Biodegradation of Undefined Complex Mixtures

M. Frenzel[1 2], S. J. Rowland[2], S.K.Burton[1] & H. M. Lappin-Scott[1]

[1]*School of Biosciences, Hatherly Laboratories, Prince of Wales Road, Exeter*
[2]*Petroleum & Environmental Geochemistry Group, SEOES, University of Plymouth, Plymouth*

1. Introduction

The fractions of petroleum which are resistant to weathering and which represent large volumes of toxic waste, comprise very complex mixtures of chemicals including hydrocarbons and carboxylic acids. Since such fractions are unresolved by conventional methods of analysis such as gas chromatography, they are often referred to as Unresolved Complex Mixtures (UCMs)[1],see figure 1a. A number of model or surrogate UCM hydrocarbons have been proposed, including alkylcyclohexyltetralins[2] (figure 1b).

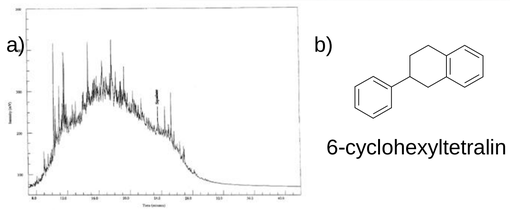


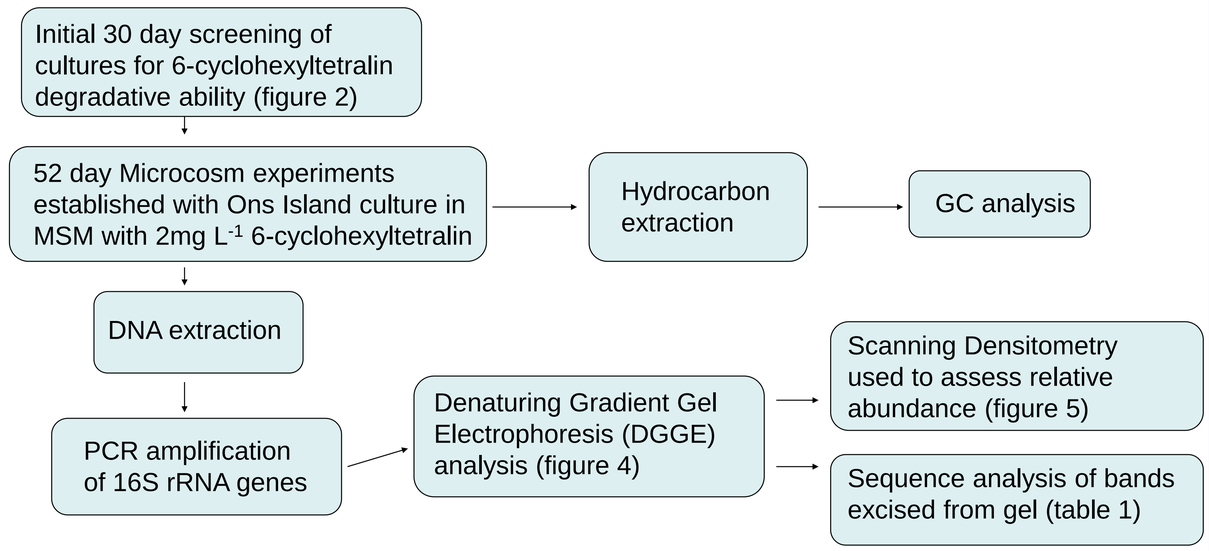
Figure 1 – (a) UCM hump typical of heavily weathered crude oil. (b) Structure of 6-cyclohexyltetralin

Aims

Our aims were to:

- Assess the degradative potential of 6-cyclohexyltetralin by a range of microbial consortia.
- Apply molecular techniques to analyse the *in situ* changes to microbial communities exposed to a surrogate UCM compound.

2. Methods



3. Results

Degradation of 6-cyclohexyltetralin was monitored over time (30 days) and quantified using gas chromatography (GC).

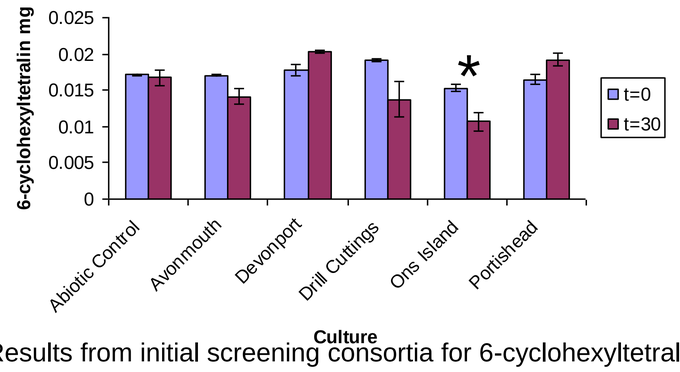


Figure 2 – Results from initial screening consortia for 6-cyclohexyltetralin degradative ability. Consortia were previously enriched from UK sites for PAH degradative abilities. Only one culture (Ons Island) exhibited statistically significant (P = 0.05) degradation of the UCM model compound when compared with abiotic controls.

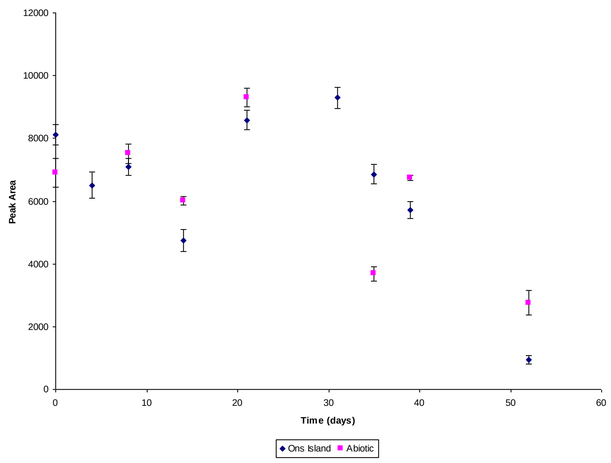


Figure 3 – Degradation of 6-cyclohexyltetralin by Ons Island consortium. Degradation by the consortium was 40% greater than abiotic losses over the time period.

DGGE and scanning densitometry analysis

DNA was extracted at specific time points during the enrichment and the 16S gene amplified by PCR using universal Eubacterial primers. PCR products were analysed by DGGE (figure 4). Scanning densitometry was used to assess the relative abundance of degraders over the enrichment period (figure 5).

Figure 4 – DGGE analysis of of 16S rRNA genes from Ons Island consortium over the enrichment period

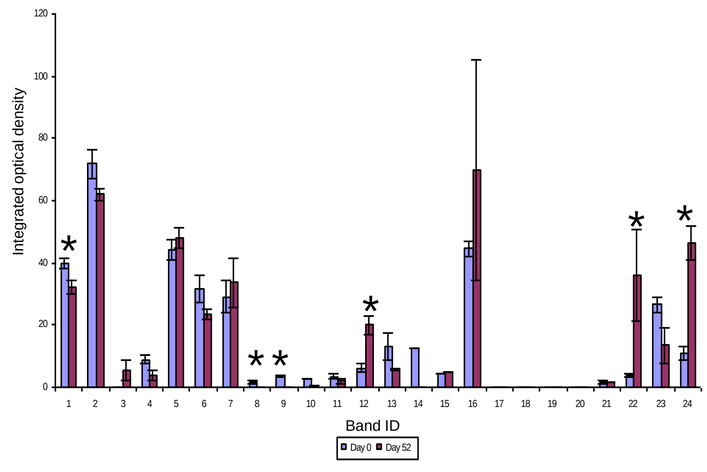


Figure 5 – Scanning Densitometry Analysis of DGGE gel. This method gives a semi-quantitative overview of the changes in microbial community composition over the course of the enrichment. The bands indicated (*) showed statistically significant (p = 0.05) changes in density, indicating that these species may be responsible for 6-cyclohexyltetralin degradation.

Sequence Analysis

Table 1 – 16S rDNA sequence analysis of bands from DGGE gel. Bands 12, 22 and 24 showed a significant increase over the enrichment period; indicating that they may be key degraders of UCM surrogate compounds

Band number	% Homology
1	90% Uncultured bacterium clone
8	91% *Bradyrhizobiaceae sp.*
9	94% Uncultured bacterium clone
12	96% *Xanthobacter sp.*
22	99% *Ensifer sp.*
24	98% *Sinorhizobium sp*.

4. Conclusions

- Observations suggest degradation of 6-cyclohexyltetralin had occurred; this holds promise for future remediation of sites contaminated with such chemicals.
- PCR and DGGE indicate that several genera (*Xanthobacter*, *Ensifer* and *Sinorhizobium*) that are potential 6-cyclohexyltetralin degraders.

5. Future Work

- Complete 16S rRNA gene sequencing.
- Apply SIP to C^{13} labelled substrates to identify both key degradative organisms and metabolites.

References

1. Gough M .A. & Rowland, S.J. (1990) Characterisation of unresolved complex mixtures of hydrocarbons in petroleum. *Nature*, **344**, 648-650.
2. Smith, E. L., Wraige E., Donkin, P. & Rowland, S. J. (2001). Hydrocarbon 'humps' in the marine environment: synthesis, toxicity, and aqueous solubility of monoaromatic compounds. *Environ. Toxicol. Chem.*, **20**, 2428-2432.

Acknowledgements

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