentration of TNT was 0.22 mM

Table 1 Observed kinetic rate constants for the reductive degradations of TNT and RDX by bio-reduced IBSMs. The data for CN32, green rust, magnetite, lepidocrocite, hematite and goethite were cited from Fig. 2 and Fig. 7

	CN32	Green rust	Magnetite	Lepidocrocite	Goethite	Hematite
TNT (h^{-1})	0.0012	0.0022	0.0039	0.0016	0.0017	0.0006
RDX (h^{-1})	0.0432	0.0757	0.1700	0.1811	0.0394	0.0495

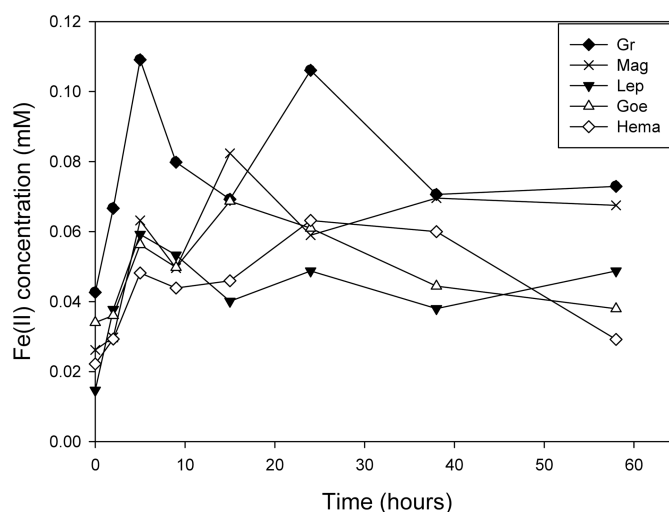


Fig. 3 Production of 3N HCl extractable Fe(II) during the reductive degradation of TNT by interaction of bio-reduced IBSMs (green rust, magnetite, lepidocrocite, goethite, hematite) (0.1 g/200 mL) and CN32 in MDM at pH 7 (PIPES)

IBSMs. Magnetite and green rust containing Fe(II) in their structures are more easily reduced by CN32 than Fe(II) deficient IBSMs (Bae and Lee 2012). Lepidocrocite, goethite, and hematite showed that relatively lower amount of Fe(II) (0.02-0.043 mM) was reduced by CN32. The bio-reduction of well crystallized minerals (i.e., hematite and goethite) has been reported to be more difficult than that of poorly-crystallized minerals (Maithreepala and Doong 2009, Roden 2003). Therefore, the results obtained by this study showed that TNT degradation was significantly affected by bio-reduction of Fe(III) to Fe(II) by CN32.

XRD and TEM analysis were conducted to investigate mineral transformation during the reductive degradation of TNT by reactivity removed IBSMs and CN32. Fig. 4 shows the XRD patterns of green rust, magnetite and lepidocrocite which exhibited high reactivity to degrade TNT compared to that of goethite and hematite. The peaks of green rust had changed to peaks of magnetite (Fig. 4(a)). This was due to the oxidation of Fe(II) to Fe(III) for reductive degradation of TNT, resulting in the change of the green rust to magnetite (Lee and Batchelor 2002b). Figs. 4(b) and 4(c) show the XRD

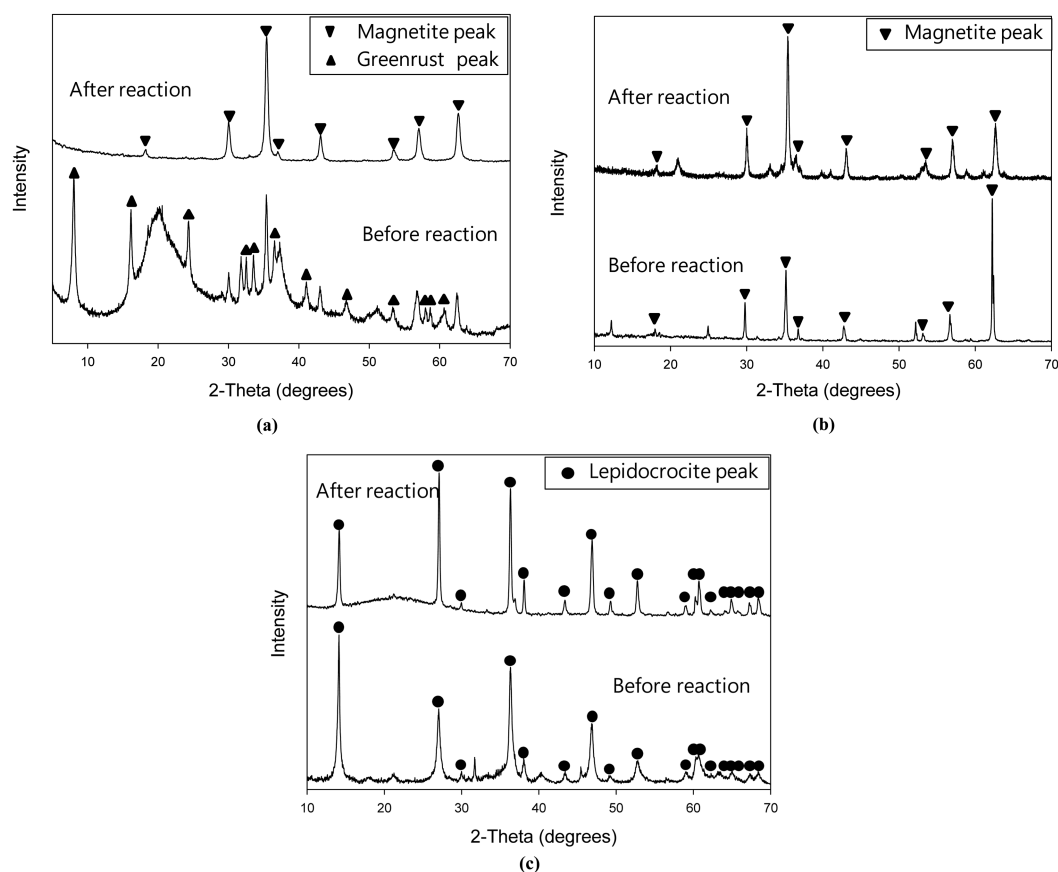


Fig. 4 (a) XRD pattern showing the transformation of green rust to magnetite during the reductive degradation of TNT by bio-reduced green rust, (b) and (c) XRD patterns showing no mineral transformations of magnetite and lepidocrocite during the enhanced reductive degradation of TNT: peaks of green rust (▲), magnetite (▼), and lepidocrocite (●)

patterns of magnetite and lepidocrocite did not significantly change during the reaction. Bae and Lee reported biotransformation of lepidocrocite and magnetite to biogenic vivianite in the presence of CN32 and phosphate in 32 d (2012). However, mineral transformation of magnetite and lepidocrocite was not observed in this study due to the absence of phosphate and the not enough time for mineral transformation of IBSMs (58 h) in this study. Fig. 5 shows the TEM images of green rust, magnetite, and lepidocrocite before and after degradation of TNT. Hexagonal shaped green rust (Fig. 5(a)) (Legrand *et al.* 2001) was transformed to spherical plate shape of magnetite (Fig. 5(b)). This also indicates that transformation of green rust to magnetite through degradation of TNT as similarly observed in XRD. A spherical shape of chemogenic magnetite (control) composed of non-uniform particles (30-100 nm) (Bae and Lee 2010) (Fig. 5(c)) and nano-sized chemogenic lepidocrocite (50-200 nm) with quadrangular and rectangular shapes (Fig. 5(d)) were not changed after the reductive degradation of TNT in this study. The results of TEM analysis are consistent with XRD analysis. The results of Fe productions, XRD, and TEM concluded that Fe(II) production during the interaction of green rust, magnetite, and lepidocrocite with CN32 was the key factor to enhance the reductive degradation of TNT in this study not by formation of reactive secondary minerals such as green rust and vivianite.

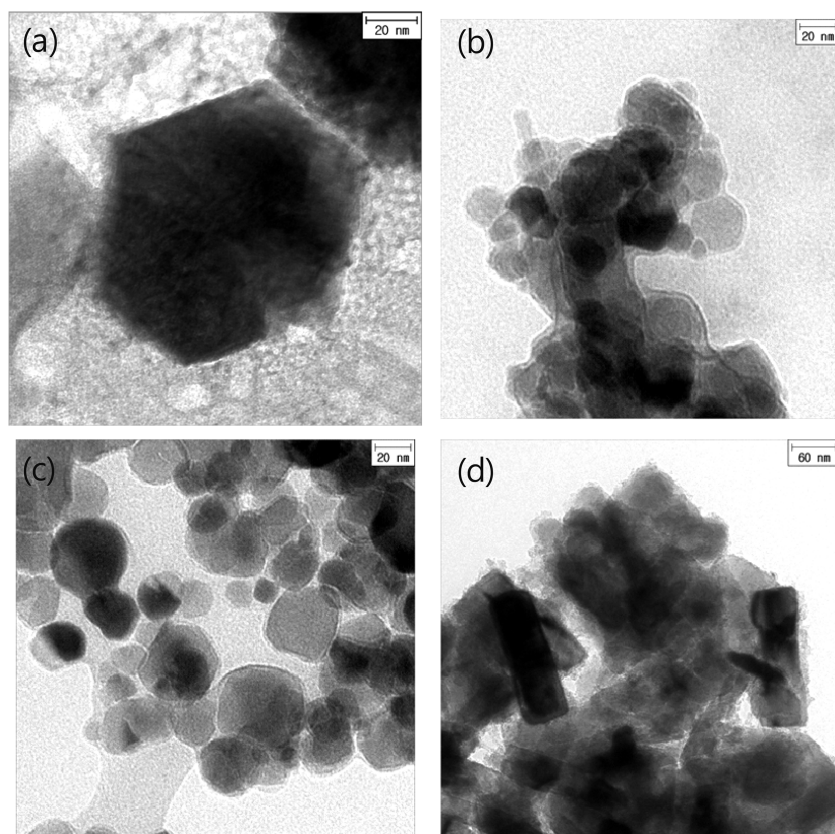


Fig. 5 TEM images of (a) green rust and (b) magnetite before and after the reductive degradation of TNT at 58 h, TEM image of (c) chemogenic magnetite and (d) chemogenic lepidocrocite after the reductive degradation of TNT showing no mineral transformations

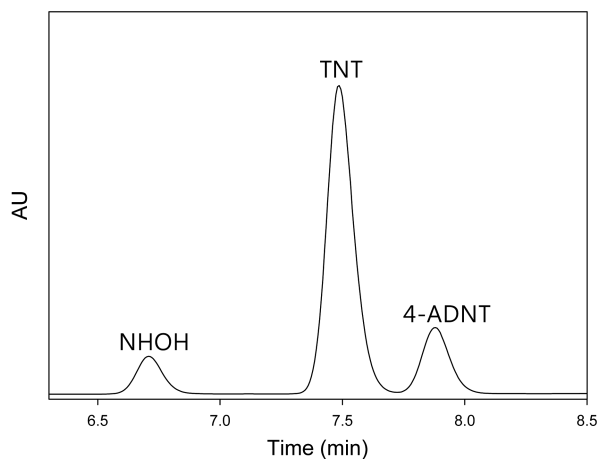
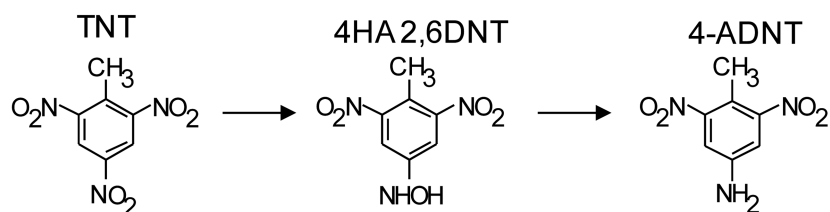


Fig. 6 HPLC chromatogram showing the production of 4-ADNT during the enhanced reductive degradation of TNT by bio-reduced green rust at 38 h

Byproduct study was carried out to investigate reaction mechanism of degradation of TNT. Fig. 6 shows the peak of 4-ADNT in TNT degradation by the interaction of the reactivity removed green rust and CN32. Borch *et al.* reported that TNT was degraded by ferrihydrite in the presence of AQDS to 2-amino-dinitrotoluene (2-ADNT), 4-ADNT, 2,6-diamino-4-nitrotoluene (2,6-DANT), 2,4-diamino-6-nitrotoluene (2,4-DANT) (2005). However, 4-ADNT increased as TNT decreased in our results suggesting that no transformation pathway to 2,4-DANT. It has been reported that degradation of TNT to 4-ADNT was much favorable compared to the 2-ADNT pathway which is consistent to the results obtained by this study (Kaplan and Kaplan 1982). Formation of 4-ADNT has been also observed in other IBSMs with CN32 in this study. Other IBSMs (magnetite and lepidocrocite) also showed the increase of 4-ADNT concentration as similar to the result of green rust indicating that the degradation pathway of TNT was same as green rust. The proposed transformation pathway of TNT by the interaction of the reactivity removed IBSMs and CN32 is followed below:



3.3 Enhanced degradation of RDX by interaction of reactivity removed IBSMs and CN32

Fig. 7 shows the kinetics of RDX degradation by the interaction between reactivity removed IBSMs and CN32. RDX degradation by reactivity removed IBSMs was enhanced by the addition of

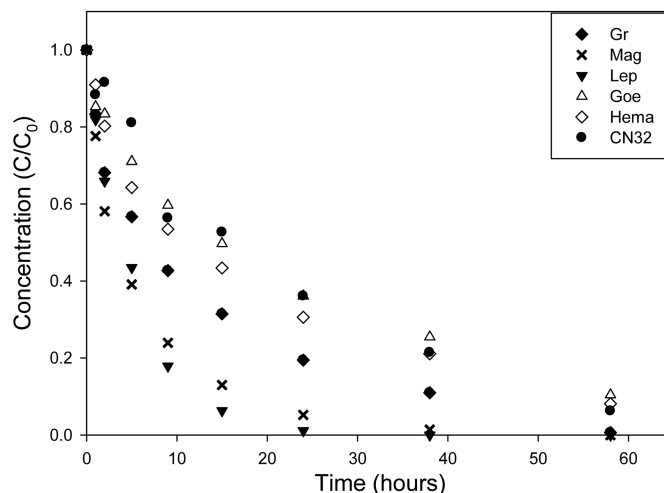


Fig. 7 Enhanced reductive degradation of RDX by bio-reduced IBSMs (green rust, magnetite, lepidocrocite, goethite, and hematite) with CN32 and only CN32 in MDM at pH 7 (PIPES). The initial concentration of RDX was 0.05 mM

CN32. Green rust, magnetite, and lepidocrocite degraded 100% of RDX in 58 h, and goethite and hematite showed more than 90 % of RDX degradation. The highest rate constant was obtained by interaction of lepidocrocite and CN32 (0.1811 h^{-1}) during RDX degradation, followed by magnetite (0.1700 h^{-1}), green rust (0.0757 h^{-1}), hematite (0.0495 h^{-1}), and goethite (0.0394 h^{-1}). However, the control (CN32 + MDM) also showed biotic degradation of RDX (90%) in 58 d induced by CN32. Kwon and Finneran has also reported biotic degradation of RDX by DIRBs such as *Geobacteraceae metallireducens strain GS-15*, *Anaeromyxobacter dehalogenans strain K*, *Desulfitobacterium chlororespirans strain Co23*, and *Shewanella oneidensis Strain MR1* (2008). The addition of CN32 to green rust, magnetite, and lepidocrocite exhibited 1.75, 3.93 and 4.19 times higher RDX degradation kinetic constants than that of biotic degradation by CN32 (Table 1). This indicates that degradation of RDX was significantly enhanced by adding CN32 in green rust, magnetite, and lepidocrocite suspensions. Kwon and Finneran also reported enhanced RDX and HMX degradation by the interaction between poorly crystalline Fe(III) oxide and several DIRBs in the presence of lactate and AQDS (2008). However, the experimental data in this research firstly showed the enhanced RDX degradation by reactivity removed IBSMs and CN32.

3N HCl extractable Fe(II) concentrations of bio-reduced IBSMs were measured to investigate the effect of Fe(II) production on the reduction of RDX (Fig. 8). 3N HCl extractable Fe(II) in the IBSM suspensions with CN32 during the reductive degradation of RDX were significantly increased in 58 h. Lepidocrocite and magnetite, which showed the fast kinetics of RDX degradation, resulted in high Fe(II) production (0.71 and 0.57 mM, respectively) in 58 h, while approximately 0.2 mM of Fe(II) was produced from green rust, goethite, and hematite. As mentioned above in the discussion of TNT degradation, the amount of bio-reduced Fe(II) by the interaction of CN32 and IBSMs was the key factor for degradation of RDX. XRD and TEM analysis were conducted to investigate mineral transformation of IBSMs by CN32 in degradation of RDX. XRD and TEM analysis (data not shown) revealed that green rust was transformed to magnetite and no significant changes were

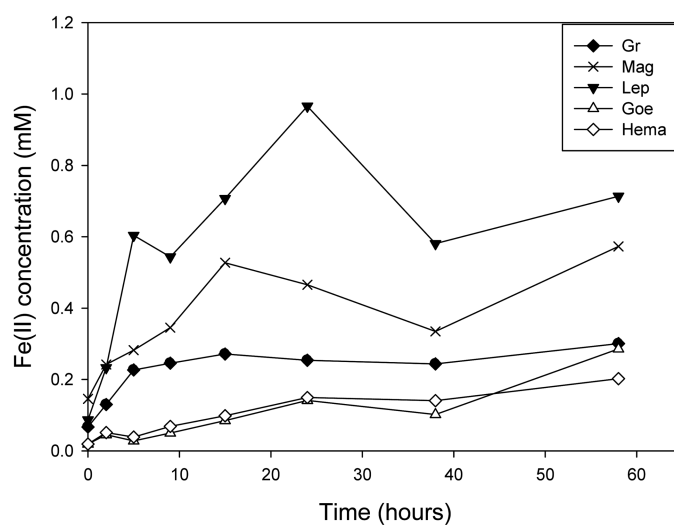


Fig. 8 Production of 3N HCl extractable Fe(II) during the reductive degradation of RDX by interaction of bio-reduced IBSMs (green rust, magnetite, lepidocrocite, goethite, hematite) (0.1 g/200 mL) and CN32 in MDM at pH 7 (PIPES)

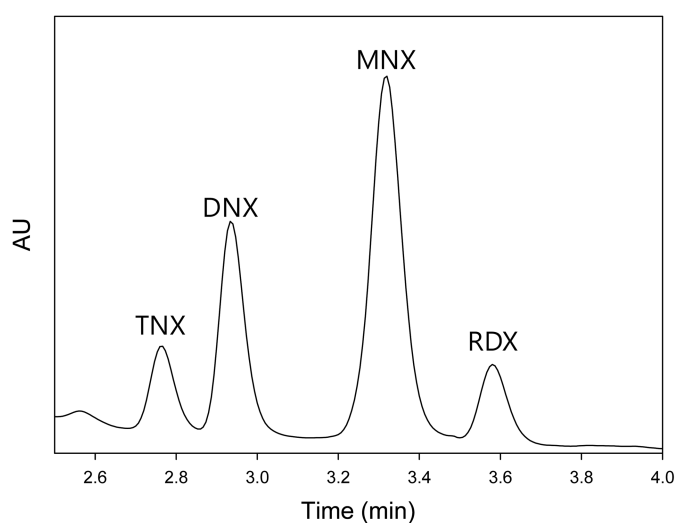
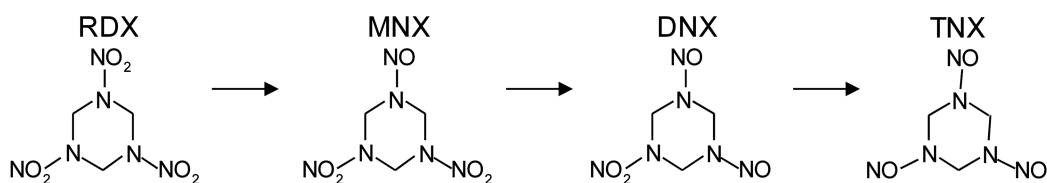


Fig. 9 HPLC chromatogram showing the production of MNX, DNX, and TNX during the enhanced reductive degradation of RDX by bio-reduced green rust at 38 h

observed in magnetite and lepidocrocite. This indicated that the enhanced degradation of RDX by addition of CN32 in IBSM suspensions was occurred by Fe(II) production not by mineral transformation which is consistent with the result obtained from TNT experiment.

Byproduct study was carried out to investigate reaction mechanism of degradation of RDX. Fig. 9 shows the peak of MNX, DNX, and TNX during the RDX degradation by the interaction of

reactivity removed green rust and CN32. This indicates that RDX was reduced to MNX, DNX, and TNX as a subsequent reduction of three nitro-on RDX to nitroso- (Kwon and Finneran 2006). It has been also reported that RDX was reduced to MNX, DNX, and TNX by magnetite and zero valent iron (Gregory *et al.* 2004, Naja *et al.* 2008). Other IBSMs (magnetite and lepidocrocite) also showed the increases of MNX, DNX, and TNX concentrations as similar to the result of green rust indicating that the degradation pathway of RDX was same as green rust case. The proposed transformation pathway of RDX by the interaction of the reactivity removed IBSMs and CN32 is followed below:



4. Conclusions

We have observed the enhanced degradation of TNT and RDX by adding CN32 to reactivity removed IBSMs. The change in total Fe(II) revealed that Fe(II) produced from the interaction of CN32 and IBSMs was the key factor to enhanced degradation of TNT and RDX. To identify mineral transformation XRD and TEM analysis were conducted during the bio-reduced IBSMs. However, only green rust was transformed to magnetite after reductive degradation of TNT and RDX and no transformation was observed by other IBSMs. TNT was reductively transformed to 4-ADNT by produced Fe(II), while RDX was reduced to MNX, DNX, and TNX. It was proved that Fe(II) can be constantly produced from the interaction of the reactivity removed IBSMs and DIRB and this produced Fe(II) can establish a redox cycle to reductively transform explosives. The result obtained from this study can provide a fundamental knowledge to develop reducing environments of IBSMs for removal of explosives by the interaction of DIRB and IBSMs when reactivity of IBSMs decreased.

Acknowledgements

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