Biodegradation of RDX through the Application of Waste Glycerol

D. Juck[1], D. Manno[1], C.W. Greer[1], M. Hendry[2] and L.-B. Jugnia[1].
[1] National Research Council Canada, Energy, Mining and Environment
[2] Department of National Defence, National Defence Garrison Petawawa
CANADA

David.juck@cnrc-nrc.gc.ca

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ABSTRACT

Energetic materials (EMs) are one of the most important groups of contaminants impacting range training areas (RTAs). Some of the challenges in addressing the management and remediation of EM impacted areas include the large areas potentially impacted by EMs, limited historical records (firing points, impact areas, ranges, etc.) prior to the mid- to late- 1990s, the dispersed nature of EM contamination and the presence of UXOs. An approach was developed to address EM contamination based on the presence and stimulation of indigenous bacterial species able to biodegrade EMs, in particular hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). The indigenous bacterial population responsible for RDX biodegradation require anaerobic (i.e. absence of oxygen) and reducing (i.e. where oxidation reactions are prevented) conditions to actively metabolize EMs. Based on lab and field pilot studies (1m^2 test plots and 100m^2), the surficial application of waste glycerol (WG), generated as a waste product of biodiesel production, was demonstrated to be an effective amendment in the generation of the desired environmental conditions. WG application decreased the oxidation-reduction potential and dissolved oxygen concentration in groundwater followed by a rapid sustained decrease in RDX concentrations to below detection limits (0.1 µg/L). This approach was then applied to a second field site (100m^2 test plots) at an active Grenade Range site with a different soil type and deeper aquifer. Results from the first year of application revealed that further optimization is required at the larger scale, but the overall approach remains very promising for the remediation of RDX impacted sites.

1.0 INTRODUCTION

Energetic materials (EMs) are one of the most important groups of contaminants impacting range training areas (RTAs). Elevated EM concentrations, typically found near target locations, are due to incomplete combustion, low order detonation events and unexploded ordnances (UXOs). These target areas impacted by EMs then become point sources for contaminant migration into the sub-surface soil and groundwater, which can have important negative impacts on sensitive environmental receptors in not only the immediate vicinity but further away from the original target area. Additionally, understanding the fate and behaviour of EMs in the local environment is essential in identifying the risks associated with the presence and migration of EMs and how these risks can be mitigated and or managed.

Some of the challenges in addressing the management and remediation of EM impacted areas include the large areas potentially impacted by EMs, limited historical records (firing points, impact areas, ranges, etc.) prior to the mid- to late- 1990s, the dispersed nature of EM contamination and the presence of UXOs. In many RTAs where hard targets such as tanks/vehicles, which have often been filled with concrete, are employed, the cost of UXO clearance and the removal of these concentrated sources of EMs can be prohibitive due to the number of hard targets throughout an RTA. When an *in situ* remediation approach is considered using traditional technologies (e.g. dig and dump, pumping and treatment of impacted groundwater, etc.), there are several important considerations which preclude their use: presence of UXOs; on-site equipment or remedial infrastructure can become damaged, either directly or indirectly, during training activities; most highly EMs contaminated areas are located in active impact areas, which maintain a high training tempo; regular access to sites for maintenance/optimization/monitoring is often very challenging as it must be scheduled around military training; and human health and safety is always a major concern when areas known to be impacted by EMs, and potentially UXOs, need to be accessed.

The outlined concerns were the driving force in developing a passive *in situ* remediation approach to addressing EM impacted soils and groundwater located at Garrison Petawawa, The approach developed was based on the presence and stimulation of indigenous bacterial species able to biodegrade EMs, in particular hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). The indigenous bacterial population responsible for RDX biodegradation require anaerobic (i.e. absence of oxygen) and reducing (i.e. where oxidation reactions are prevented) conditions to actively metabolize EMs.

2.0 TREATMENT APPROACH

2.1 Laboratory Assays

The fundamental treatment approach was developed through lab scale column experiments and is based on the surficial application of a carbon source to EM impacted soils originating from a demolition range (Jugnia et al., 2017). The initial hypothesis was that the application of a carbon source would stimulate the activity of aerobic bacteria (i.e. oxygen consuming bacteria), which in turn would create anaerobic (i.e. absence of oxygen) and reducing conditions in the soil. The soil from the demolition range was a coarse sandy soil containing low concentrations of organic matter (1-2%). Two carbon sources, (waste glycerol [WG], derived from the commercial production of biodiesel, and cheese whey), were initially tested in mineralization batch assays which measured the complete degradation of RDX into CO_2 and H_2O. These assays revealed that the addition of the 2 carbon sources increased the rates and extents of RDX mineralization over un-amended control soils. Soil column studies with the same demolition range soil were then performed using WG as the carbon source, at low and high concentrations (0.5 and 3.8 g waste glycerol/kg soil, respectively). The experiments revealed that RDX degradation was stimulated at both WG concentrations in the soil columns and that the approach showed promise as a remediation technology.

2.2 Field Validation – Demolition Range

Validation of the approach was then performed at the demolition range over several years (Jugnia et al., 2018, Jugnia et al., 2019). Test plots of 1 m^2 and 100m^2 treated areas plots were established in the EM impacted area and WG or distilled water (control plots) was applied to the soil surface. The impact of the WG application on EMs was determined by following the concentrations of RDX and 2,4-DNT in the soil pore water during the study with 1m^2 test plots, and in groundwater collected from monitoring wells (samples collected at approximately 6.4 to 8.6m below surface) surrounding the 100m^2 treatment plots. Additionally, the impact of WG application on the dissolved oxygen (DO), pH, oxidation-reduction potential (ORP) and specific conductivity (SC) were measured in the groundwater during each sampling campaign and the overall impact of WG application on the indigenous microbial populations and ecotoxicity were also determined.

The waste glycerol was able to percolate down to the groundwater (3.7m to 5.7m below ground surface) in the

100m[2] treatment plots and generated the desired anaerobic reducing conditions. The concentration of RDX in soil pore water and down-gradient groundwater samples decreased to below detection limit (0.1 µg/L) over time (but not in the control plots treated with distilled water) (Figure 1). There was also an increase in volatile fatty acid (VFA) and total organic carbon (TOC) concentrations in the down-gradient groundwater monitoring wells. There was no impact on 2,4-DNT concentrations in either the soil pore water with the addition of WG and additional results (not shown) suggested that 2,4-DNT was not mobile in this system. Microbiological community analysis of samples from groundwater monitoring wells, looking at 16S rRNA gene sequences, demonstrated that the anaerobic reducing conditions genereated favoured the growth and activity of bacterial groups such as *Geobacter, Clostridiun, Klebsiella* and *Bacteriodales* which have been previously identified as being able to degrade RDX under anaerobic conditions (Kwon et al., 2014, Livermore et al., 2014). Ecotoxicological assays on soil (earthworm lethality and phytotoxicity [rye seed germination]) and leachates (Microtox and algae growth inhibition [*Pseudokirchneriella subcapitata*]) indicated that there was a dose-related deleterious effect on the organisms tested but which was not statistically significant.

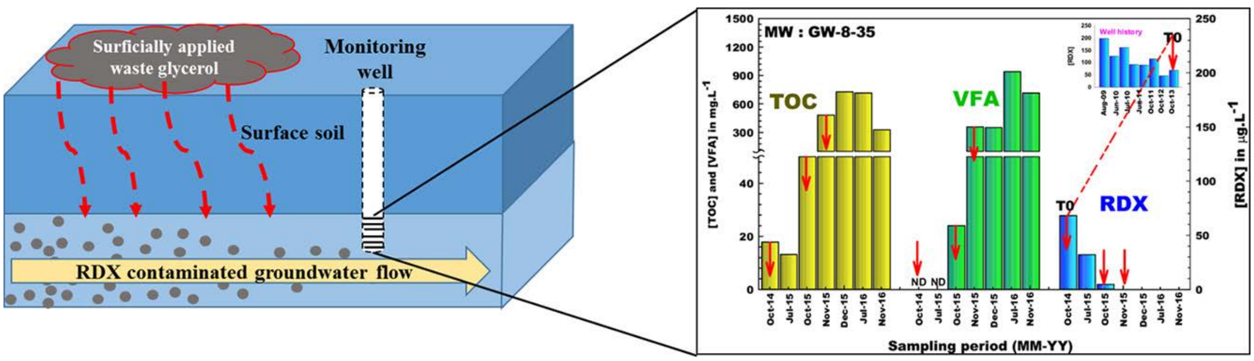

Figure 1. Evolution of TOC, VFA and RDX concentrations over time after the surficial application of waste glycerol at the demolition range.

2.3 Larger Scale Field Trials– Grenade Range

The applicability of the developed technique to treat a different matrix as well as a larger area was tested at a second site at Garrison Petawawa, an active grenade range. The soil type in this area was a medium fine sand which extended down past the top of the groundwater at ca. 9m. The approach taken in this area was to establish 2 experimental plots (10m x 10m) located within the active grenade range, with each plot centred on the two principle impact zones. The monthly treatment schedule consisted of applying 350L of WG (diluted to 1,000L total with water) on the test plot or 1,000L of water on the control plot. The first treatment was applied in June 2019 and continued until October 2019, for a total of 5 treatment applications.

Two wells (GR-6 and GR-7) were installed, just before treatment started, approximately 11m down-gradient of the groundwater flow direction from the edge of the test and control plots, respectively, and a previously installed nested well (GW-DF-GR-4-12 and -15) which has historically demonstrated the movement of EMs from the active grenade range site, was also sampled. The depth of groundwater sampling in GW-GR-6, GW-GR-7, GW-DF-GR-4-12 and GW-DF-GR-4-15 was ca. 8.8m, 8.8m, 12m and 15m, respectively. These wells were sampled for the following chemical analyses: EMs, (HMX, RDX, MNX, DNX, TNX, 2,4-DNT, 2,6-DNT, TNT, 2-ADNT, 4-ADNT, NG, PETN, TNB, 1,3-DNB and NB), TOC and total sulfates analysis. Samples were obtained from each MW using dedicated tubing. A flow-through cell connected to a YSI 600XL field meter (YSI, Inc., Yellow Springs, OH) was utilized to measure field geochemical parameters (DO, pH, ORP, temperature and SC). Sampling was conducted only after field parameters were stable based on low-flow sampling guidelines (USEPA 2017). The groundwater samples were collected monthly from June 2019 (start of treatment) until November 2019 (one month after last treatment application).

The results of the water quality parameters, in particular DO and ORP, over the 6 month course of monitoring, are shown in Figure 2. The results observed in GW-GR-7 (adjacent to the control plot) suggested that at the beginning of the treatment, the groundwater in this area had a reduced DO concentrations and had a slightly lower ORP than in the other wells. This subsequent sampling campaigns revealed that both DO and ORP in GW-GR-7 shifted to more closely resemble the conditions in the other monitoring wells. The other water quality parameters (pH, SC and temperature) did not reveal any significant differences between the different monitoring wells (results not shown). The DO and ORP results suggested that the WG treatment had not reached the groundwater over the 5 months of treatment application and the desired anaerobic and reducing conditions were not created in the deep subsurface.

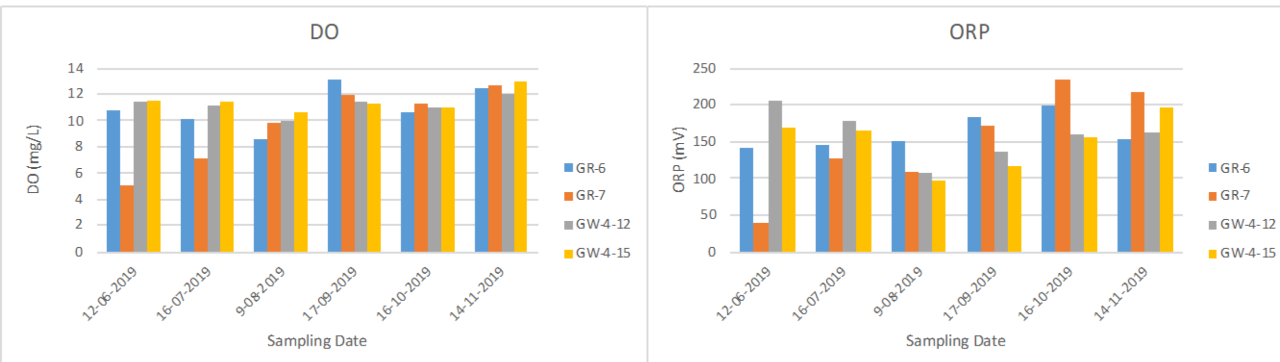

Figure 2. DO and ORP values of groundwater samples from the Grenade Range.

The results of the chemical analysis for EMs revealed that of the 15 compounds being examined, only 5 were observed in the groundwater samples, and in the case of PETN and TNT, were only observed in 1 and 2 samples, respectively (Figure 3). There were no significant decreases in RDX groundwater concentrations over time, and in fact increased in concentration in the last sample from GW-GR-6 (well adjacent to the treatment area). This may be due to a washing effect of the treatment application. Concentrations of RDX in GW-GR-7 were approximately 10 fold higher than in GW-GR-6. MNX concentrations in GW-GR-7 were present at low levels in all but one of the samples and suggested that in this portion of the aquifer, natural attenuation of RDX was possibly occurring, as MNX is a by-product of RDX biodegradation. In monitoring wells GW-DF-GR-4-12 and -15, which have previously been observed to have elevated concentrations of RDX, only one sample collected from GW-DF-GR-4-12 during the first sampling campaign was impacted by RDX. Concentrations of TOC, VFAs and total sulfates, which are indications that the WG treatment is present at sufficient concentrations to elicit a significant impact, did not significantly differ between monitoring wells or over the course of the sampling period (results not shown).

Having been the first year of treatment at a larger scale, there are several explanations as to why there was no measurable impact of the WG application on important environmental conditions (i.e. DO and ORP) or biodegradation of RDX. The overall rainfall measured at Garrison Petawawa was significantly less during the months of July, August and September (39, 34 and 72% of average monthly rainfall, respectively), possibly reducing the movement of the WG into the subsurface. The quantity of WG glycerol used during each treatment application (350L) may be insufficient to generate the desired conditions in the groundwater located almost 9m below surface. Additionally, the WG may be moving through the subsurface but will require a longer time period before it will reach the groundwater.

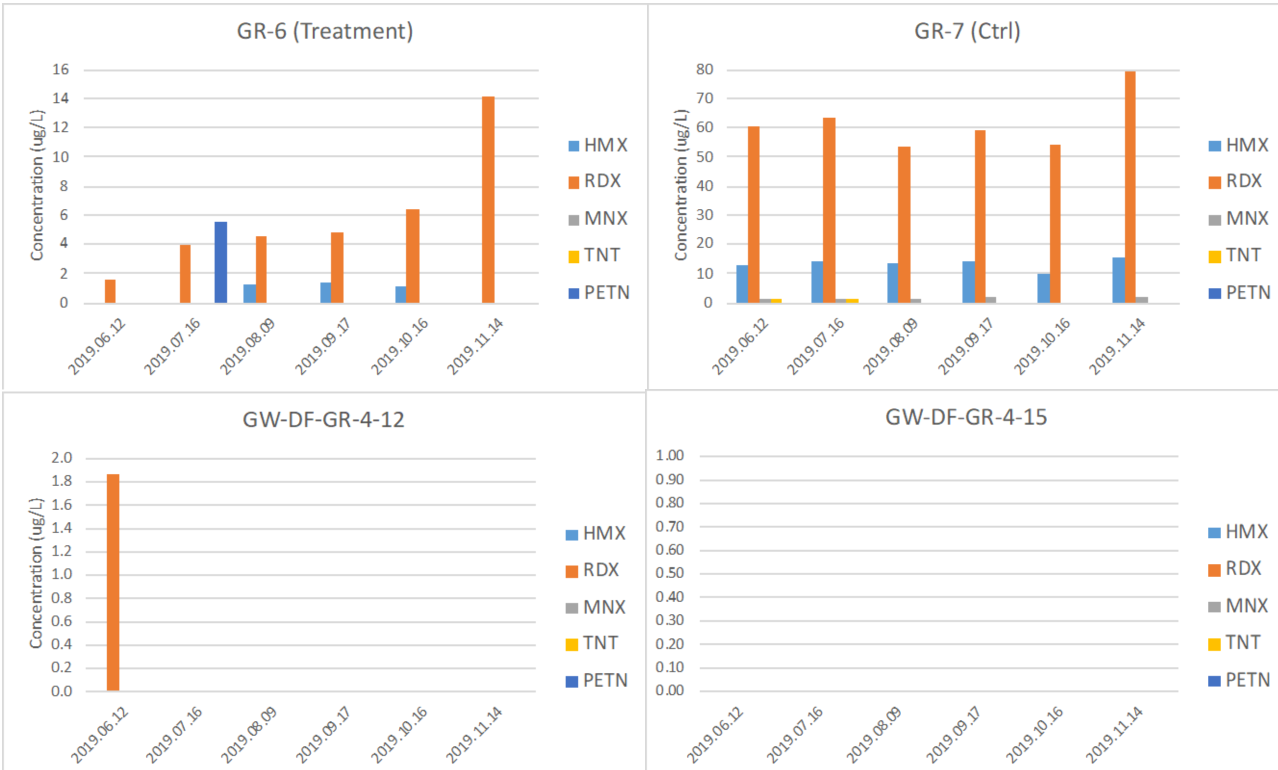

Figure 3. EM chemical analysis for groundwater samples.

3.0 CONCLUSIONS

The surficial application of WG in an EM impacted site was demonstrated in the lab and at a small pilot scale field plot to have a positive impact on the generation of subsurface environmental conditions (anaerobic and reducing) which stimulated the biodegradation of microbial species able to biodegrade RDX. This was evidenced by a decrease in DO and the generation of reducing conditions, concomitant with the disappearance of RDX and the transitory production of MNX. Application of this treatment approach, over the course of one summer season, at a larger scale in a different environment did not produce the same results as observed in the smaller scale experiments, but this is not an unexpected result due to the differences between the sites. Further optimization of the treatment approach (e.g. larger quantities of WG, larger volumes of total treatment solutions, addition of nutrient amendments, etc) are planned for future field trials. The overall approach is very promising for the treatment of EM impacted soils located within active training ranges using the surficial application of a carbon source (waste glycerol) to stimulate the biodegradation of RDX.

4.0 REFERENCES

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