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Alkali Hydrolysis of Trinitrotoluene

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Abstract

Data for alkali hydrolysis of 2,4,6-trinitrotoluene (TNT) in aqueous solution at pH 12.0 under static (pH-controlled) as well as dynamic (pH-uncontrolled) conditions are reported. The experiments were conducted at two different molar ratios of TNT to hydroxyl ions at room temperature. The TNT disappeared rapidly from the solution as a first-order reaction. The complete disappearance of aromatic structure from the aqueous solution within 24 h was confirmed by the ultraviolet-visible (UV-VIS) spectra of the samples. Cuvet experiments in a UV-VIS spectrophotometer demonstrated the formation of Meisenheimer complex, which slowly disappeared via formation of aromatic compounds with fewer nitro groups. The known metabolites of TNT were found to accumulate only in very small quantities in the liquid phase.

Index Entries: 2,4,6-Trinitrotoluene; alkali; hydrolysis; ultraviolet-visible spectra; metabolites.

Introduction

2,4,6-Trinitrotoluene (TNT) has been a widely used explosive since the Russian-Japanese war in 1905. Its production, processing, and extensive use have made it a major soil and groundwater contaminant all over the world *(1)*. Its concentrations in contaminated soil have been reported to range from 10 mg/kg (~47 μ*M*ol/kg) to 12,000 mg/kg (~56,000 μ*M*ol/kg) *(2)*; contaminations as high as 35,000 mg/kg have also been observed. Aqueous waste streams containing TNT concentrations as high as 70 mg/L

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(330 μ*M*) have been reported *(3)*. At these concentrations, TNT is highly toxic, mutagenic, and potentially carcinogenic. The bacterium *Vibrio fischeri* has been found to lose 50% of its luminescence in the presence of a 2–4 μ*M* concentration of TNT *(4)*. Earthworms lose weight at low concentrations of TNT and die at 320 mg/kg of soil *(5)*. TNT uptake by animal receptors can occur by skin contact, by inhalation, or by swallowing contaminated soil and water. Ingested TNT in small amounts manifests in headache, anemia, and skin irritation. In larger amounts, TNT can damage the liver, eyes, and nerves *(6)*. The products of TNT metabolism in human body interact with macromolecules (like DNA) causing genetic defects *(7)* and cancer *(8)*. For Reuber H35 HIIE rat hepatoma cells, an LC_{50} value of 4 μg TNT/ml medium was observed [9]; Chinese hamster ovary cells had an LC_{50} of 24 μg/mL of TNT. As a result, methods for rapid removal of TNT from contaminated soils and aqueous solutions are needed.

Both chemical and biological methods for treatment of TNT-contaminated soils and groundwater have been explored *(2,10–15)*. Of these, biological methods are preferred because of their environmental appeal and mild operating conditions. A preferred goal of the biological methods is a high extent of mineralization, which involves conversion of the contaminant into carbon dioxide. Unfortunately, the chemistry of the TNT molecule prevents a high degree of mineralization. The strongly electron-withdrawing nature of nitro groups causes a negative inducing effect on the C-N bond and makes the oxygen atoms on the nitro groups highly electron rich. The presence of multiple nitro groups on the ring depletes the electron density of the ring, making it a strong π acceptor, and allows the ring in nitroaromatics to undergo π-π or *n*-π interactions with suitable electron donors *(16)*. These electron donor-acceptor interactions result in TNT reacting with the soil and other natural organic materials in its environment and thus influence its mobility, bioavailability, and reactivity in nature. The high electron density on the three symmetrically placed nitro groups in 2,4,6-TNT hinders an electrophilic attack on the ring, resulting in the observed low extent of chemical or enzymatic oxidation of ring. At the same time, the nitro groups are highly susceptible to reductive transformation. These factors are primarily responsible for the observed reductive transformations of nitro groups even under otherwise oxidative environments. The reduced electron density of the aromatic ring in TNT suggests that the ring may be subjected to nucleophilic attack. This nucleophilic attack has been reported at the carbon atoms containing the nitro groups as well as at the carbon atoms in the ortho position relative to the carbon atoms containing nitro groups *(17)*. The nucleophilic attack on the carbon attached to the nitro group forms the classic Meisenheimer complex in which the nucleophile and the nitro group both are still attached to the same carbon atom before elimination of the nitro group. The second nucleophilic attack takes place because, although nitro groups deactivate all positions on the ring, the carbon atoms in the ortho position relative to the

carbon having the nitro groups become the most deactivated through resonance stabilization. As a result, the π electrons move away from the ortho carbon toward the nitro group outside the nitro aromatic ring. This deactivation makes the ortho carbons susceptible to nucleophilic attack.

Indeed, the reactions of TNT with hydroxide ions (a strong nucleophile) under basic conditions have been reported *(18,19)*. Only recently have some investigations of TNT with hydroxyl ions in aqueous solutions been reported as a potential treatment technology for TNT-contaminated soils and groundwater *(20–22)*. Emmrich *(20)* presented the kinetics of disappearance of TNT under alkaline conditions in aqueous solution of TNT but did not report any intermediate formation. Qasim et al. *(22)* calculated the minimized formation energies (MNDO) and steric energies (MM2) of a number of hydroxylated nitro aromatics. The minimized formation energies of 1-hydroxy-2,4,6-TNT and 2-hydroxy-2,4,6-TNT molecules were calculated to be 83.1 and 94.2 kcal/mol, respectively, compared with 75.3 kcal/mol for 2,4,6-TNT. At the same time, minimized steric (MM2) energies of these hydroxylated compounds could not be calculated, suggesting that these could be activated complexes under suitable conditions. On the other hand, the MNDO-minimized formation energy of 3-hydroxy-2,4,6-TNT was calculated to be 27.8 kcal/mol, and its minimized steric energy was estimated to be 33.8 kcal/mol. This is only slightly smaller than that of 2,4,6-TNT (36.7 kcal/mol), indicating that 3-hydroxy-2,4,6-TNT could be a stable intermediate in the nucleophilic attack of hydroxyl ions on TNT. Following the logic of Vorbeck et al. *(17)*, all the hydroxylated TNTs will be classified as Meisenheimer complexes in this article. All should result in the appearance of color in the visible range in solution.

We have undertaken an investigation of the kinetics of hydrolysis of 2,4,6-TNT in aqueous solutions under alkaline conditions with the goal of enhancing mineralization of TNT by indigenous microorganisms present at contaminated sites. We report on several interesting results involving the dynamics of reaction of 2,4,6-TNT with $Ca(OH)_2$ at pH 12.0.

Materials and Methods

Aqueous solution of 2,4,6-TNT containing 50 mg of TNT/L (pH 7.0) was obtained from the US Army Engineer Research and Development Center (USAERDC) in Vicksburg, MS. The stock solution was stored in a dark bottles in a refrigerator to prevent any photon-induced transformation. For experiments involving lower TNT concentrations, the stock solution obtained from USAERDC was diluted using filtered deionized water. Both these solutions are referred to as stock solutions herein.

A saturated solution of $Ca(OH)_2$ was used to adjust the pH of the TNT solution. The saturated solution was prepared by dissolving $Ca(OH)_2$ in boiling deionized water followed by cooling and filtering the solution through a 0.2 μm filter to remove any crystals. The pH of the saturated

$Ca(OH)_2$ solution was measured by titration with oxalic acid solution and was found to be 12.55.

The experiments were conducted in a beaker at room temperature (25–27°C). The reactions were initiated by the addition of a predetermined amount of saturated $Ca(OH)_2$ solution to 100 mL of TNT stock solution in a beaker. The solution was magnetically stirred with a Teflon-coated magnetic bar. A pH probe in the liquid in the beaker was used to continuously monitor the pH of the solution. During the reaction, the solution pH would drop owing to consumption of hydroxyl ions. During "dynamic" experiments, the pH was simply monitored but not controlled. In "static" experiments, the pH in the reaction mixture was controlled by automatic addition of saturated $Ca(OH)_2$ solution using a pH controller and a peristaltic pump. The pH control was within ±0.1 pH units. A digital balance on which the $Ca(OH)_2$ feed solution was placed measured the amount of $Ca(OH)_2$ pumped. The reaction chamber (beaker) was covered with aluminum foil to prevent any exposure to light. Five-milliliter samples were withdrawn from the beaker at different times. A drop of concentrated HCL was added to each sample to stop the reaction. The samples were stored in a refrigerator pending analysis.

TNT and its metabolites were analyzed by high-performance liquid chromatography (HPLC). The solvent system containing 55% methanol and 45% water was pumped (Series 4 pump; Perkin-Elmer) at a flow rate of 0.6 mL/min through a 20-µL injector loop and a C_{18} reverse-phase column for separation of the components. A ultraviolet-visible (UV-VIS) detector (Shimadzu SPD-10A) at 254 nm was used to monitor the eluted components, and the peaks were analyzed using a Spectra-Physics (SP4270) integrator. All the samples were filtered through a 0.2-µm filter before injection. The compounds were identified by peak retention times, and the peak areas were converted into concentrations using standard curves. The compounds used for peak identification were 2,4,6-TNT, 1,3,5-trinitrobenzene, 4-amino-2,6-dinitrotoluene, 2-amino-4,6-dinitrotoluene, 2,4-dinitrotoluene, 2,6-dinitrotoluene, 2-nitrotoluene, and 4-nitrotoluene.

The samples in several experiments were analyzed in a Cary 50 Bio UV-VIS spectrophotometer, in which UV-VIS spectra in the 190 to 700-nm range were measured using a 500-µL quartz cuvet. Some shorter-term experiments were conducted in the cuvet itself as well. In such experiments, the pH of the TNT solution was adjusted, and the solution was immediately placed in the cuvet and in the spectrophotometer. A number of UV-VIS spectral scans were taken over the next several hours, and the formation of different compounds was established with the help of UV-VIS spectra of pure compounds and information available in the literature.

The concentrations of nitrate and nitrite in the samples were measured using a dionex ion-exchange chromatograph. The samples were pretreated through an IC-Ag filter to remove the interfering chloride ions. The separations were conducted using an IonPack AG4A 4-mm precolumn, HPLC

CS3 column 1, and IonPack AS4A 4-mm column 2, followed by a conductivity detector for peak detection and an integrator for calculation of peak areas. The peak areas were converted into concentrations using standards.

All the experiments were repeated at least two times, and the reproducibility was judged good.

Results and Discussion

Figure 1 presents the results of "static" experiments with a 50 mg/L stock solution of TNT at pH 12.0. A substantial amount of saturated solution of $Ca(OH)_2$ had to be added to the stock solution to bring its pH to 12.0. As a result, the initial concentration of TNT in the final solution was about 25 mg/L (0.011 mmol/L). In addition, some $Ca(OH)_2$ solution was added to the system in order to control the pH at 12.0. However, significant amounts of $Ca(OH)_2$ solution were needed only toward the end of the experiment. Hence, it was not necessary to account for the effect of dilution by the $Ca(OH)_2$. These concentrations of TNT and of (nitrite + nitrate) are presented in Fig. 1 for two static experiments. In both the experiments, TNT concentration in the liquid was observed to drop rapidly and was almost nondetectable after 6 h of experimentation. At the end of this period, the amount of total nitrite + nitrate released in the solution was almost 0.5 mol/mol of TNT. The stock solution had a small amount of 1,3,5-trinitrobenzene (0.4 μmol/L), and its concentration in the liquid fluctuated around this value during the experiment. Small amounts of 2,6-dinitrotoluene and (2- and 4-) amino-dinitrotoluenes were also detected by HPLC, but their concentrations never exceeded 0.2 μmol/L, which was insignificant compared with a 110 μmol/L initial concentration of TNT in the solution. None of the other products given in Materials and Methods were detected by either HPLC or gas chromatography mass spectroscopy, which was used for a few selected samples. These observations are supported by the UV-VIS spectra for several samples from the static experiments, which are presented in Fig. 2. The UV-VIS spectra confirm that no intermediate product accumulated in the liquid, indicated by the absence of any peak other than the one for TNT at about 227 nm. The disappearance of this peak along with the absence of any other peak in 9- and 18-h samples indicates complete loss of aromatic structure in these samples.

Figure 3 presents the results of a cuvet experiment at an initial pH of 12.0. The first spectrum, taken after 1 min of mixing $Ca(OH)_2$ with TNT solution, showed essentially pure TNT. Spectra at subsequent time intervals showed a temporal increase in peak absorbance at 450 nm that was later replaced by a significantly smaller peak at 340 nm. The absorbance at 450 nm peaked after 31 min of addition of alkali and mostly disappeared after 5 h. The TNT peak at 227 nm monotonically decreased and mostly disappeared after 5 h. This sequential appearance and disappearance of the absorption peaks in the UV spectrum is indicative of formation of aromatic

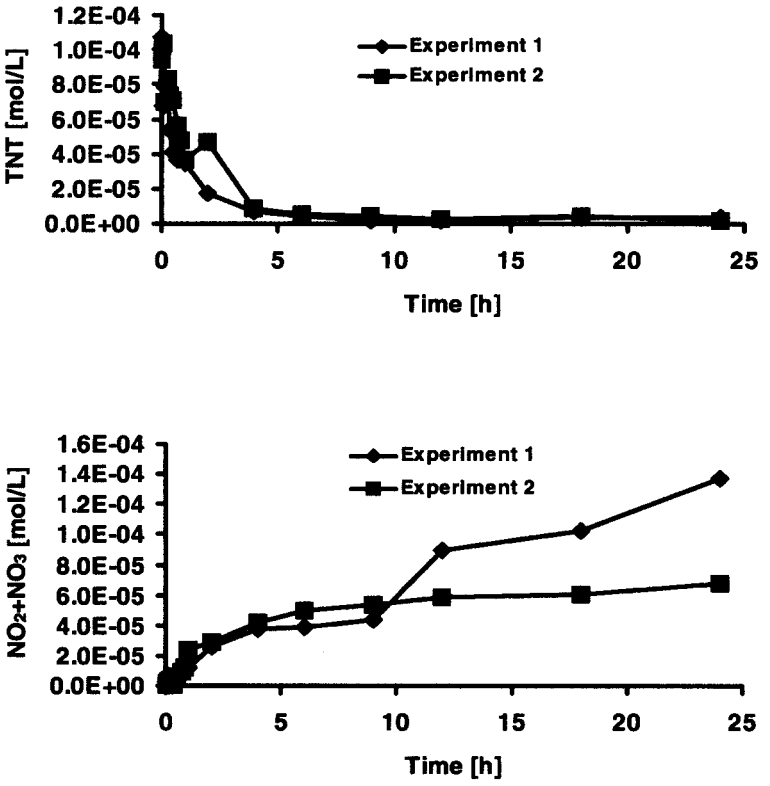

Fig. 1: Static experiment involving reaction of TNT with $Ca(OH)_2$ at pH 12.0. Initial TNT concentration = 25 mg/L.

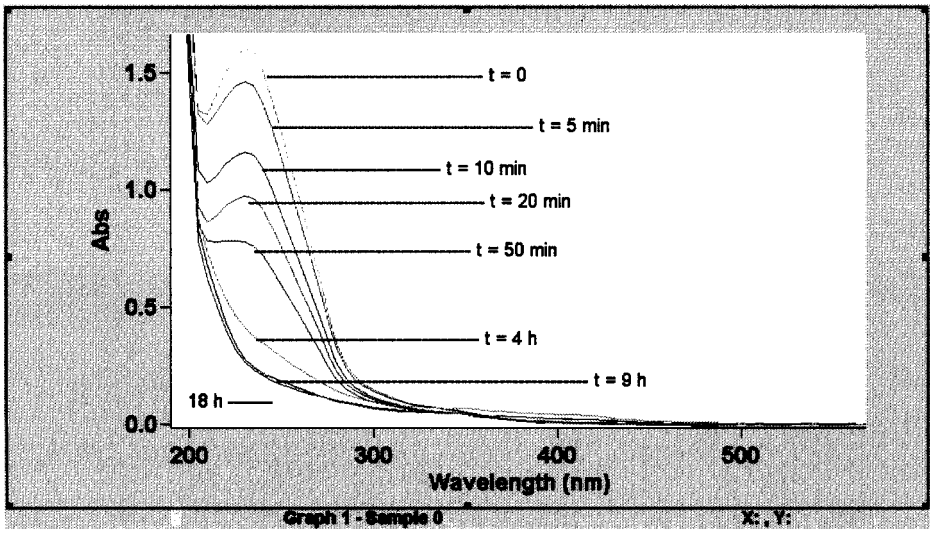

Fig. 2: UV-VIS spectra of samples collected from a static experiment at pH 12.0. Initial TNT concentration = 25 mg/L.

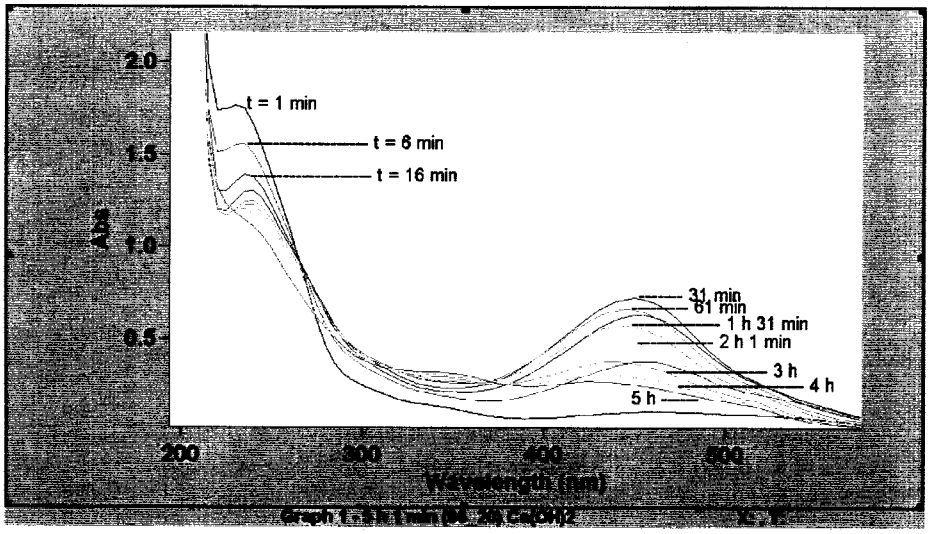

Fig. 3: UV-VIS spectra from a cuvet experiment starting at pH 12.0. Initial TNT concentration = 25 mg/L.

intermediates of reaction. Since the observations of beaker experiments indicate formation of color in solution on addition of alkali and also the appearance of nitro groups in solution, it is postulated that the reaction takes place via formation of a Meisenheimer complex. Based on the contributions of hydroxyl, methyl, and nitro groups to absorption peaks in UV spectra, the compound exhibiting a peak at 450 nm is postulated to be a dihydroxy-dinitro toluene, but this has not yet been independently confirmed. Over a period of time, this intermediate was further converted into still another intermediate aromatic compound before loss of ring structure. This loss of aromatic structure has been postulated based on observations that the long-term samples show no color and their spectra show no absorbance maximum in the UV-VIS range. Since only 0.5 mol of nitrite + nitrate/mol of TNT degraded was measured in the solution, it is expected that the solution contained some nitrogen-bearing compounds. A recent publication by Arienzo *(11)* involving reaction of TNT with calcium peroxide and calcium hydroxide in aqueous solution reported the release of 3 mol of nitrite + nitrate/mol of TNT but no CO_2 formation. This suggests formation of soluble organics as a result of hydrophilic reactions. The discrepancy in the amount of nitro groups released is a subject of ongoing investigation.

Several features of UV-VIS spectra from the cuvet experiments are interesting and worthy of comment. First, these spectra suggest formation of an intermediate product that does not show up in the other static and dynamic experiments conducted in beakers. This appears to be a result of sample processing when the experiments were conducted in a beaker. The aqueous solution in a beaker turned pink when $Ca(OH)_2$ was added to the

TNT solution. The pink color turned orange about 5 h into the experiment, and the color intensity continued to decrease. Moreover, the samples collected from the beaker were acidified with HCl in order to stop the alkali hydrolysis. On acidification, the pink color of the samples also turned orange. These observations suggest that color-forming complexes were formed in beakers as well. However, the samples were stored in a freezer for several days before their analysis by HPLC and UV-VIS spectroscopy. Additionally, the samples were filtered through a 0.2-µm nylon disk filter prior to injection into HPLC and UV-VIS spectroscopy. The filtered samples were colorless since the color was retained in the filter. This removal of color during sample-processing steps is the most likely reason for the observed differences. This is currently being explored.

Second, the nature of intermediate products formed in this experiment is fundamentally different from the hydride-Meisenheimer complex reported by Vorbeck et al. *(17)* in aerobic cultures of mycobacterium strain HL 4-NT-1 metabolizing TNT with 4-nitrotoluene as the sole nitrogen source. The hydride-TNT complex reported by Vorbeck et al. *(17)* showed twin adsorption peaks at 260 and 500 nm, whereas the two intermediates produced here have single adsorption maxima at 450 and 340 nm, respectively. The adsorption maxima of the different compounds and intermediates investigated in this work were as follows: 2,4,6-trinitrotoluene, 226 nm; 1,3,5-trinitrobenzene, 225 nm; 4-amino-2,6-dinitrotoluene, 225 and 360 nm; 2-amino-4,6-dinitrotoluene, 220 and 358 nm; 2,4-dinitrotoluene, 245 nm; 2,6-dinitrotoluene, 240 nm; 4-nitrotoluene, 280 nm; 2-nitrotoluene, 261 nm.

In light of HPLC analysis of the samples, it would appear that one of the intermediates observed in the cuvet experiments is either the TNT anion or a TNT-hydroxide postulated by Qasim et al. *(22)*. Formation of a TNT anion under alkaline conditions has been postulated in the literature. However, its formation in aqueous solutions has been questioned. The observations of Fig. 3 are supported by the observations of alkali reactions of picric acid by Qasim (personal communications).

Figures 4 and 5 present the results of dynamic experiments. These experiments were started at pH 12.0, but pH was not controlled. Hence, pH of the solution dropped during the course of the experiments. This represents a situation more likely to be observed in a treatment scenario. The solution pH and the concentrations of TNT and total (nitrite + nitrate) in the different samples in experiments with an initial TNT concentration of 25 mg/L are presented in Fig. 4. As in static experiments, TNT concentration dropped rapidly with almost no detectable amounts in TNT solution beyond 5 h. In both experiments, pH dropped very slowly in the beginning. Perhaps as a result of this, the analyses showed trends almost identical to those observed with static experiments. pH in the solution had dropped to 10.0 or less by h 24, when the experiments were terminated. Most of the drop in pH occurred in the latter part of the experiment, whereas the TNT was transformed most rapidly in the initial hours. This may indicate formation of intermediates, none of which, however, showed up in HPLC and

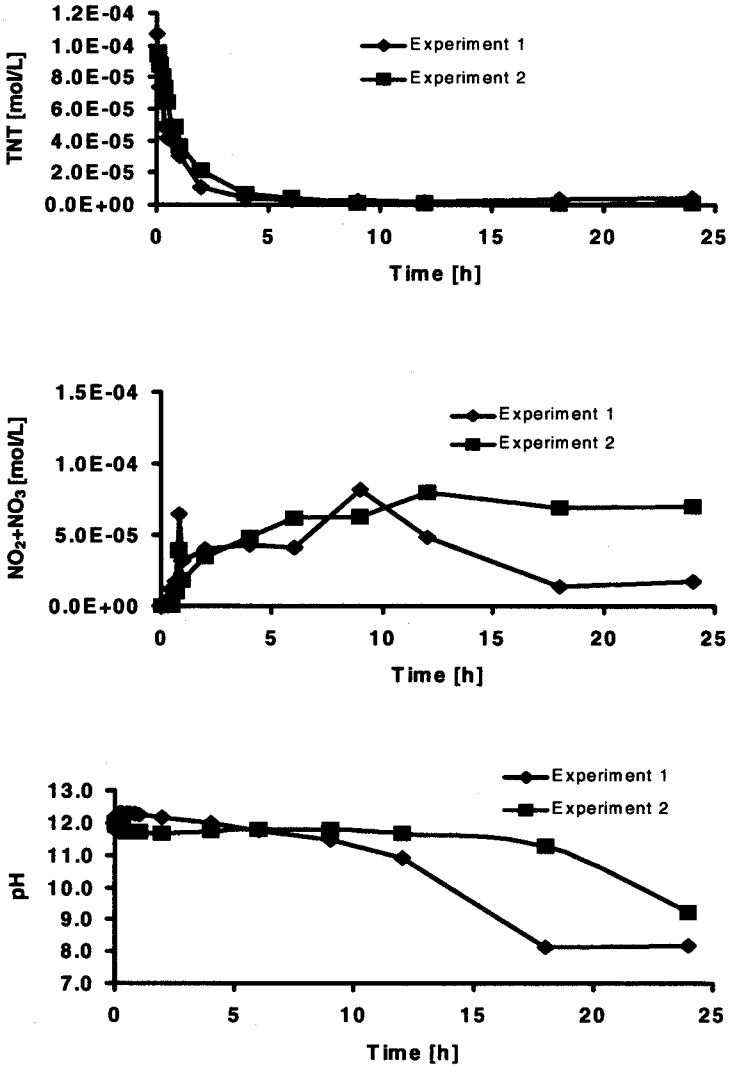

Fig. 4: Dynamic experiment involving reaction of TNT with $Ca(OH)_2$ starting at pH 12.0. Initial TNT concentration = 25 mg/L.

UV-VIS spectra (Fig. 5). Once again, a pink color developed as soon as alkali was added to the TNT solution, and it slowly faded away after 5 h. The color was adsorbed by the filter and thus was removed during sample preparation prior to analysis. The ratio of (nitrite + nitrate) formed and TNT consumed was again about 0.5 by h 5. More detailed analysis of nitrate/nitrite data was not undertaken because a brown gas formed in several samples, indicating loss of nitrites as NO_2. This observation was universal and we are trying to overcome this issue. The UV-VIS spectra of the samples (Fig. 5) suggest that aromatic structure was destroyed in the course of the reaction and none remained by the 6 h sample.

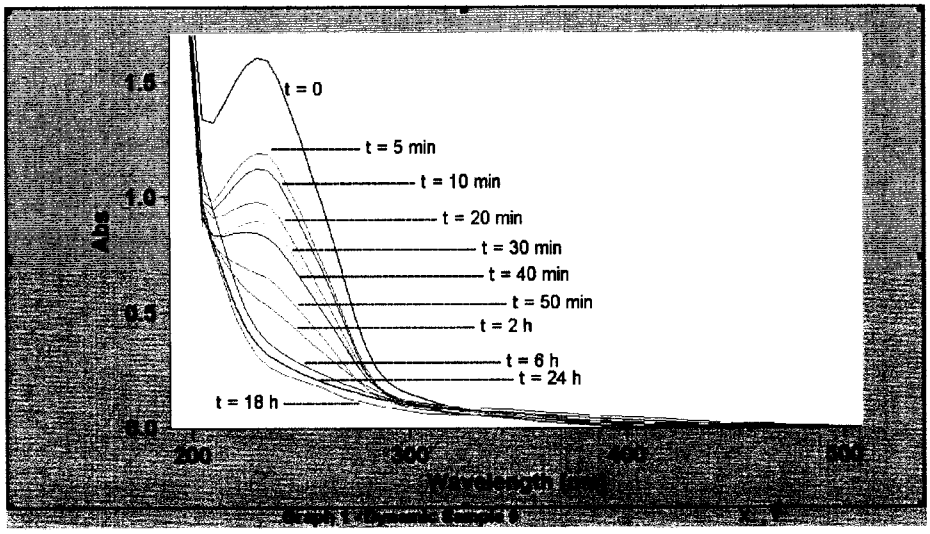

Fig. 5: UV-VIS spectra of samples from a dynamic experiment starting at pH 12.0. Initial TNT concentration = 25 mg/L.

Figure 6 presents the results of dynamic experiments in which initial TNT concentration was 2.5 mg/L. pH in the solution remained more or less constant for the first 10 h and then dropped rapidly to 9.0 or below. TNT concentration also dropped rapidly to under 10% by the 6 h sample and was barely detectable after that. Nitrate/nitrite concentration was very small and could not be detected in all but a few samples. We believe that it was on the verge of the instrument's detection limit. The main significance of these results is the demonstration that TNT disappears from solution in a first-order fashion. Data for the first 6 h from all the experiments mentioned in Figs. 1, 4 and 6 have been plotted in Fig. 7 on a semilog plot. The first 6 h were selected, since pH remained about 12.0 for the first 6 h even in dynamic experiments. This plot shows that TNT transformation at pH 12.0 is first order with a rate constant equal to 0.61 h^{-1}. The value of this rate constant is of the same order of magnitude but higher than the value reported by Emmrich *(20,21)*. The reason for the differences is being explored in the continuing research. It should be pointed out that Emmrich *(20,21)* has also reported different values for different systems.

Conclusions

Experimental data for hydrolysis of TNT in aqueous solutions have been reported at pH 12.0. The experimental observations suggest that the alkali transformation of TNT takes place through formation of color-producing intermediates that are further transformed to nonaromatic compounds. The reaction at pH 12.0 was quite fast, with 25 mg/L of TNT transformed into nonaromatic compounds within 6 h. This is very encouraging from the perspective of the project goal of enhancing mineralization

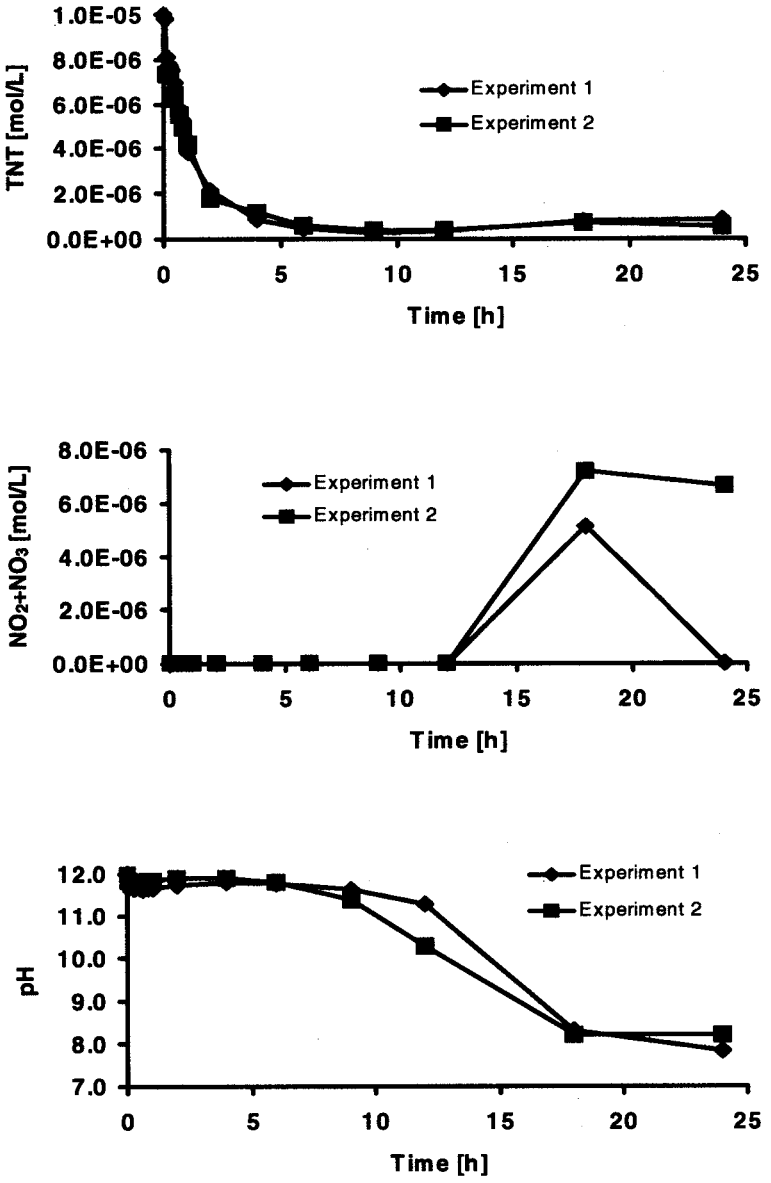

Fig. 6: Dynamic experiment involving reaction of TNT with $Ca(OH)_2$ starting at pH 12.0. Initial TNT concentration = 2.5 mg/L.

of TNT by subjecting it to alkali. Significant amounts of nitrite were also released, indicating that the resulting compounds are not only nonaromatic, but also less nitrated. The presence of nitrate in the nonaromatic compounds is, however, not considered a drawback in light of the easily leaving character of nitro groups. Thus, oxidative attack on the exposed carbon backbone may even be enhanced by the electron-withdrawing nature of nitro groups.

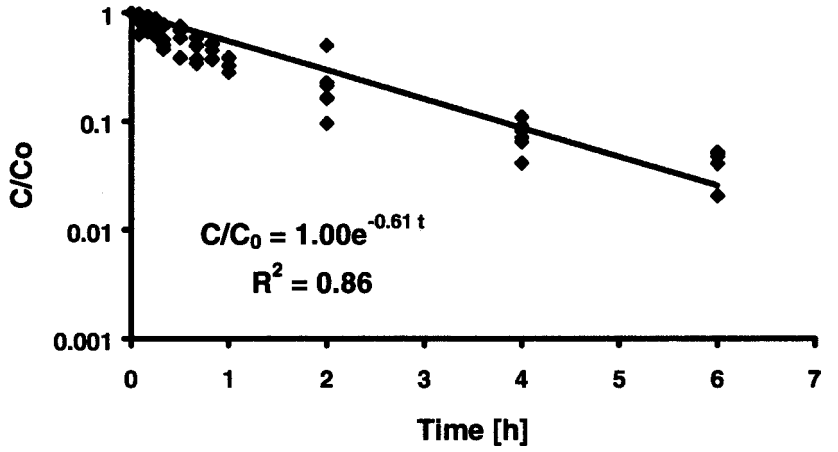

Fig. 7: First-order fit of experimental data at pH 12.0.

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