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BIOREMEDIATION OF 'THE HUMP': ASSESSMENT OF THE BIODEGRADATION OF A TOXIC AROMATIC HYDROCARBON UNRESOLVED COMPLEX MIXTURE (UCM) AND MEASUREMENT OF REMAINING TOXICITY

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Crude oil is a complex mixture of chemicals, many of which exhibit significant resistance to weathering processes (*e.g.* biodegradation) when released into the environment. Components of this resistant fraction which are typically unresolved by conventional analytical methods such as gas chromatography (GC) and often referred to as unresolved complex mixtures (UCMs) or 'humps, are toxic (e.g. to marine organisms such as mussels; Donkin *et al*., 2003). 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 (Booth *et al*., 2007a). Methods now need to be found to remove or reduce these toxic UCMs in the environment. One approach is use of bacteria to remediate the UCMs by biodegradation.

In the present study, an aromatic hydrocarbon UCM fraction was isolated from an in-reservoir degraded Venezuelan Tia Juana Pesado crude oil. The toxicity of the fraction was determined using a *Vibrio fisheri* Microtox® test and invertebrate toxicity investigated using feeding rate assays with mussels (*Mytilis edulis*). The toxic UCM was then subjected to aerobic biodegradation by a natural bacterial consortium enriched from marine sediments collected at Whitley Bay, UK, and previously reported to degrade alkylcyclohexyltetralins proposed as good models for some toxic aromatic UCM compounds (Booth *et al*., 2007b). The microbial community composition was assessed by PCR-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) of the 16S rRNA gene. The biodegradation of some of the UCM hydrocarbons was determined and quantified using GC-mass spectrometry (GC-MS) and GCxGC-ToF-MS. The latter provided a technique for assessing which aromatic UCM components were susceptible to biodegradation by the consortium and which were still

resistant. The microbial consortium was indeed able to degrade some of the recalcitrant toxic oil fractions and thus seemingly has potential for 'hump' bioremediation.

However the toxicity of the resulting degraded fraction was again determined using the *Vibrio fisheri* Microtox® test and mussels. Thus, changes in toxicity resulting from the microbial alteration of the original aromatic UCM composition via biodegradation could be determined and the true potential for bioremediation, rather than bioalteration, assessed.

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