Oil Retention and Microbial Ecology in Porous Pavement Structures

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Introduction

Traditionally, stormwater runoff from impermeable surfaces has been intercepted and discharged swiftly to sewer systems and watercourses. Rapid growth of urban and industrial areas has resulted in an associated increase in impermeable surfaces such as roofs, highways and paved surfaces. This continual expansion and infilling is placing an increased burden on existing drainage networks and urban watercourses. During periods of heavy rain, large volumes of runoff may exceed the capacity of sewer systems, resulting in risks of flooding to property and to human health. In addition, pollutants deposited on impermeable surfaces may be entrained by stormwater flow, concentrated within sewer systems, and discharged to aquatic ecosystems with little or no treatment. Urban stormwater can contain toxins, such as heavy metals, oil and other hydrocarbons, whilst average levels of suspended solids may exceed that of untreated sewage .The majority of pollution in urban stormwater originates from non-point or diffuse sources. These are notoriously difficult to locate and quantify and may include wet and dry atmospheric deposition from industrial and domestic properties; traffic emissions; decomposed litter; de-icing salts; vegetative residues; pet faeces; and soil losses.

The construction of permeable highways as an alternative to impermeable surfaces has been shown to be an effective method of stormwater source control. The main design criterion for infiltration systems has usually been the reduction of peak discharge through the retention of stormwater flow. To date although some effort has been directed towards the use of such systems for the treatment of retained pollutants there has been little thought put into gaining a fundamental understanding of the microbiological processes taking place. Previous research has demonstrated the ability of a permeable pavement to retain suspended solids (Pratt *et al*,1996).

In situ bio-remediation (microbial degradation) has been shown to be a potent technique for the breakdown of contaminants into less harmful forms, particularly in the fields of contaminated land remediation and oil-spill clean up. Research initiated at

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Coventry University has been targeted towards using this technology to degrade petroleum-derived hydrocarbons within the sub-base of a full scale, laboratory model permeable pavement structure (PPS) (Pratt *et al*, 1996, 1999).

To date, studies have indicated that the use of the permeable pavement structure as an *in situ* aerobic bio-reactor for the breakdown of petroleum-derived hydrocarbons is feasible. Flourishing microbial populations have been established for a period of approximately 30 days after dosing laboratory model pavement structures with high loadings of mineral oil, previously seeded with commercially available microbe and nutrient mixes (Brownstein, 1997) but it was the work of Bond *et al* (1999) which first showed that by replacement of liquid fertiliser with a readily available horticultural slow release fertiliser one could stabilise the microbial population and thus use as a long term treatment device was viable. The system can also, with modification, be used as a storage element for re-use of roof water etc.

It is hoped that the system will offer a long-term solution for the reduction of automobile-derived hydrocarbons on urban paved surfaces, which are washed into watercourses untreated via separate, storm sewers and perhaps more importantly allow the regulators to accept that where geological conditions permit the infiltration of water from large parking surfaces can be done without threat to the environment.

The aim of the ongoing research described in this paper is threefold:

1) To determine how much oil can be retained by a non-respiring PPS structure and thus produce targets for the required rate of degradation to achieve no breakthrough during the lifetime of a structure.

2) To investigate whether a microbial population, subjected to chronic, low level, hydrocarbon contamination will persist long-term within the structure under simulated field conditions. Research has concentrated on the effects on microbial survival of nutrient and water availability within the structure and their means of supply.

3) To develop methods by which the microbial ecology of the structure could be better understood with the aim of enhancing the performance of the structure even further. In this case research has been aimed at both biochemical and molecular biological approaches and it is the latter which is reported here.

Experimental

Determination of oil and grease

Throughout this study analysis of oil and grease in effluents were performed to American Society for Testing and Materials method D3921-85 (ASTM, 1985) modified as previously described. The average recovery for this technique was found to be 84.85% (SD 5.75, n=12), and all results have been recovery-corrected.

Non-biologically-mediated retention studies

The aim here was to study structures to find breakthrough capacity and compare them with traditional parking surface materials. Models of a PPS based on the design of Pratt (Brownstein, 1995; Pratt *et al*, 1996, 1999) The pavement comprised pre-formed concrete blocks of a design known as CeePy blocks, and used previously, bedded on clean pea gravel, with vertical drainage provided through gravel filled inlets between the blocks (Figure 1). A geotextile membrane (Exxon Terram 1000) separated the block bed from the underlying sub-base, comprising 400 mm depth of washed 20-50 mm granite. The entire structure rested on an additional geotextile underlay, supported by a stainless steel mesh. The structure was set up in steel/aluminium boxes having a plane area of 400mm x 400 mm and a height of 600 mm. Each box had an open bottom with a 300 mm deep pyramidal funnel for the collection of the effluent samples. At the funnel outlet, a tap was fixed, preventing unintentional effluent loss. Only a 310mm x 310mm area of the surface was used for oil application.

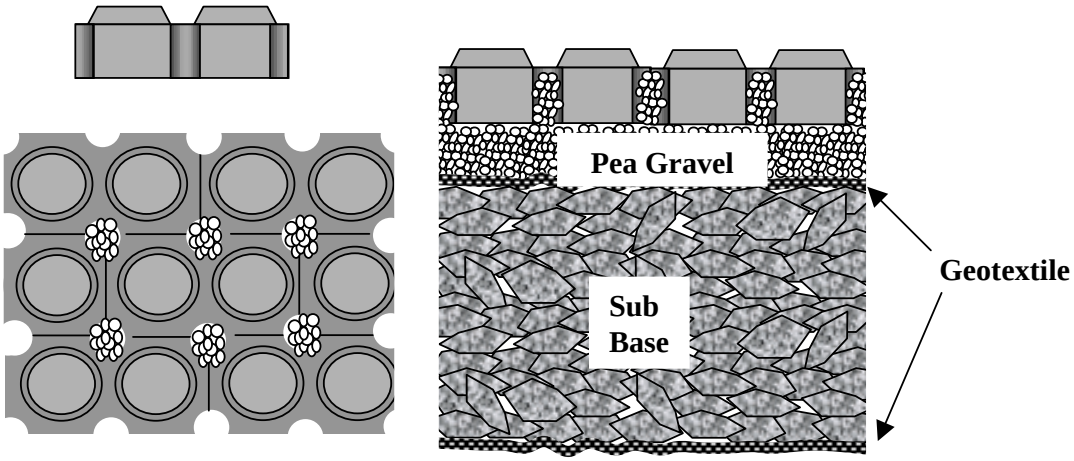

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Figure 1 General arrangement of the porous parking surface

It was intended here to study retention and not degradation of oil. Previous work (Bond *et al* 1999) had shown that without addition of inorganic nutrients significant degradation did not occur. The rig materials were washed before assembly and no inorganic nutrients were provided. Provision was made to manually remove gas samples from the interior of the models by means of a syringe to check for elevated carbon dioxide within the rigs. This would have indicated that significant microbial activity was taking place. In fact no elevated levels were detected during the experiment indicating that nutrient starvation had been successful in inhibiting biological activity.

Two typical highway materials, asphalt and concrete, were chosen for investigation alongside the PPS. Eight identical aluminium trays were manufactured (310mm x 310 mm plan area, 50mm deep) from 1 mm thick aluminium sheet. On one side of each tray, a 310 mm wide effluent channel was fixed. This channel was used to collect the effluent from the trays and direct it to the collection bottles.

Mature asphalt blocks from a redundant area of pathway, not previously exposed to oil were cut into rectangular pieces. The asphalt blocks, supported on a suitably thick layer of sharp sand were placed inside the trays as close as possible to their front sides. The slight gaps between the asphalt structures and the sides of the aluminium trays were filled with a sharp sand and finally sealed with multi-purpose silicone sealant. Four sets of this apparatus were constructed

For the concrete experiments grade C40 concrete was chosen. This was cast directly into the aluminium trays

These structures were used as a subject of 63-day study (Newman *et al* 1998) where a total of only 320σl of oil were added to each structure. Whilst it was possible to show that, when exposed to relatively low additions of oil the retention capability of the PPS was excellent compared to the traditional structures. Because of the fact that the PPS systems were nowhere near saturation and that new concrete may behave differently to old concrete, it was decided to carry out this high-loading experiment after the concrete had had a period of maturation.

Rainfall events and oil addition

For a nine-week period, three rainfall events per week were simulated (using a device previously described by Bond *et al* (1999)) on each structure. The rainfall intensity was set at 13 mm/h for duration of approximately 28 minutes, applying an amount of 263.5 ml distilled water to each pavement per rain event.

Oil was applied (simulating crank-case leakage) to each structure (except the controls, one per structure type) by means of previously calibrated oil drippers described previously (Newman *et al* 1998) using drippers placed randomly using two throws of a 6 sided die to identify the co-ordinates for placement. The amount of oil added per event was increased in a stepwise manner (starting at 0.8ml per oil application and increasing to 20ml) over the experiment in an attempt to try to reach saturation potential of the PPS in a reasonable period. Oil was applied on the morning prior to each rain event.

All effluents were analysed for oil and grease using the method described above. The results are presented as a plot of cumulative mass of oil and grease added against cumulative mass of oil and grease detected in effluent over the days of the experiment (mean values of all replicates) for all structures. The data is also presented as a table giving the total proportion of oil and grease retained for each structure type over the experimental period. The additions made previously in the study by Newman *et al* (1998) are included in this data.

Ongoing long-term bioremediation study

Although, as will be seen below in table 1 and figure 3, the structure will retain oil for effectively it is clear that the system will eventually either saturate with oil or clog due to oil deposits on the geotextile. The aim of encouraging bioremediation of the oil *in situ* is to prevent this situation occurring before the end of the 15 year design life of the structure.

This experiment was an extension of previous studies reported by the group (Pratt et al 1996,1999). The details of the experiments are thus covered only briefly. The aim was to study the long term behaviour of a model PPS system. A representative section of the pavement was constructed as described above (except that the depth of sub base was 600mm and the plan area was 600mm x 600mm) in a steel/glass box.

In this study the oxygen and carbon dioxide in the air spaces within the model were measured at the pea gravel bed and sub base levels, respectively. Concentrations were measured on a cyclic basis as previously described (Bond *et al*,1999). The data presented below in figure 5 is the sub-base data which has been smoothed according to the method described by Bond (1999).

Lubricating oil additions of approximately 3.3g per week were applied except during periods of holidays and staff changeover. The upper line in figure 6 shows the actual rates at which oil was applied during the period of the experiment. Rainfall events at 1.6mm/hr were applied on average once every 3.5 days (approximating to the mean for London (Wallén 1970)) except during deliberate drought periods as discussed below. All effluents were analysed for oil and grease using the method described above. The results are presented as a plot of cumulative mass of oil and grease added and cumulative mass of oil and grease detected in effluent against time, over the whole 1205 days of the experiment.

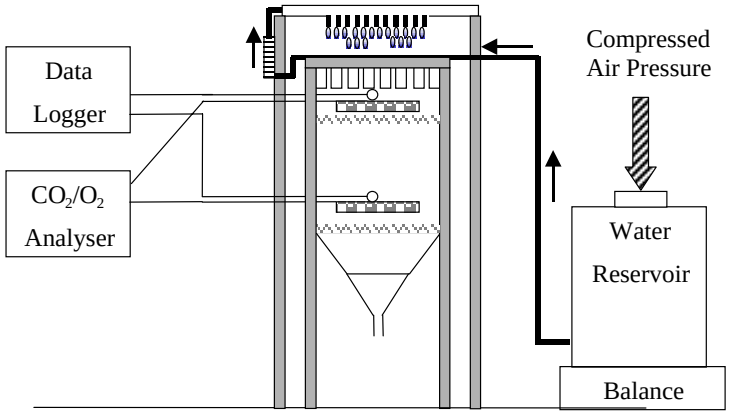

Figure 2. Rainfall simulation system and monitoring components.

Molecular Biology

The final part of this paper is a brief introduction to the attempts the group is making to understand the microbiological processes taking place in the structures. If we are to optimise the microbiological capabilities of the rig through changing it's design there are two approaches. The first is the one we have taken so far, an empirical approach in which we change parameters and measure responses. This is, by itself, an inefficient approach and it would be far better to understand the microbial ecology of

the system. The first step in understanding this is to know how many different species are present and ideally, what they are.

The problem with traditiona1 microbiological approaches is that many species of bacteria will not grow under laboratory conditions and thus the microbial diversity observed may seem simpler than it actually is. The aim of this initial experiment was to demonstrate whether it is possible to distinguish, if the bacterial community present in the effluent (and thus in the experimental rig itself) from a rig which has been operated for over 1200 days is different from the bacterial community in the commercially available seed which was used to inoculate the rig and initiate biodegradation.

The Polymerase Chain Reaction (PCR) was used to amplify the 16s ribosomal RNA gene (Giovannoni *et al*, 1990) from DNA from cells collected from the effluent from the test rig and from the original innoculum. This technique although simple in principle is very complicated in practice since each different sample type requires optimisation for a number of parameters. First of all the correct DNA extraction procedure (Bead beating (BB) and Freeze Thawing (FT); Bateson and Ward, 1995) must be chosen.

Then depending on the type of information required a decision must be made on the types of primers to use. These short lengths of single stranded DNA are used as starting and finishing points for the part of the DNA to be amplified. After selecting the primers the temperature and conditions for the multiplication by the PCR reaction is also critical. For these experiments 'universal' ITS Primers, 16S-Primers and Primers for a fragment of the 16s region have been chosen. A suitable set of conditions for the separation and visualisation of the PCR product originating from each bacterial species must be chosen using Agarose Gel-Electrophoresis (Sambrook *et al*, 1989). This was the work carried out in this part of the project.

Results and Discussion

Retention Experiment

The results of the retention experiment are shown graphically in figure 3 below. It is clear that the PPS is considerably more retentive of the oil than the traditional structures.

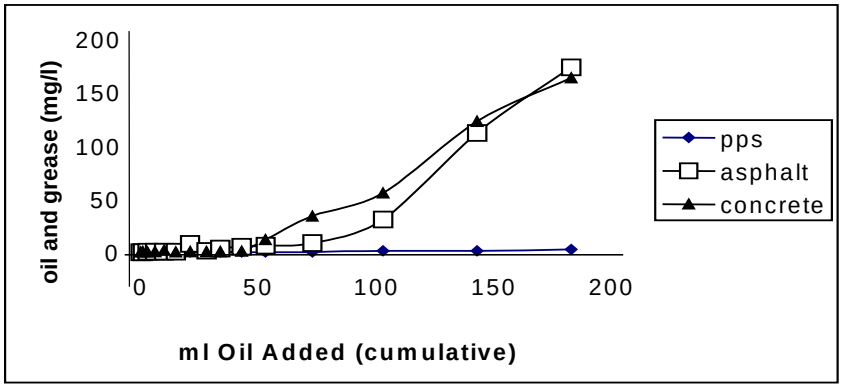

Fig 3 Graph of cumulative volume of oil added against concentration of oil released for PPS, asphalt and concrete (means of three replicates) Horizontal graph axis set at y= –5mg/l for clarity.

Clearly the traditional structures release considerably more oil than the PPS. Since the PPS line is so close to the horizontal axis that it seems almost flat the data was re-
This data is plotted as figure 4 below on a larger scale for the PPS only. If we take an arbitrary figure of 0.25mg/l oil and grease in the effluent as defining significant breakthrough **(**Balades *et al*, 1995) we can see that breakthrough occurs at about 100ml of added oil. This corresponds to an oil application of roughly 500ml per m^2. At the point where 100ml of oil had been added to the traditional structures the concrete and asphalt structure effluents were approximately 25mg/l and 50mg/l respectively. At the end of the experiment after the addition of 183ml of oil the concentration in the effluents from the asphalt and concrete were in the region of 150mg/l oil and grease with free oil clearly present in the samples. At this point the concentration in the PPS effluent was less than 1mg/l.

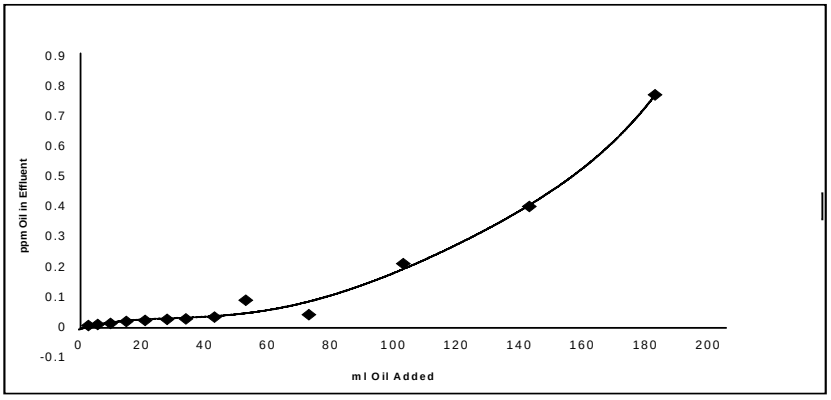

Fig 4 Graph of cumulative volume of oil added against concentration of oil released for PPS only

The total proportions of oil and grease relaesed from the structure as a percentage of the total 183ml applied in the 9 weeks was as shown in table 1.

Structure Type	**Mean% Retained**
Porous Pavement	99.6 %
Asphalt	49.6%
Concrete	70.2%

Table 1 Oil retention capability of test structures

The retention capabilities of the PPS structure was thus very impressive, sufficient to indicate that the system would, at least in the short term, be well capable of protecting either ground or surface waters into which the PPS drainage is permitted to discharge.

The next important question is whether or not we can biodegrade the oil in the structure at a sufficiently fast rate to prevent the retention properties of the PPS being overloaded. Following initial experiments in which the best commercially available fertiliser was found to be Osmocote Plus, a horticultural fertiliser which was developed from the fertiliser used to bioremediate crude petroleum contamination in the Exon Valdis disaster, this was studied by Bond(1999) using sealed respirometer systems in a kinetic study in which it was estimated that oil could be broken down

sufficiently fast to ensure that at the normal rate at which oil drips onto a typical road surface the system would not saturate within the design life of the structure.

The remaining question was whether it was possible to maintain the biofilm within the structure for an extended period and whether, over the long term, the oil retaining properties of the structure would be maintained. The results of the first 1220 days of the experiment are shown in figures 6 & 7.

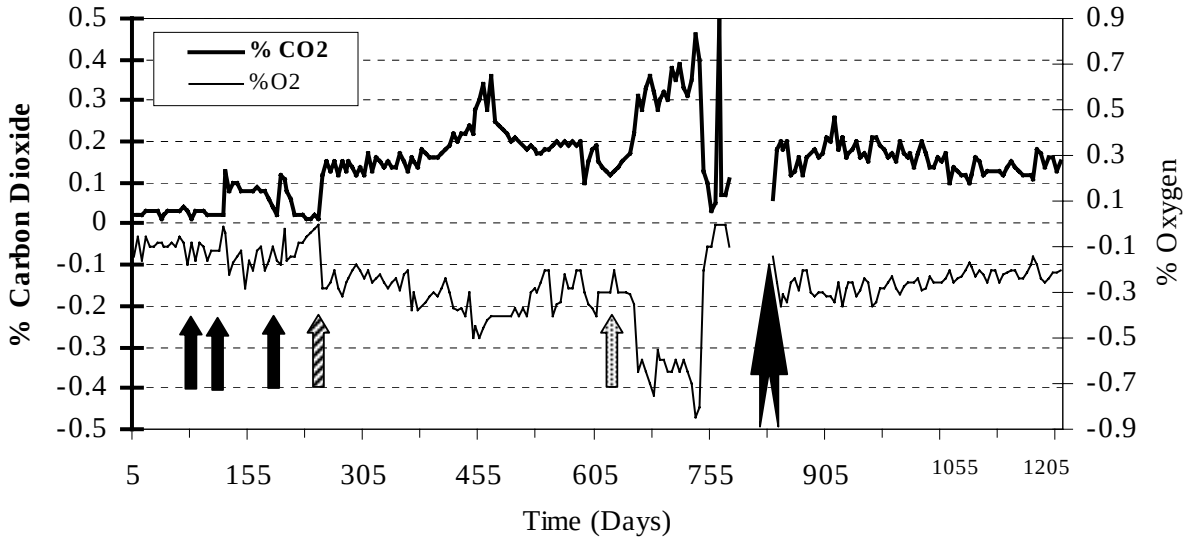

Fig 6 Gas monitoring results: Liquid additions ➡ Osmocote Addition ⇨
Replacement of Osmocote with Osmocote Plus ⇨ End Of Drought ➡

Figure 6 shows the results of monitoring carbon dioxide and oxygen in the sub base of the structure. From the date when the Osmocote was added there is an elevated carbon dioxide and reduced oxygen in the structure (this continues to the time of writing without addition of extra fertiliser). The rapid fall after day 755 was due to an artificially induced drought in the structure started on day 650 but even after over 150 days of drought the system recovered quickly when rain was again applied. The spike in carbon dioxide (not mirrored by a dip in oxygen), just after the drought, may have been due to an instrument malfunction or possibly due to a release of carbon dioxide from carbonates in the structure as dying microorganisms released acids when they decayed.. There is, unfortunately, a gap in the data which represents data loss from the computer just after the drought period. The two short duration initial peaks in the early days of the experiment were typical of previous observations when a liquid fertiliser is used, rapid increase in activity followed by rapid fall off until a fresh application is made. Oil applications were started on day 19 and a commercial bacterial seed was added on day 48 along with liquid fertiliser. Further liquid fertiliser applications were made on days 118 and 183 The slow release NPK fertiliser Osmocote was added on day 237 and the improved fertiliser Osmocote Plus which also contains trace elements was added on day 605

It can be seen that the activity reaches a peak about 200 days after slow release fertiliser application but that the fall off is very slow. A further, (higher) peak in activity is reached about 150 days after Osmocote Plus activation. But even after approximately 57 weeks with rain (i.e. ignoring the days of the drought) there is still an activity in the test rig which is as good as any which was achieved with the liquid fertiliser. It is estimated that the activity could be maintained at an acceptable level for at least 1 year for a single fertiliser application.

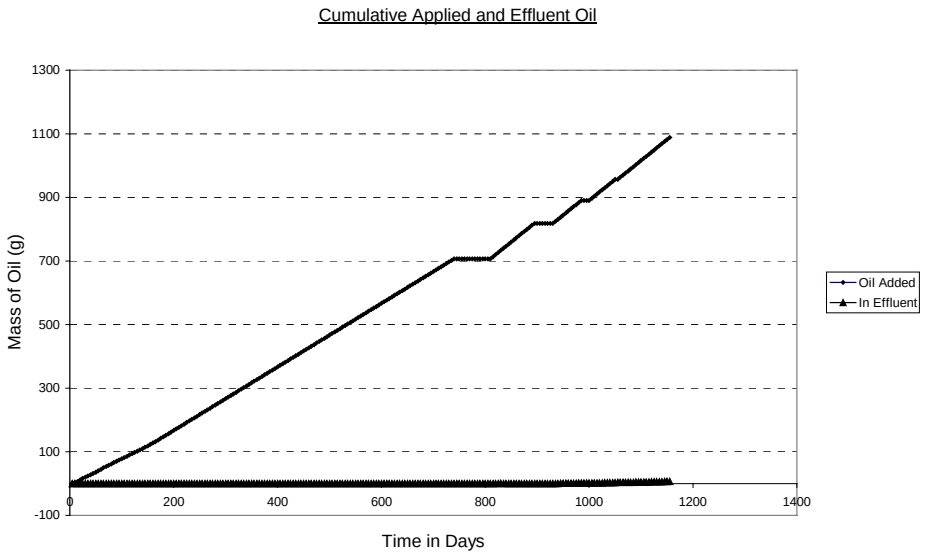

Fig 7 Graphs of cumulative mass of oil added (upper line) and released in effluent (lower line) For First 1150 days of experiment.

There is a gradual decline in the last 300 days shown and it is not known whether this is due to nutrient decline or some effect of the drought . Experiments are underway to determine this at the time of writing prior to repeating the drought experiment to replace the lost data.

Figure 7 shows that the proportion of oil released from the structure is still minute compared to the structure despite over 1 litre of oil now having been added.

Construction is currently underway for a large scale outdoor car park with gas monitoring data so that a long term study can be carried out in realistic conditions. The indoor model experiment is continuing and 1400 days of this experiment including details on microbial activity, nutrient release and microbial assemblages in the effluent will be reported at the Novatec Conference in Lyon in 2001 (Pratt *et al* 2001).

Molecular Biology.

Figure 8 below is as a photograph of PCR products after analysis by agarose gel electrophoresis. This is presented to illustrate the basic results obtained in this field so far.

Lanes 1 and 2 show the change of diversity over time in the long term rig. In this method each band represents a region of DNA between the ribosomal RNA genes which is free to vary by mutation. Therefore, in general each bacterium has a different sized gap between the genes and hence a different band size. The number of bands present indicates the number of dominant bacteria present in the sample. It can be seen that the effluent from the long term experimental rig has more DNA bands than the original innoculum. It is interesting to note how this technique clearly shows that by culturing the bacteria, the apparent diversity is reduced (see lanes 8 and 9 and 10 and 11). This indicates that, as we expected, traditional microbiology will be of little use in trying to understand the processes

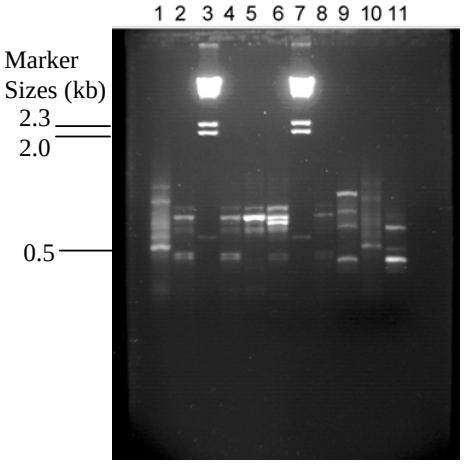

Figure:8
PCR amplification products for ITS Primers.

Lane 1: long term rig (BB);
Lane 2: innoculum (BB);
Lane 3: ƒç -*Hind*III marker;
Lane 4-6: innoculum (BB purified, BB, FT);
Lane 7: ƒç -*Hind*III marker;
Lane 8-9: innoculum (direct sample, cultivated sample);
Lane 10-11: long term rig (direct sample, cultivated sample).

occurring in the pavements. Lane 3, 4 and 5 compare the different DNA extractions. It was found that the “freeze thaw” method used here performed better than the “bead beating” method. The next stage in this work will be to take the process further to understand what bacteria are represented in each band and also to quantify the increase in diversity in the population over time using a variety of more advanced techniques. In this way a better picture of the microorganisms present and active in the rigs will be gained.

Conclusion

The work presented here confirms previous results indicating that porous pavements based on the CeePy block can be used successfully to both trap and biodegrade oil which is accidentally released onto parking surfaces. This is due to a combination of retention and biological breakdown both of which are required if the process is to be practicable. The techniques of molecular biology should be capable of enhancing our understanding of the microbial ecology so that the process can be optimised further. Meanwhile developers should be aware that the remarkable flood control properties of these systems can be used with the confidence that stormwater discharged from such surfaces to ground or surface waters will not cause pollution and thus lead to liability.

Acknowledgement

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