Changes in Isomeric Composition of LAB Cable Oil Associated with a Range of Terminal Electron Acceptors

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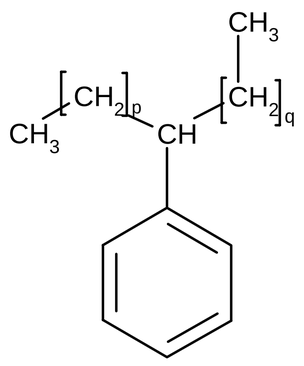
Abstract

Synthetic linear alkylbenzenes (LABs) are used as insulating oils in underground electricity transmission cables. Following accidental damage to the cables or a failure of the cable system, oil may leak into the surrounding soil. The cable is normally repaired quickly and the majority of the oil is recovered, but access to soil containing residual contamination may be hampered by proximity to other buried services, so minimally invasive techniques for remediation are sought. Laboratory experiments have been designed to investigate LAB degradation under anaerobic conditions, as these are known to pertain in the field. Preliminary results indicate, for the first time, that microbial growth can occur with nitrate as a terminal electron acceptor (TEA) and LAB as the sole carbon source. LAB degradation was not evident under sulphate-reducing and methanogenic conditions. GC-MS data show that the isomeric composition of oil degraded under nitrate-reducing conditions is altered in a way that differs significantly from oil that has been degraded by aerobic microorganisms. Ongoing work, aimed at confirming these findings, includes repetition of the flask culture experiments, greater replication and the inclusion of an internal GC standard to allow the degradation to be fully quantified. Cable-oil degrading microorganisms (CDMs) will be isolated and identified, and the pathways of degradation delineated.



p, q = 0 - 7
p + q = 7 - 10

Figure 1. Generalized structure of LAB in cable oil.

Methods

Samples of unused cable oil supplied by National Grid Company plc. were exposed to microorganisms from an LAB-contaminated soil with a variety of terminal electron acceptors. Cable oil is a synthetic mixture of about 20 LAB isomers (not including stereoisomers) with the general structure shown in Figure 1.

Flasks containing 20ml Bushnell-Haas medium plus 0.1ml LAB as the sole carbon source were inoculated with organisms cultured from cable oil-contaminated soil (Johnson *et al* 2001). Aerobic (AER) and killed control (CON) flasks were incubated aerobically in a shaker/ incubator. Other cultures containing either no additional TEA (MET), nitrate (NIT), sulphate (SUL) or both nitrate and sulphate (ANA) were incubated anaerobically under a 80% N_2/10% CO_2/5% H_2 atmosphere.

Three flasks from each treatment were harvested at intervals and the LAB extracted by shaking in *n*-hexane. 5µl of 1C12 LAB was added immediately prior to the extraction step to serve as an internal GC standard. The organic fraction was analysed by GC-MS:

HP5890 Series II Gas Chromatograph. **Column:** HP5 (5% polysiloxane), 0.32 mm i.d.; 0.25µm film thickness; 30 m length. **Carrier gas:** He. **Injection:** 1µl; splitless; 250°C. **Oven:** 50°C for 2min, increase at 10°C/min, 100°C for 2 min, increase at 2.5°C/min, 150°C for 2 min, increase at 5°C/min, 300°C for 1 min. **HP5972 Mass Selective Detector:** 280°C; scan m/z 35-500 at ~1 s^{-1}.

Typical chromatograms from a preliminary experiment (annotated with alkyl chain lengths and position of the phenyl group) without the addition of a standard are shown in Figures 2 & 3. The standard is, conveniently, eluted in the gap between 3C13 and 2C13, and thus does not interfere with any of the peaks of interest.

Peaks in the chromatograms of fresh LAB and CON flask extracts were identified as being identical by a library search, and by examining the mass spectra directly. Peaks in the experimental flasks were identified by comparison to the CON chromatograms, with particular attention to retention times.

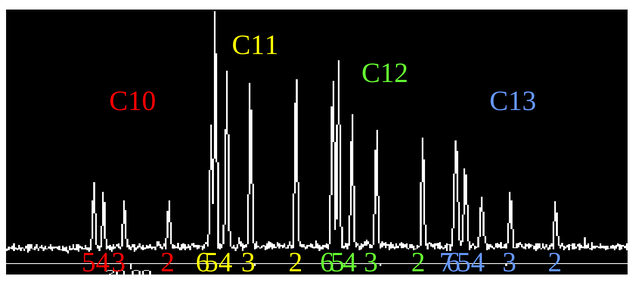


Figure 2. Typical GC of fresh LAB cable oil. Annotated with alkyl chain length and position of phenyl group.

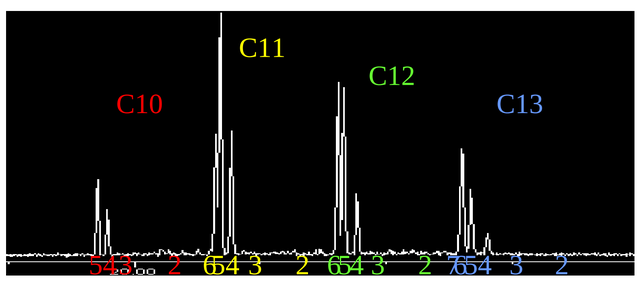


Figure 3. GC of LAB cable oil exposed to nitrate as a TEA (after 13 weeks).

Results and Discussion

Many, though not all, flasks in each of the anaerobic treatments displayed microbial growth. This, with the long lag phase, suggests that the inoculum contained rather low numbers of viable cable-oil degrading organisms. Growth took the form of a cream-coloured pellicle at the oil/water interface. The SUL and MET flasks showed similar, though less marked growth than the ANA and NIT treatments.

As expected, the AER flasks all showed growth, but there was not the distinct pellicle seen in the anaerobic cultures. Rather, the oil dispersed into sub-millimetre globules. It is likely that this was a result of the production of biosurfactants by aerobic CDMs, since the oil in the CON flasks, exposed to the same shaking regime, but not inoculated, did not become dispersed.

The volume of oil recovered from the flasks is plotted in Figure 4. The rather low recovery rate from the CON flasks may be due to volatilisation, or sorption mechanisms.

The most commonly used indicator of LAB degradation in environmental samples (e.g. for LAB residues in aquatic sediments, used as a marker for contamination with LAS detergents) is the ratio between “internal” and “external” isomers of C12 LAB (Equation 1), and this has been adopted for this study. The variation in I:E ratio in flasks from each treatment is shown in Figure 5.

$$I:E = \frac{[6C12 + 5C12]}{[4C12 + 3C12 + 2C12]}$$

Equation 1. Definition of I:E isomeric ratio (Naming convention nCm*, where* n = *position of benzene ring;* m = *length of alkyl chain).*

The LAB and I:E data represent the mean of three flasks from each treatment harvested per time point. No distinction was made between flasks with visible growth and those without, so there is a relatively large error (not shown). However, the data are consistent with biodegradation of LAB in the AER, ANA and NIT flasks.

Nitrate data (not shown) are inconclusive, but nitrite analysis indicates that nitrite is produced in the NIT flasks. Interestingly, the same is not true in the ANA flasks and it has been suggested that molybdate, added to the NIT flasks to inhibit sulphate reduction, may interfere with the reduction of nitrite to gaseous nitrogen.

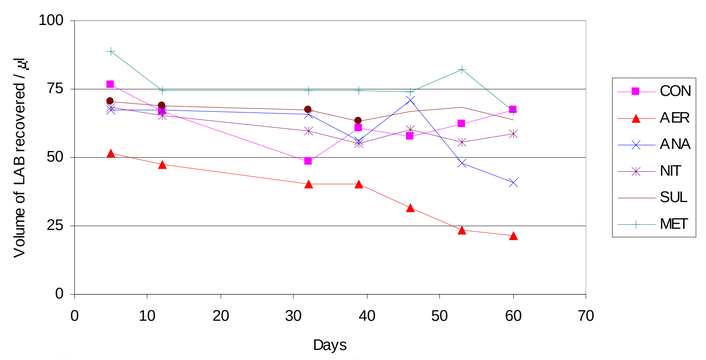


Figure 4. Volume of LAB recovered from flasks containing different TEAs.

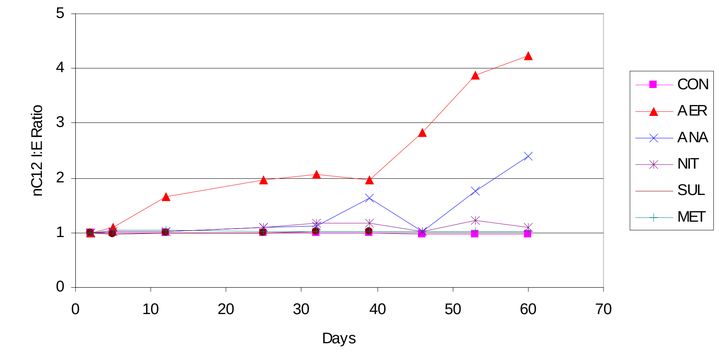


Figure 5. Variation in n*C12 I:E ratio in LAB exposed to different TEAs.*

It is likely that growth in the SUL and MET flasks uses nitrate present in the basic Bushnell-Haas medium as a TEA, though sulphate reduction and methanogenesis have not been ruled out.

No breakdown products have yet been identified. This may be because the LAB is degraded within single cells, or that breakdown products are water-soluble and so not extracted into the *n*-hexane. Samples of the aqueous phase have been retained for further study.

The experiment continues, but the data so far are strongly suggestive of LAB utilisation as a carbon and energy source by facultative anaerobes, using nitrate as a terminal electron acceptor.

Acknowledgements

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Reference

Johnson, S.J., Barry, D.A., Christofi, N., and Patel, D. (2001) Potential for anaerobic biodegradation of linear alkylbenzene cable oils: Literature review and preliminary investigation. Land Contamination & Reclamation ***9****, 269-291.*

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