# Overcoming the hump: biodegradation of recalcitrant oily wastes

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## 1. Introduction

Oil fractions which are resistant to weathering and represent large volumes of toxic waste comprise very complex mixtures including hydrocarbons and carboxylic acids. 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). The acids therein are also often referred to as naphthenic acids (NAs), though they too are often unresolved and unidentified. A number of model UCM hydrocarbons and NAs have been proposed, including alkylcyclohexyltetralins[2] and alkylcyclohexylalkanoic acids[3].

Study aims:

1) To synthesise two model UCM hydrocarbons and NAs to high purity for use in biodegradation studies.

2) To undertake biodegradation experiments with an enriched consortium (Whitley Bay, UK) and compare degradation rates of surrogate compounds.

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

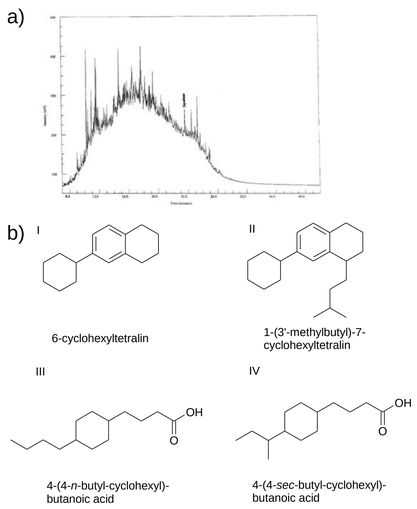
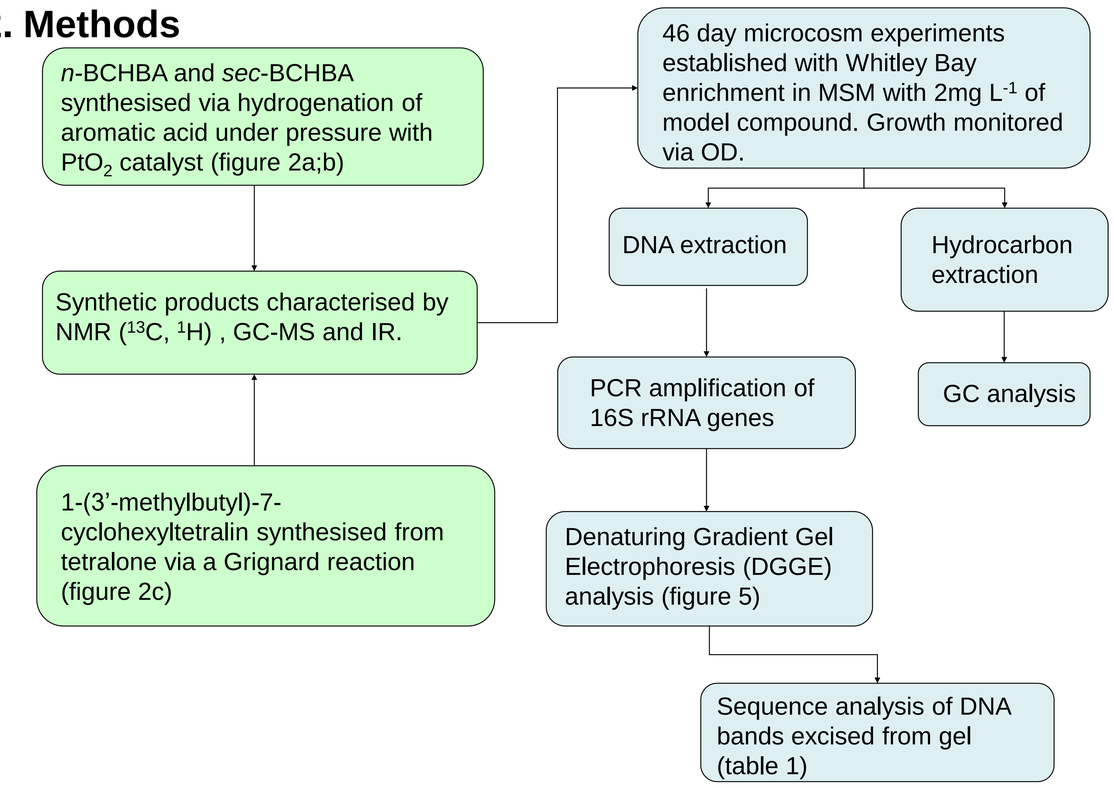



**Figure 1** (a) UCM hump typical of heavily weathered crude oil.

(b) Chemical structures of the alkylcyclohexyltetralins (I-II) and alkylcyclohexylalkanoic acids (III-IV) used in this biodegradation study.

## 2. Methods




## 3. Results

### Synthesis

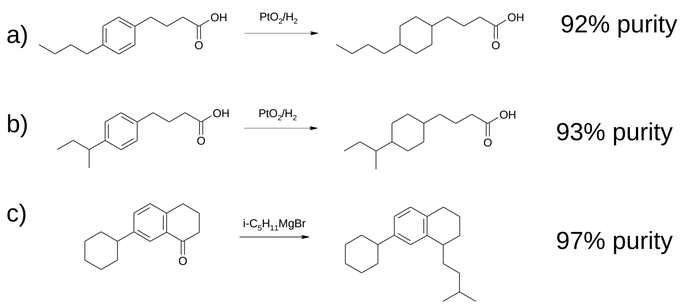



**Figure 2** Synthetic pathways of model compounds (a) n-BCHBA (b) sec-BCHBA (c) 1-(3'-methylbutyl)-7-cyclohexyltetralin

## 3. Results

### Biodegradation with consortium isolated from Whitley Bay, UK

Microcosm experiments were established and enriched in media containing one of the four model compounds. Degradation of each substrate was monitored over time (46 days) and quantified using gas chromatography (GC-FID). Growth was monitored by measuring the optical density of the cultures at each time point (figure 4).

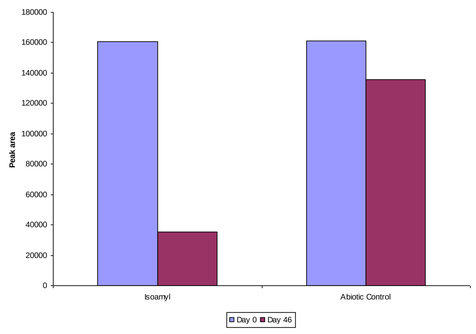



**Figure 3** Degradation of 1-(3'-methylbutyl)-7-cyclohexyltetralin by Whitley Bay consortium.

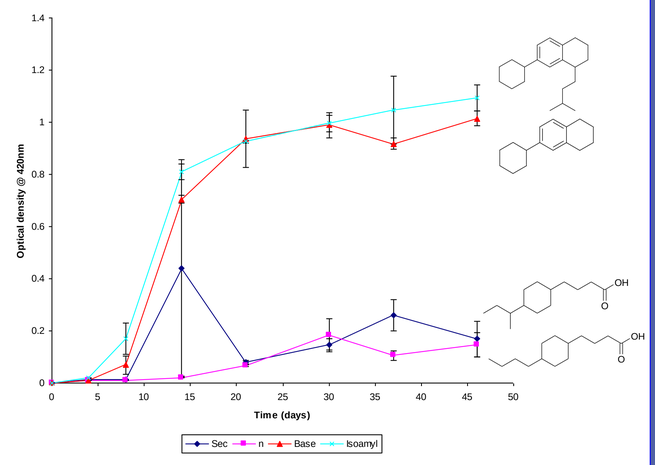



**Figure 4** Growth of Whitley Bay consortium on each model compound.

### DGGE 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 5).

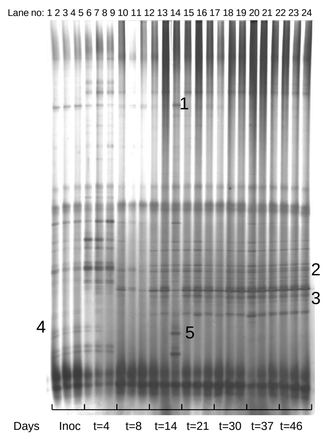



**Figure 5** DGGE analysis of 16S rRNA genes from Whitley Bay consortium enriched on 1-(3'-methylbutyl)-7-cyclohexyltetralin

### Sequence Analysis

| Band number | % Homology |
|---|---|
| 1 | 100% *Rhodococcus erythropolis* |
| 2 | 98% α-proteobacterium |
| 3 | 97% *Arthrobacter sp.* |
| 4 | 99% *Actinobacteridae sp.* |
| 5 | 98% *Acidobacterium sp.* |

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

## 4. Conclusions

- Data consistent with 1-(3'-methylbutyl)-7-cyclohexyltetralin degradation holds promise for future remediation of sites contaminated with these recalcitrant chemicals.
- Characterisation of degradation enrichments is continuing, however OD data suggest no evidence for degradation of *n*-BCHBA or *sec*-BCHBA
- PCR and DGGE of the consortium have identified several genera

## 5. Future Work

- Construct clone libraries for complete 16S rRNA gene sequencing from enrichments.
- Apply Stable Isotope Probing (SIP) to $^{13}C$ labelled substrates to identify 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.
3. Smith, B.E. (2006). PhD Thesis, University of Plymouth.

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