E.R.A.S.E. Explosive Remediation by Applied Synthetic *E. coli*

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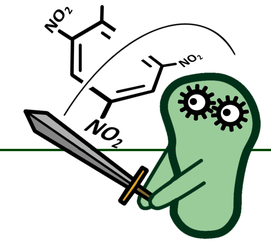
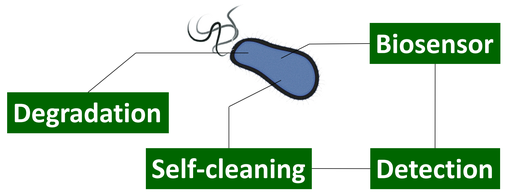


Introduction

Environmental pollution by explosive waste from Trinitrotoluene (TNT) and nitroglycerin (NG) is a widespread, long-term health problem (Fig. 1A and B). Current remediation strategies are impractical and expensive (Fig. 1C).[1, 2]

Figure 1. The problem with Trinitrolouene (TNT) and nitroglycerin (NG). A. TNT is a highly toxic compound with many detrimental health effects. **B.** The problem is global. **C.** Current decontamination practise produces its own problems.

We propose a bacteria capable of bioremediation that targets the toxic compounds TNT and NG, converting them to harmless products (Fig. 2, below).



The bacteria should also monitor the presence of the target pollutant, killing itself as toxicity falls.

Discussions with stakeholders indicated a need for improved detection systems.

As kill switches and reporter systems are already available we focussed on two aims:

1. **Identify enzymes to degrade TNT/NG to harmless products.**
2. **Identify a genetically encoded detection system.**

In addition, we sought to build a model which would help us to design, analyse and debug our biological system. Specifically it must:

- Inform the biological design.
- Inform the choice of experiments.
- Be receptive to empirical observations.
- Should explain how our system works.

The Modelled System

We modelled our system on four key levels:

1. The Biochemical Level

The primary determinant of TNT/NG degradation will be the activity of our enzyme(s).

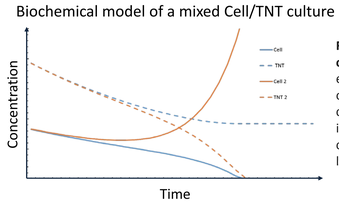


Figure 3. The influence of enzyme kinetics on degradation rate. In the blue cells, the enzyme reaction rate is slow and the outcome is failure to clean up all TNT and cell death. However, in the orange cells the increased enzyme rate allows TNT concentration to be reduced to non toxic levels.

2. The Cellular Level

In this model cells grow and are destroyed at a rate proportional to the TNT concentration. Survival at a given level of TNT is dependant on cell density as well as the kinetic properties of the enzymes.

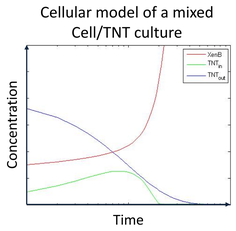


Figure 4. Cell growth and TNT concentration over time. Cells grow and are destroyed at a rate proportional to the TNT concentration. Survival at a given level of TNT is dependant on cell density as well as the kinetics properties of the enzymes.

3. A Stochastic Model

We created a more complex simulation, modelling the life and death of the cell population over time. Although this model did not guide any experiments it contributed to our spatial model.

4. A Spatial Model

Finally, our most sophisticated model also takes into account the position of each individual bacterium, the level and toxicity of TNT at that positon, population growth and a kill-switch.

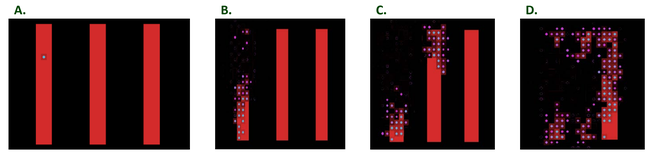


Figure 5. The combined model. By combining all levels of the model we were able to explore different scenarios. **A.** The starting condition. A single cell in one region of TNT. **B.** If the distance between regions is too great cells cannot cross to new areas of TNT due to the activity of the kill switch in the absence of TNT. **C.** and **D.** The shorter distance between islands allows TNT to spread to other areas of TNT.
Red: Area of TNT. Black: No TNT. Colour intensity is proportional to TNT concentration.

Degradation

We identified two enzymes, XenB and NemA[3], which may degrade both TNT and NG. We constructed two expression cassettes to test this (Fig. 6) and found that cells expressing the enzymes rapidly catalysed TNT (Fig. 7).

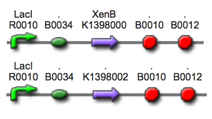


Figure 6. XenB and NemA expression constructs. Constructs were designed using GeneDesigner (DNA2.0) using sequences from GenBank. Sequences were synthesised and codon optimised for *E. coli* by DNA2.0 using their GeneGPS technology to increase their expression. Enzymes were His(x6) tagged to allow for purification by Ni^{2+}-affinity chromatography.

Time after addition of TNT (min)	Sample		
	XenB	NemA	Top10
40			
80			

Figure 7. Response of cells expressing NemA or XenB to addition of TNT in the media. Colour change was rapid in cells expressing NemA or XenB. Carried out in LB media with 1.47 mM TNT.

Purified NemA protein degraded TNT in the presence of cofactors (Fig. 8).

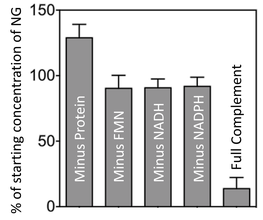
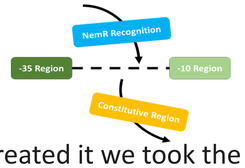


Figure 8. In vitro activity of purified NemA against TNT. TNT loss after 15 minutes incubation at RT was determined using Raman spectroscopy. Assays included buffer (50 mM Tris-HCl pH7.5) plus different combinations of protein (0.047 mg) and cofactors FMN (0.2 mM), NADH (0.2 mM) or NADPH (0.2 mM).

Detection

We successfully created a synthetic promoter based on the *nemR* regulatory region which varies in expression depending on the concentration of TNT.



To created it we took the NemR binding "box" found within NemR regulators and inserted it between the -35 and -10 regions of a constitutive Anderson promoter (BBa_J23100).

Our natural promoter, based on the entire *nemR* region, showed no specificity for TNT.

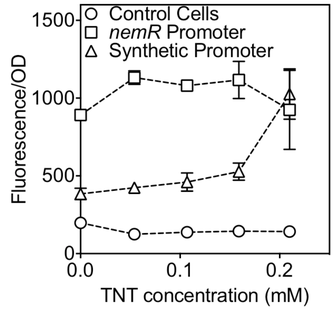


Figure 11. Fluorescence response to increasing concentrations of TNT in the media. Cells were grown in 96-well plates in a modified minimal media to minimise autoflourescence. Cells were: control (TOP10), *nemR* promoter (TOP10 with BBa_K1398004) or the synthetic promoter (BBa_K1398007).

iLOV Characterisation

We characterised BBa_K660004, which encodes the fluorescent protein iLOV. iLOV was introduced to the Registry by Glasgow 2011 but was not characterised. We were able to demonstrate its function in *E. coli* (Fig. 12A, as well as determining its excitation/emission spectra (Fig. 12B).

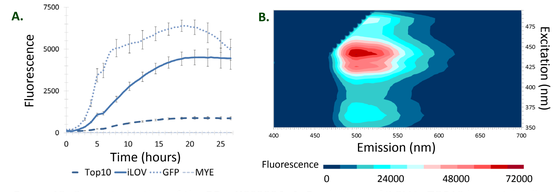


Figure 12. Characterisation of the BBa_K660004. A. Production of iLOV in TOP10 bacterial cells using the *nemR* promoter relative to Top10 control cells, media and GFP. **B**. Excitation and emmision spectra for iLOV. Peak excitation wavelength, 449±1 nm, peak emission at 494±2 nm.

iLOV is a viable alternative to GFP and has several benefits, including its reduced size compared to GFP, meaning it can be synthesised for a lower cost.

Conclusions

We designed and created a synthetic promoter comprised of two completely separate sequences that has the capability to be used as a biosensor in a bioremediating organisms. We have also successfully characterised two enzymes capable of degrading TNT and NG *in vivo* which could be used in a bioremediator.

References

1. Meyns B, *et al*. Water, Air, and Soil Pollution. **2002** 138:37-49.
2. Jiamjitrpanich W, *et al*. Journal of Environmental Science and Health. **2010** 45:263-274.
3. Ramos J et al. Current opinion in biotechnology. **2005** 16(3):275-281.

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