Biodegradation of Aromatic Unresolved Complex Mixtures (UCMs): A Comparative Study Using an Isolated Aromatic UCM and Synthetic Model UCM Hydrocarbons

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Summary

C**yclohexylalkyltetralins**, which are believed to be good model hydrocarbon compounds for components of aromatic **unresolved complex mixtures (UCMs)** have been synthesised in this laboratory. To validate their candidacy as components of the UCM they have been subjected to rigorous experimentation based upon known properties of the UCM (toxicity, solubility, photodegradation). This study has monitored the **biodegradability** of both an isolated aromatic UCM and the synthetic model compounds using the bacterium strain ***Pseudomonas fluorescens,*** a known aliphatic and aromatic hydrocarbon degrader.

1. Introduction

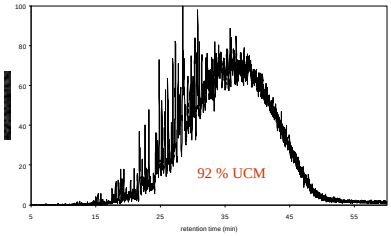

Figure 1: Gas chromatogram of biodegraded Tia Juana Pesado (Venezuela) Crude Oil: Aromatic fraction (TJPARO1), isolated by open column chromatography.

- Unresolved complex mixtures (UCMs) of hydrocarbons are an almost universal feature of environmental samples contaminated with weathered residues of petroleum [1].
- The UCM is considered to be relatively inert to microbial degradation [2] and, consequently, it is a dominant feature of the gas chromatograms of biodegraded crude oils (Fig. 1).
- Alkyltetralins have previously been identified as important aromatic UCM components in several biodegraded and refinery feedstock oils [3,4].
- The most toxic component of the UCM is the monoaromatic fraction [5,6].
- The synthesis of proposed 'average' model structures (Fig. 2, I-VI) [7,8] of monoaromatic UCM hydrocarbons has allowed a comparative toxicological evaluation to be made [5].
- Exposure of *Mytilus edulis* to compounds (I-III) exhibited similar toxicity effects (>70% reduction in filtering rate) to those observed after exposure to aromatic UCM.

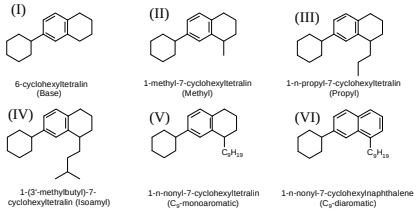

Figure 2: Chemical structures of the synthesised alkyltetralins (I-VI) used in the biodegradation experiment.

2. Aims

- To provide further evidence for the occurrence of the proposed model compounds within the aromatic UCM, by investigating the biodegradability of the compounds compared to that of an aromatic UCM fraction of Tia Juana Pesado (TJPARO1) unresolved biodegraded crude oil.

3. Methods

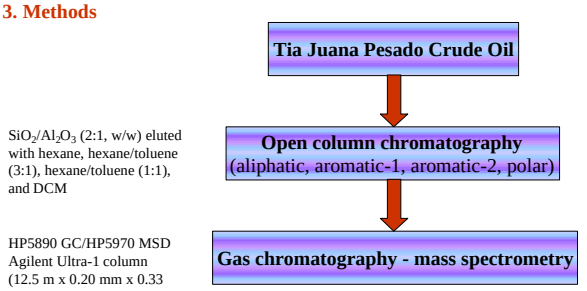

Figure 3a. Schematic of experimental protocol - open column chromatography

- The biodegradation experiments are based upon methods previously employed [9,10].

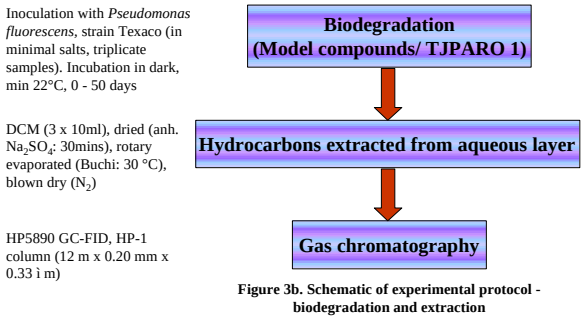

Figure 3b. Schematic of experimental protocol - biodegradation and extraction

4. Results

- At each sampling point an aliquot of bacteria was taken and streaked onto an agar nutrient plate. Growth observed after 24 hours confirmed that the bacteria remained viable and that they had not been contaminated by other bacterial species.
- Optical density measurements, taken to determine whether the bacterium population was increasing, indicated no utilisation of hydrocarbons.
- The results of the biodegradation of the model compounds can be seen in Figure 4(a), where the concentration of the lower molecular weight compounds has decreased. Figure 4(b) shows the maximum degradation of each of compounds I-VI after incubation for 50 days (mean ± σ, n=3).
- The control samples show that all losses of hydrocarbons can be attributed to abiological effects. It is proposed that evaporation rather than photooxidative processes are responsible since the samples were kept in dark .

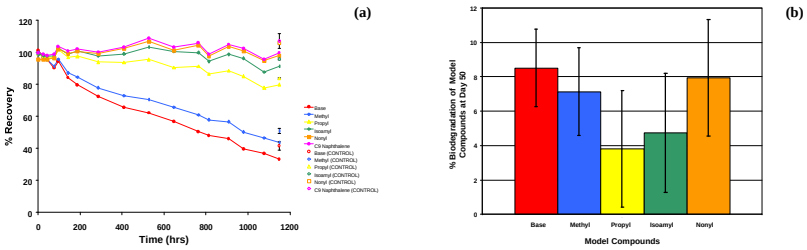

Figure 4. (a) Summary of biodegradation data obtained herein show the percentage recovery of the alkyltetralins (I-VI) and controls over a 50 day period. Data for abiotic controls are also shown. (b) Summary of the biodegradation of compounds I-V (mean ± σ, n=3) at day 50 compared to the control values.

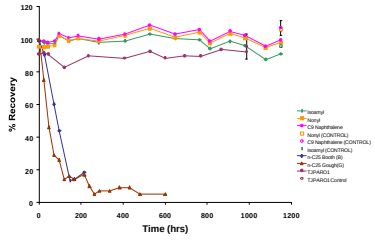

Figure 5. Percentage recoveries of the compounds (I-VI) and Tia Juana Pesado aromatic UCM hydrocarbons (TJPARO1) after incubation *with P. fluorescens* for up to 50-days period. Values have been adjusted to account for any compound loss during extraction, based upon the recovery data of the *n*-C_{25} internal standard.

- The results of the biodegradation experiment are summarised in Figure 5, which shows the mean proportion of compounds (IV-VI) and the aromatic UCM recovered in triplicate experiments after incubation for 50 days with *P. fluorescens.* Data for abiotic controls (n=3) are shown. Also shown for comparison are data for the degradation of *n*-C_{25} in the present study (B) and in an earlier study (G) by [2].

5. Conclusions

- The decrease in the concentration of compounds I and II is mainly attributed to evaporation.
- Under the conditions used herein, the synthetic compounds I-VI were as resistant to biodegradation as the aromatic UCM hydrocarbons remaining in TJP crude after it had been biodegraded naturally over geological time.
- The similar responses to biodegradation suggest that these synthetic compounds are reasonable surrogates for some monoaromatic UCM components.
- This work is consistent with the chemical oxidation studies of UCM compounds and with the toxic action of the synthetic compounds, both of which resemble the results of studies of biodegraded oil aromatic UCMs [3, 4, 5, 6].

6. Further Study

Whilst there has been previous work investigating the biodegradation of tetralin [11,12,13], the resistance of the present synthetic tetralins to a pure culture of *P. fluorescens* is perhaps to be expected. Further experiments should investigate whether this is the case with mixed cultures of bacteria under a wider variety of conditions.

Acknowledgements

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