Microbial toxicity and degradation of a monoaromatic crude oil fraction

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Introduction

Oil fractions which are resistant to weathering and represent large volumes of toxic waste comprise very complex mixtures of hydrocarbons. Since such fractions are unresolved by conventional methods of analysis including gas chromatography (GC), they are often referred to as Unresolved Complex Mixtures (UCMs) or 'humps'[1] (Figure 1a). Recent studies have used comprehensive two-dimensional GC time-of-flight mass spectrometry (GCxGC-ToF-MS) to resolve and characterise many thousands of the toxic compounds occurring in UCMs[2]. A number of model or surrogate UCM hydrocarbons have been proposed, including alkylcyclohexyltetralins and have been shown to be degraded by a bacterial consortium isolated from Whitley Bay UK[3]. It would now be appropriate to test the biodegradation potential of this consortium with a UCM isolated from crude oil, and to assess changes in toxicity resulting from the microbial alteration of the original aromatic UCM composition.

Study aims:

1) To isolate an aromatic fraction from Venezuelan Tia Juana Pesado (TJP) crude oil for use in biodegradation studies.

2) To undertake biodegradation experiments with an enriched consortium (Whitley Bay, UK).

3) To apply molecular techniques to analyse the *in situ* changes to microbial communities exposed to the isolated UCM.

4) To test the toxicity of the isolated fraction by microtox® and mussel feeding rate assays before and after microbial exposure to assess the potential for bioremediation.

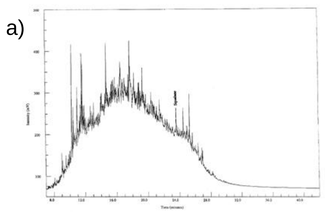
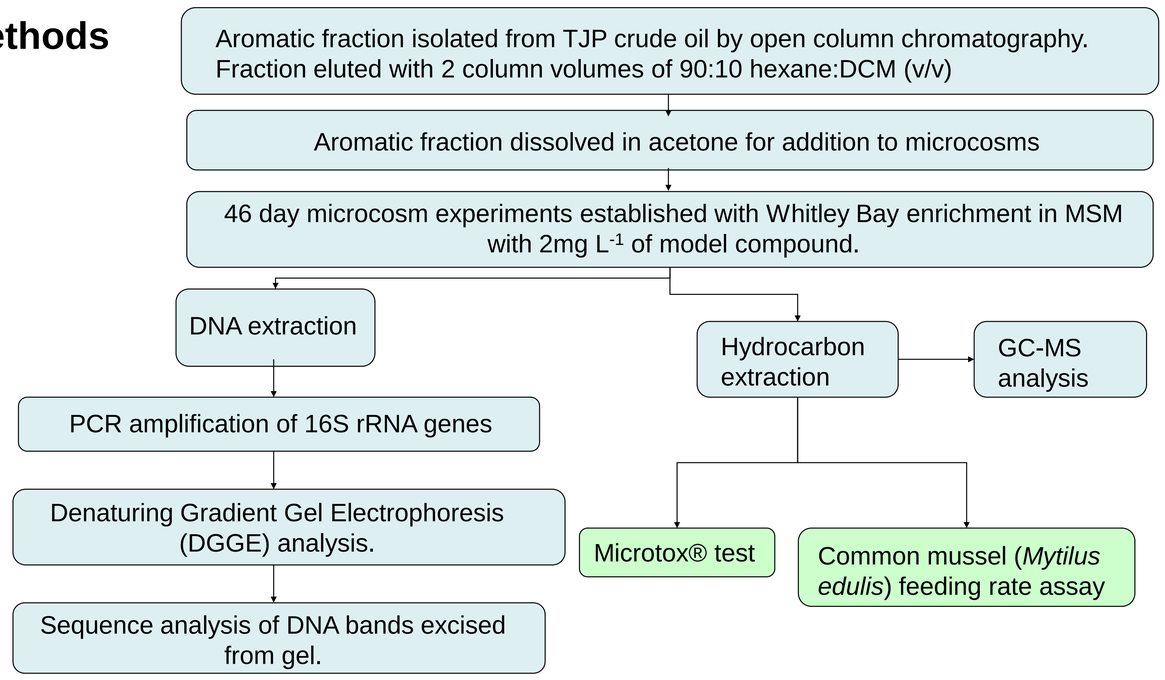


Figure 1 UCM hump typical of heavily weathered crude oil.

Methods



Results

Biodegradation with consortium isolated from Whitley Bay, UK

Microcosm experiments were established and enriched in media containing 200 mg of TJP aromatic fraction dissolved in acetone (200 µL). Degradation of the fraction was monitored over time (46 days) and quantified using gas chromatography-mass spectroscopy (GC-MS).

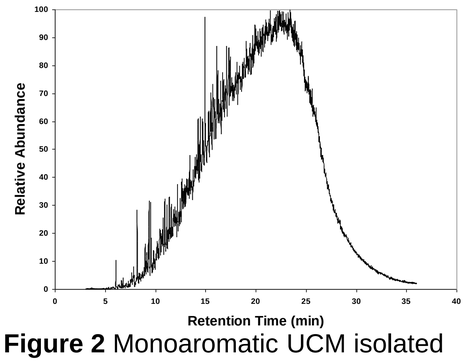


Figure 2 Monoaromatic UCM isolated from TJP crude oil

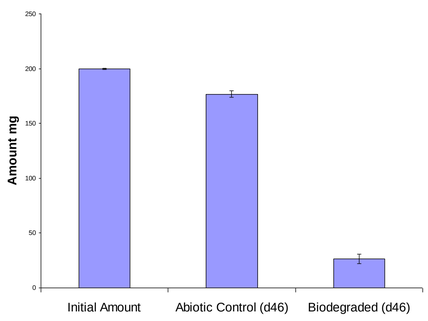


Figure 3 Recovered amounts of monoaromatic fraction after 46 days exposure.

Results

Toxicity

Common mussels (*Mytilus edulis*) were exposed for 24 hours to the undegraded fraction (abiotic), biodegraded fraction, acetone (solvent control), or seawater (control). Feeding rates on algal cells were measured by coulter counter.

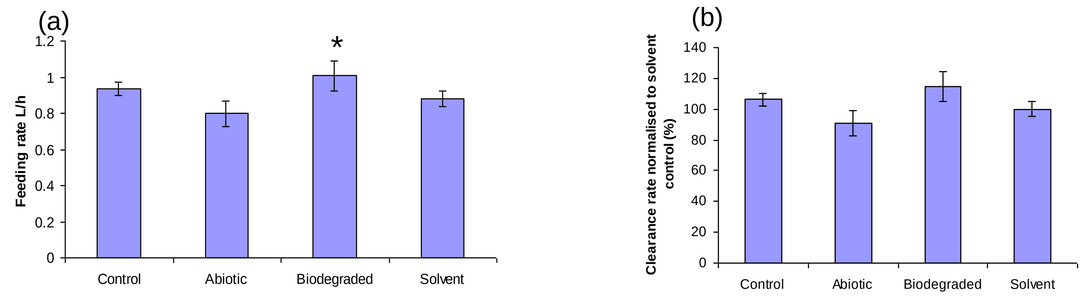
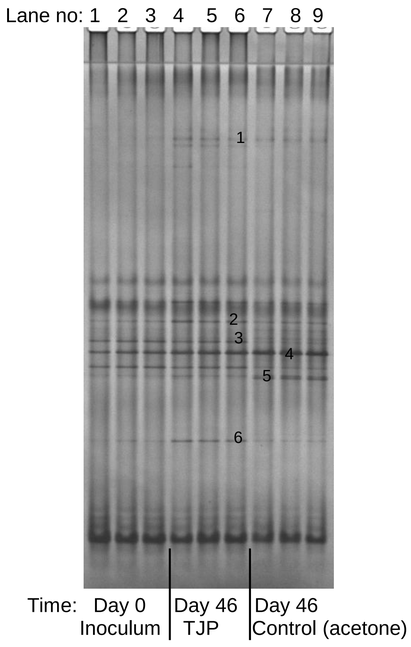


Figure 3 (a) Feeding rate of *M. edulis* exposed to oil fractions. Feeding rates of mussels exposed to the biodegraded fraction were significantly higher than those exposed to the undegraded fraction (b) Clearance rate of *M. edulis* mussels normalised to solvent control.

DGGE community analysis

DNA was extracted during the enrichment and the 16S rRNA gene amplified by PCR using universal Eubacterial primers. PCR products were analysed by DGGE (figure 4). Identified members of the bacerial consortium are listed in Table 1.



Sequence Analysis

Band number	% Homology
1	99% *Ochrobactrum sp.*
2	93% *Bosea sp*
3	94% *Defluvibacter*
4	96% *Pandoraea sp.*
5	99% *Uncultured bacterium clone.*
6	98% *Rhodococcus sp.*

Table 1 16S rDNA sequence analysis of bands from DGGE gel.

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

Summary and Implications

- Over 75% of the monoaromatic fraction was degraded by the Whitley Bay consortium
- Feeding rates of mussels exposed to the degraded oil fraction are significantly higher ($P<0.05$) than those exposed to the undegraded oil fraction.
- Aerobic biodegradation significantly reduced the toxicity of the isolated UCM to invertebrates.
- Bioremediation is a viable method of reducing UCM pollution in the environment

Future Work

- Alkaline saponification extraction of mussel tissue to establish which compounds are taken up by these organisms during exposures.
- Mass-balance measurements of CO_2 production during degradation.

References

1. Gough M .A. & Rowland, S.J. (1990) Characterisation of unresolved complex mixtures of hydrocarbons in petroleum. *Nature*, **344**, 648-650.
2. Booth, A., Sutton, P.A., Lewis, C.A., Lewis, A.C., Scarlett, A., Wing Chau, Widdows, J. and Rowland, S.J. (2007) Unresolved complex mixtures of aromatic hydrocarbons: Thousands of overlooked persistent, bioaccumulative and toxic contaminants in mussels. *Environmental Science and Technology 41*, 457-464.
3. Booth, A.M., Aitken, C., Jones, D.M., Lewis, C.A., and Rowland, S.J. (2007) Resistance of toxic alkylcyclohexyltetralins to biodegradation by aerobic bacteria. *Organic Geochemistry* (In press)

Acknowledgements

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