Bio Tube - Full AEA Report

Initial results from BIO Tube trials for the Environmental Protection Agency

We have recently been involved in the initial trials carried out by AEA Technology for the Environmental Protection Agency involving Biotechnics Bio Tubes and products from other manufacturers developed to carry out the bioremedation of oil in industrial oil water separators.

The initial trials involved testing our product in comprehensive bottle tests and the results look very promising.

Test method and results follow:

1. FLASK SET-UP

For each product tested, a total of 30 flasks were set-up as follows:

- 6 killed controls (200 ml), 3 with waste engine oil and 3 with fresh diesel;
- 18 test flasks (200 ml), 9 with waste engine oil and 9 with fresh diesel;
- 6 "Microtox™" control flasks (120 ml), 3 with waste engine oil and 3 with fresh diesel.

A rain water simulant was used as the liquid phase in all the flasks. The composition of the rain water simulant is presented below:

- Sodium carbonate at 0.142 mmol/l
- Sodium chloride at 0.600 mmol/l
- Sodium nitrate at 0.016 mmol/l
- Sodium sulphate at 0.096 mmol/l

Both the waste engine oil and the fresh diesel were placed on a filter paper and added to the flasks. This prevented the contaminant from adhering to the sides of the flasks and thereby avoided limiting the surface area available for biodegradation. In the killed controls and the test flasks the amount of either waste engine oil or fresh diesel was 50 mg in a final liquid phase volume of 200 ml. In the "Microtox™" controls 30 mg of either waste engine oil or fresh diesel were added to the flasks on a filter paper, in a final volume of liquid phase of 120 ml. In the case of

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Bio Tubes (Biotechnics product) the contaminant was not added to a filter paper but rather to the surface of the liquid phase allowing the oil sorbent present in the products to play its role.

Sterility of the killed controls was obtained by adding mercuric chloride to the flasks at a final concentration of 300 mg/l. These were designed to enable an estimation of any changes in the absence of biological reactions.

Both the killed controls and the test flasks were incubated at 20ºC in a temperature controlled orbital incubator at 100 rpm.

The "Microtox™" controls were designed to provide a "killed control" for the Microtox™ acute toxicity test (see Section 2.5.1.4). The mercuric chloride killed controls could not be used for this testing since the mercuric chloride itself is highly toxic. Therefore, inhibition of biodegradation was achieved by flushing the flask headspace with nitrogen gas before incubation in an anaerobic jar at 20ºC without agitation. The purpose of this was to reduce the biodegradative events to a minimum by removing oxygen, an essential ingredient of the main oil degradation process.

2. BIOTECHNICS MATERIALS

Bio Tubes product exists as fibres of oil sorbent, micro-organisms and nutrients (AEAT-3801). For this trial the manufacturer supplied a sachet of dry bacterial cultures and nutrients, and two types of blown polypropylene absorbents, a "fibrous" and a "floc" type. Following the manufacturer's advice, the oil absorbent fillings were added to cover only part of the surface of the liquid phase. To cover half of the liquid phase surface, 0.45 g of the "floc" sorbent and 0.20 g of the "fibrous" sorbent were placed in the killed controls and test flasks. In the "Microtox™" controls, 0.20 g of the "floc" sorbent and 0.10 g of the "fibrous" sorbent were added.

The mix of dried bacterial cultures and nutrients provided was rehydrated prior to its addition to the all flasks at a final dilution of 1/900.

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3. SAMPLING STRATEGY AND ANALYSES

For each product tested the 30 flasks were divided in 3 lots as follows:

- Those analysed on Day 1:
  - 2 killed controls, 1 with waste engine oil and 1 with fresh diesel
  - 6 test flasks, 3 with waste engine oil and 3 with fresh diesel
  - 2 "Microtox™" controls, 1 with waste engine oil and 1 with fresh diesel

- Those analysed on Day 7:
  - 2 killed controls, 1 with waste engine oil and 1 with fresh diesel
  - 6 test flasks, 3 with waste engine oil and 3 with fresh diesel
  - 2 "Microtox™" controls, 1 with waste engine oil and 1 with fresh diesel

- Those analysed on Day 28:
  - 2 killed controls, 1 with waste engine oil and 1 with fresh diesel
  - 6 test flasks, 3 with waste engine oil and 3 with fresh diesel
  - 2 "Microtox™" controls, 1 with waste engine oil and 1 with fresh diesel

The sampling strategy on these sets of flasks is detailed in Table 1.

Set of flasks	Flask denomination	Analysis
Day 1	Killed controls	Total CO_2 in all flasks COD in all flasks Oil chemistry in 2 flasks (1 waste, 1 diesel)
	Test flasks	Total CO_2 in all flasks COD in all flasks Oil chemistry in 2 flasks (1 waste, 1 diesel) Microtox™ in 2 flasks (1 waste, 1 diesel)
	"Microtox™" controls	Microtox™ in all flasks

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Day 7	Killed controls	Total CO_2 in all flasks COD in all flasks Oil chemistry in 2 flasks (1 waste, 1 diesel)
	Test flasks	Total CO_2 in all flasks Oil chemistry in 2 flasks (1 waste, 1 diesel) Microtox™ in 2 flasks (1 waste, 1 diesel)
	"Microtox™" controls	Microtox™ in all flasks
Day 28	Killed controls	Total CO_2 in all flasks COD in all flasks Oil chemistry in 2 flasks (1 waste, 1 diesel)
	Test flasks	Total CO_2 in all flasks COD in all flasks Oil chemistry in 2 flasks (1 waste, 1 diesel) Microtox™ in 2 flasks (1 waste, 1 diesel)
	"Microtox™" controls	Microtox™ in all flasks

The carbon dioxide concentrations in the flask headspace were measured using a Perkin Elmer 8500 gas chromatograph. The method used a stainless steel column 72.6 cm long packed with Chromosorb 102, 60/80 mesh (Supelco). The oven temperature was 100 °C and the injector temperature was set to 250 °C. The detector was set to 'medium' and the detector temperature was set at 175 °C. The mobile phase was Helium. In each case, prior to gas chromatography, the instrument was calibrated with carbon dioxide standards at appropriate concentrations. The volume of headspace gas injected at each time point was 1000 µl.

For the Total CO_2 measurements, the liquid phase was acidified to pH<3 by addition of concentrated hydrochloric acid and allowed to equilibrate at 20ºC, 100 rpm for an hour before measurement. This method brings the equilibrium constant for the distribution of carbon dioxide between the liquid and gas phases in the flasks to 1, allowing determination of the total carbon dioxide concentration in the flasks.

The COD was determined on 10 ml samples of the liquid phase by the Analytical Service Group of AEA Technology plc. This was to determine if the biological processes increased the oxygen demand of the water. Such an event at full scale could be problematic to receiving waters and may form the basis of consents.

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The Extractable Organic Matter (EOM), GC derived alkanes (C_{11} to C_{35}), Total Petroleum Hydrocarbon (TPH), GC-MS derived selected aromatics and hopane quantification were determined by the Fossil Fuels and Geochemistry division of the University of Newcastle Upon Tyne. The results of these analyses will provide information on the extent of the hydrocarbon biodegradation in the flasks in support of the information obtained from the carbon dioxide measurements.

The Microtox™ acute toxicity basic test on liquid phase samples was determined by CHEMEX International plc. The results of this test will show whether the promotion of hydrocarbon degradation by the different products will increase the toxicity of the liquid phase towards the microbial community of the receiving waters. This is a further measure of potential impacts on receiving waters quality and may be used for the basis of a consent.

4. RESULTS

4.1 Oil chemistry and CO_2 results

4.2 COD results

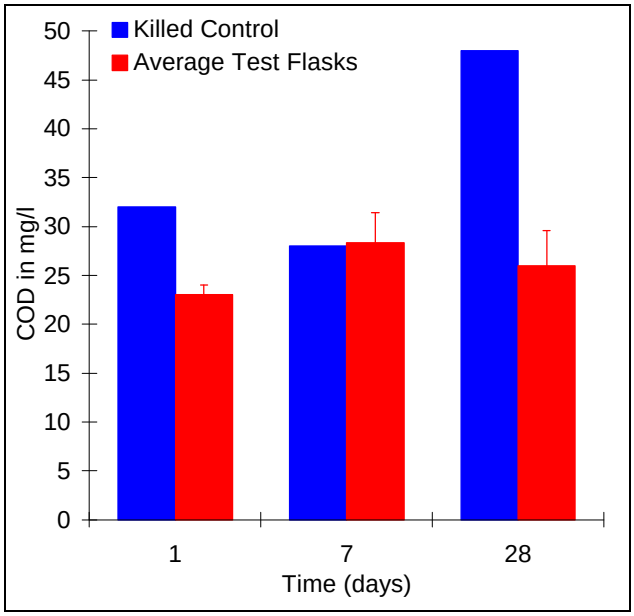

Figure 1. Chemical oxygen demand in the liquid phase for the waste engine oil

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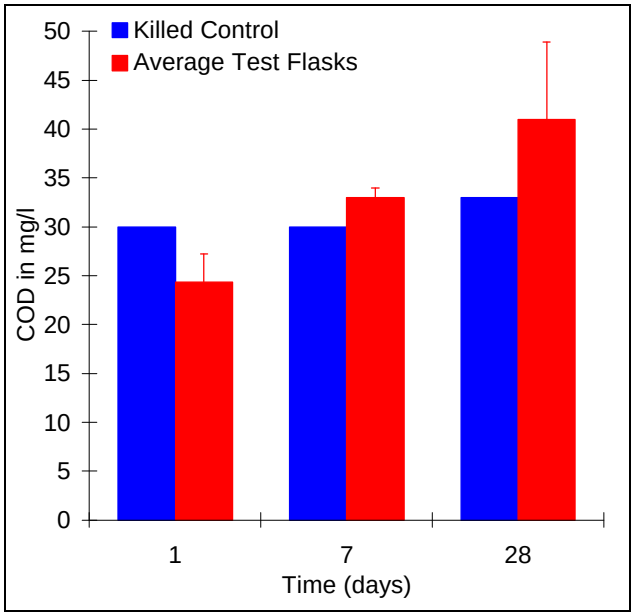

Figure 2. Chemical oxygen demand in the liquid phase for the fresh diesel

4.3 Microtox results

The results are expressed as the percentage of diluant added to the sample to produce the test solution that induces a 50% reduction of the light emitted by the test bacteria (EC_{50}). Therefore, the higher the percentage of diluant in the sample, the higher the toxicity is.

The Microtox™ control was not agitated in the same manner as the test flasks. This has an impact on the processes of release and evaporation of compounds from the filter and thus on the toxicity. Therefore, comparisons between controls and tests can only be viewed as qualitative. However, the evolution of toxicity in controls and tests can be compared.

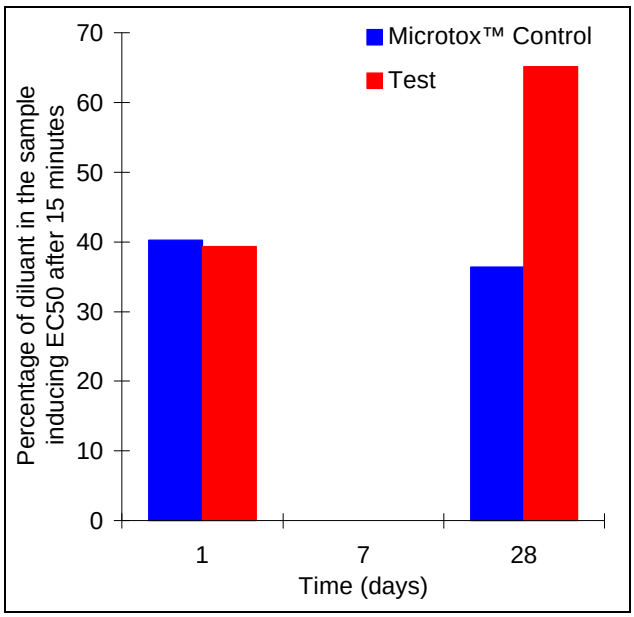

Figure 3. Toxicity of the liquid phase for the waste engine oil

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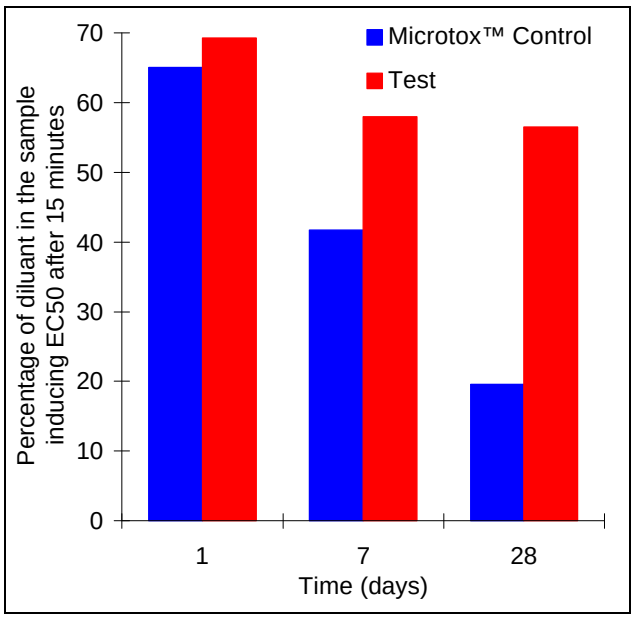

Figure 4. Toxicity of the liquid phase for the fresh diesel

5. SUMMARY

The tests demonstrated that none of the bioremediation products were harmful or toxic to the environment.

The next phase of the project will be field trials which will involve testing the Bio Tubes in field separators. These trials are due to start in May.

We will let you have the results of the field trials when they become available.

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