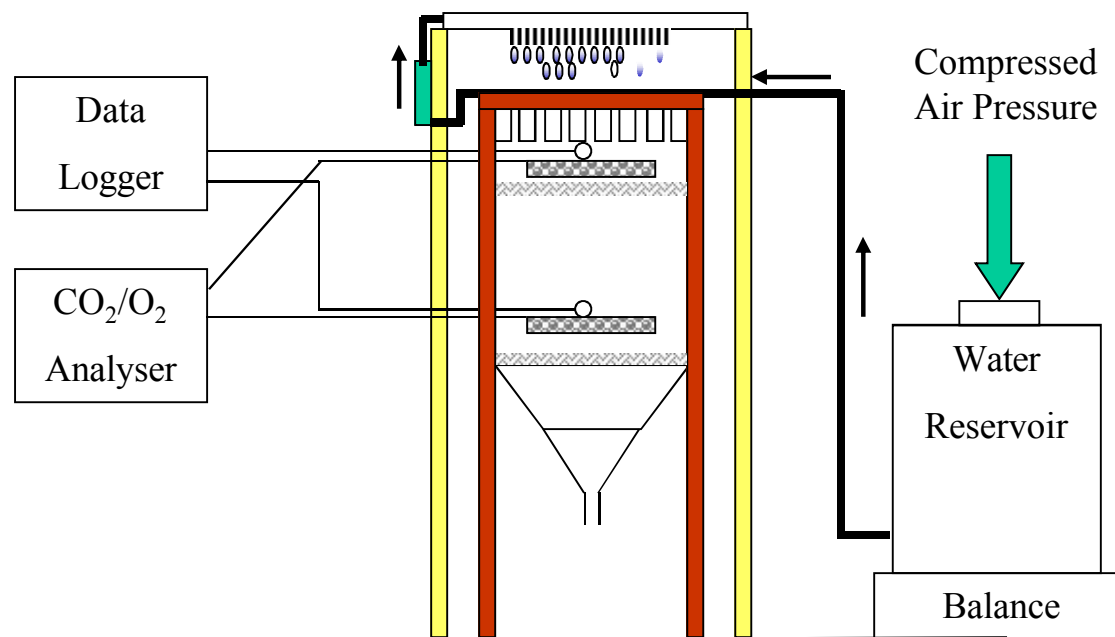




Pervious Pavements Coventry's Interdisciplinary Approach





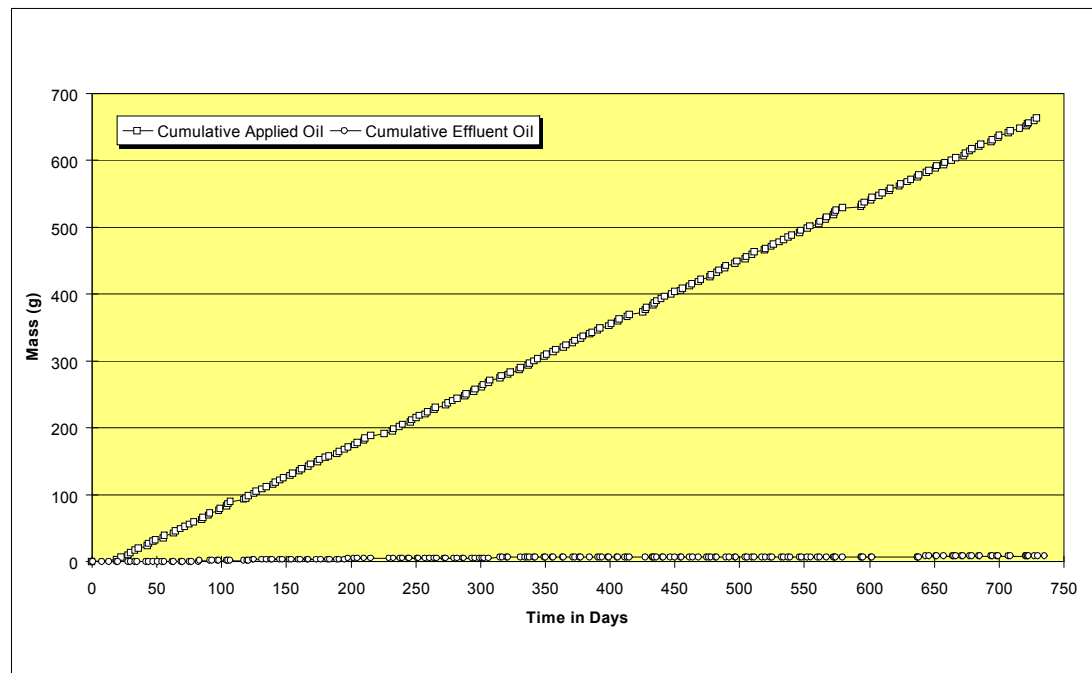
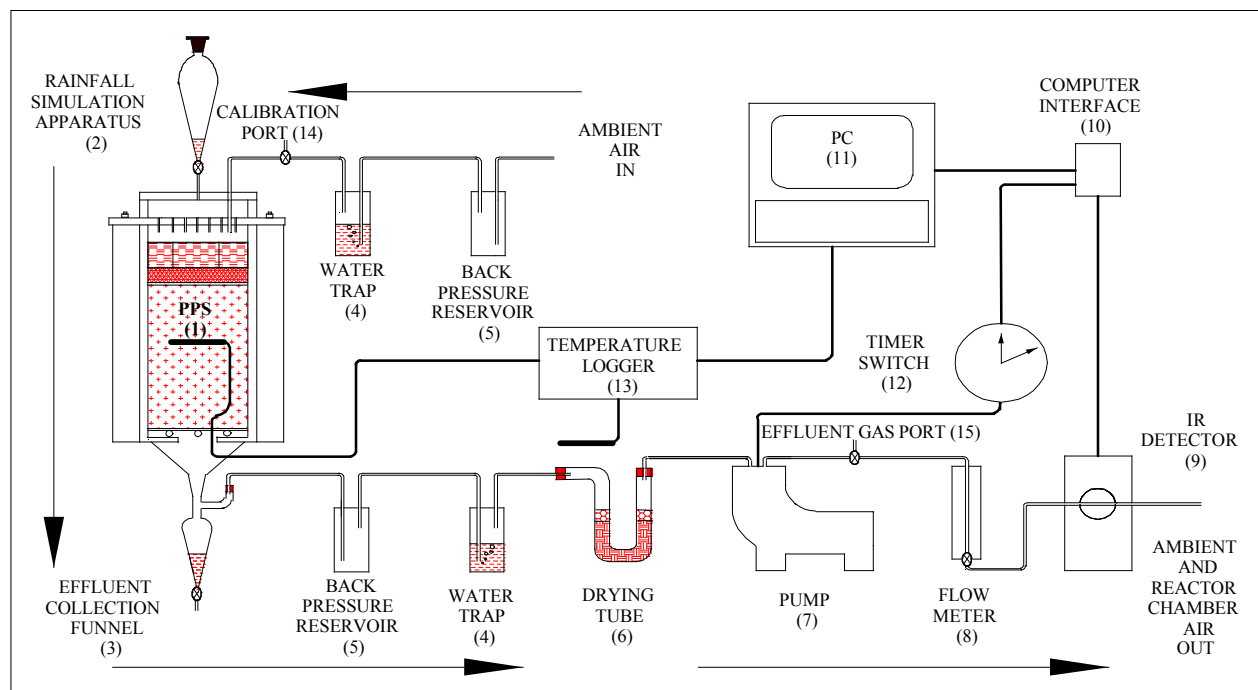
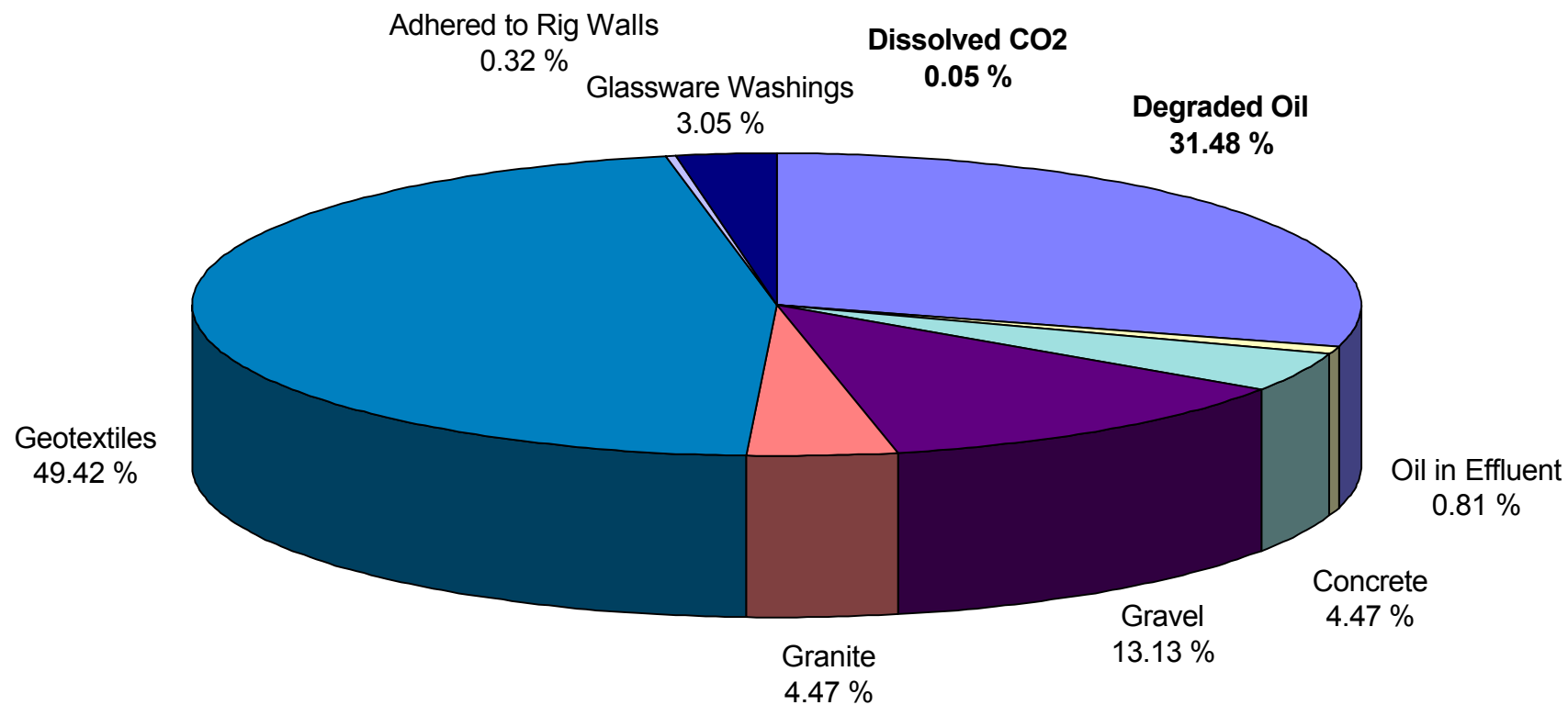


Figure 4.19. Cumulative Oil Application and Recovery Masses for Large Rig PPS



Small Rig Design for Total Carbon Mass Balance Studies: Principle of Operation



5550 mg Oil Added to Structure, Along with 1.522g of Osmocote Plus Fertilizer.
Rig Broken Down After 78 Days. Total of 1789 mg Oil Degraded (1747 mg if control subtracted).
Recovery = 107.22 % of added oil.

Fate Of Oil In The System

Large Spillages



Compacted sand bedding layer below installation



PERMAVOID™ Pad within geomembrane

Effect of 150 ml/m²



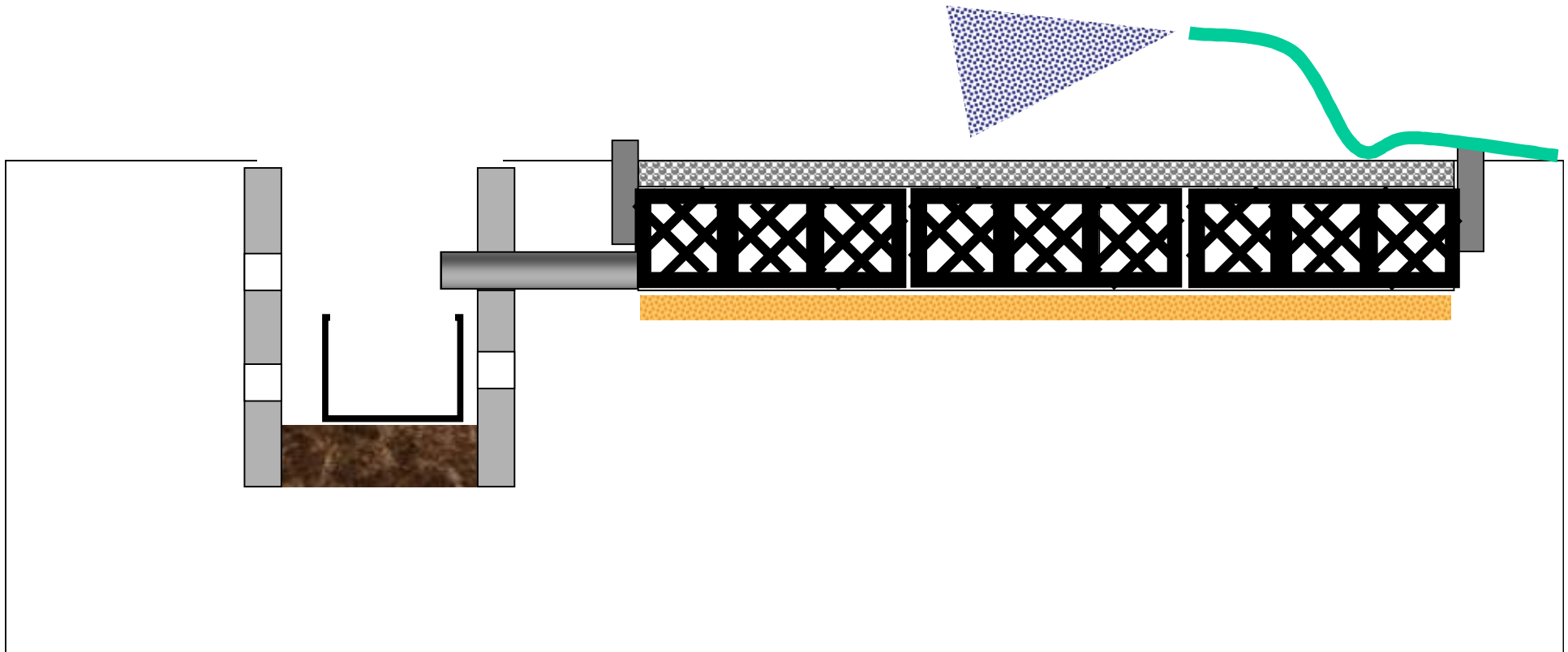
5 litre Experiment





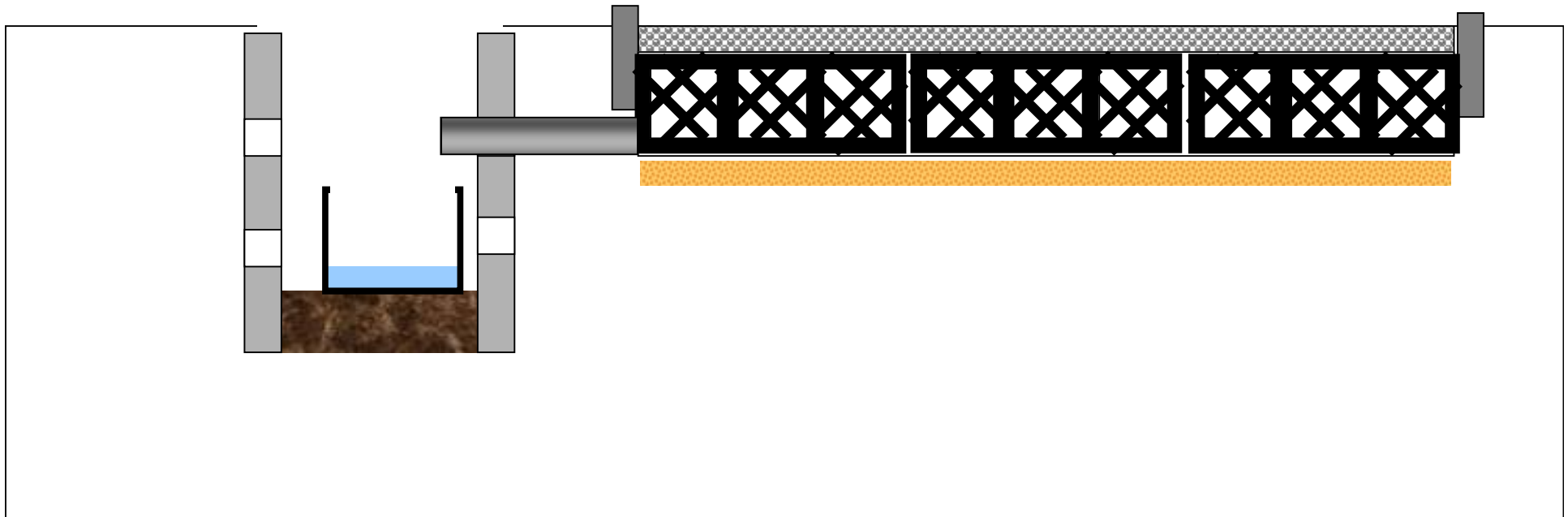
Experiment

13mm Rain Applied



Experiment

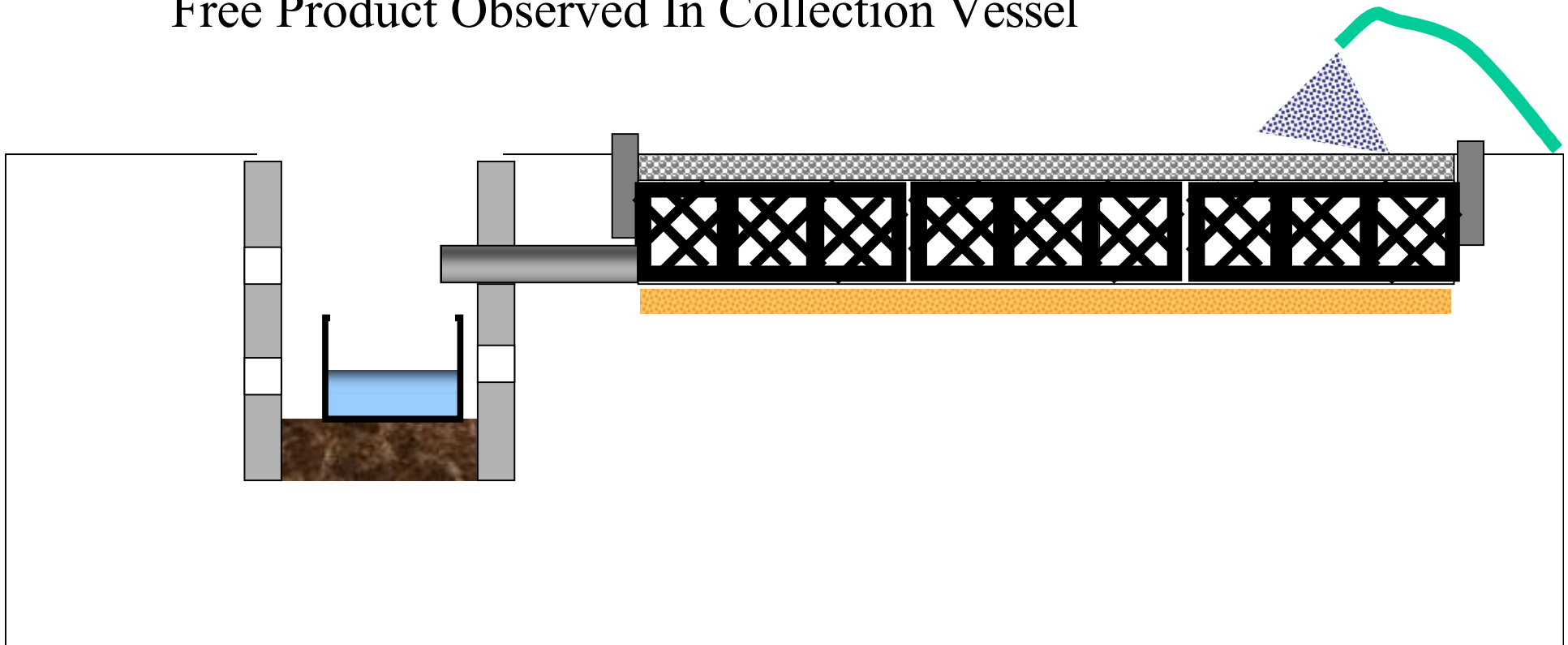
System allowed to drain overnight



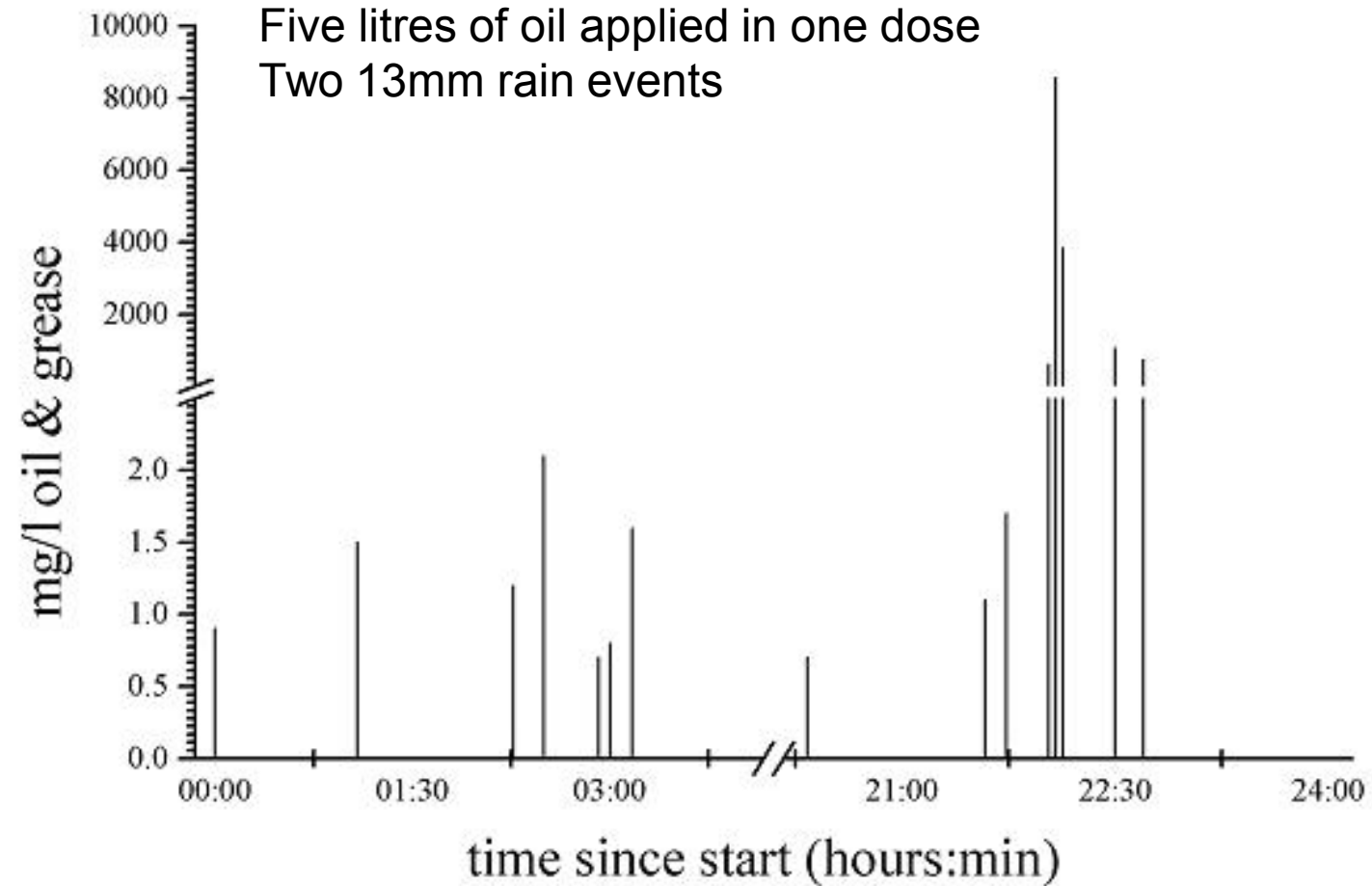
Experiment

Another 13mm “Rain”

Free Product Observed In Collection Vessel



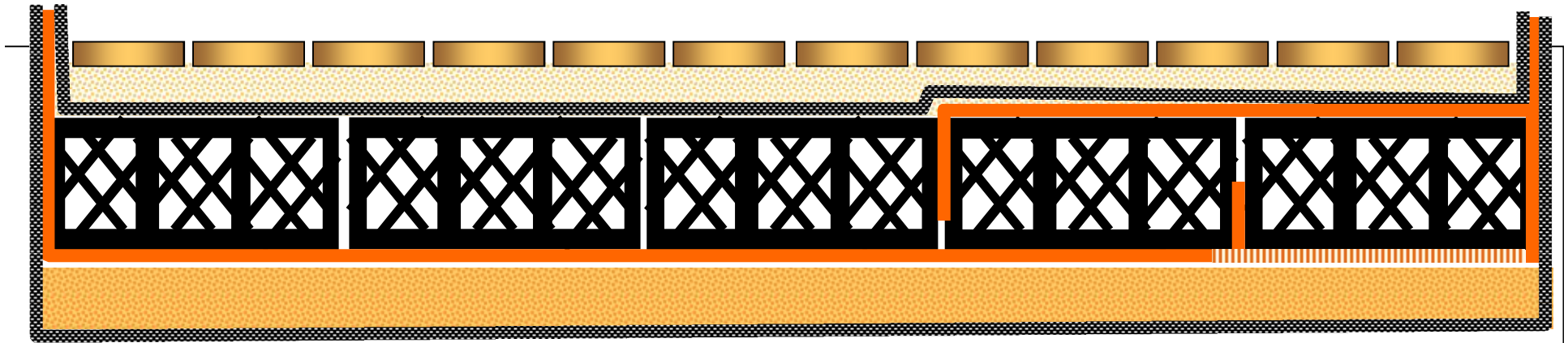
Four bay car park-pervious block paving
Five litres of oil applied in one dose
Two 13mm rain events



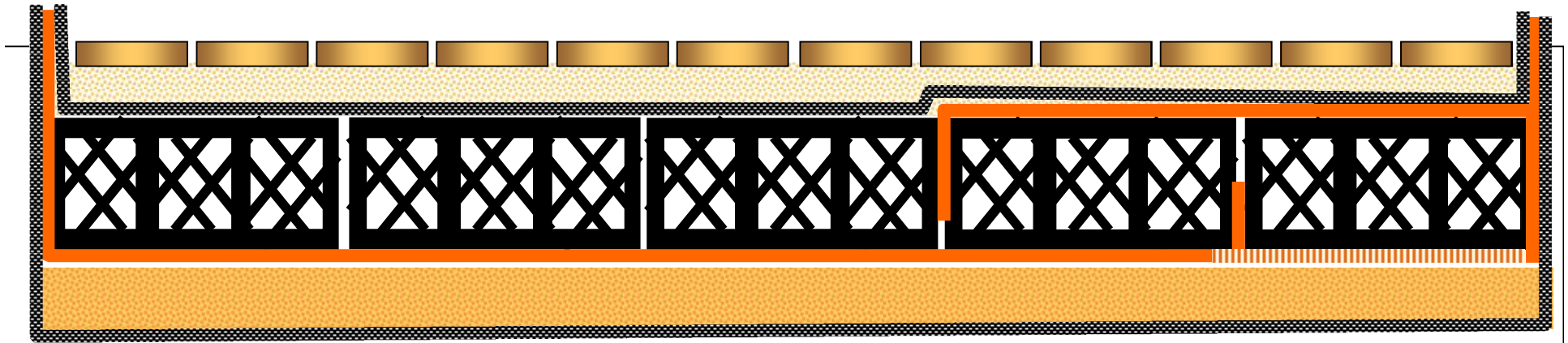
The Answer- Incorporate a Separator Into the Pavement



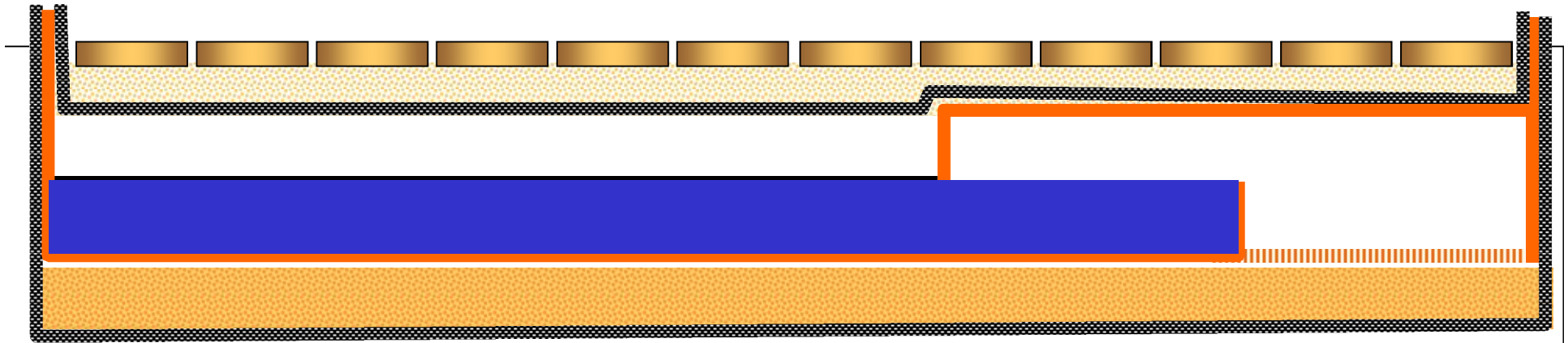
The Answer- Incorporate a Separator Into the Pavement



The Answer- Incorporate a Separator Into the Pavement



How It Works



Flume Experiments



Mechanisms of Pollution Prevention

- Primary
 - Sorption on gravel layer
 - Filtration by gravel layer
 - Sorption on Geotextile
 - Filtrationon Geotextile
- Secondary
 - Oil trapped as floating body
 - Oil sorption on secondary geotextile
 - Ability to recover free product
 - Biodegradation on water body

The Porous Pavement as a Bioreactor

Requirements For Biodegradation

- Sufficient retention time to allow degradation to proceed.
- Organic food supply.
- Microorganisms.
- Oxygen.
- Inorganic nutrients NPK(trace elements).

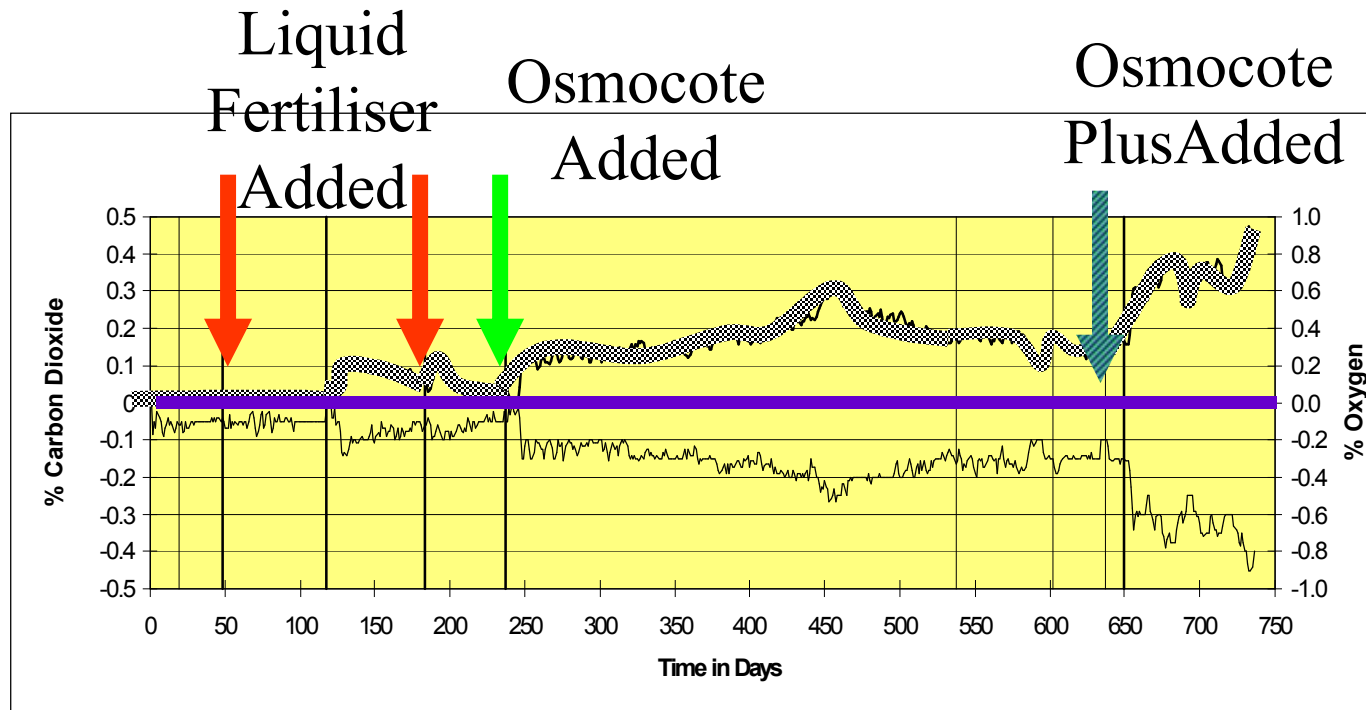
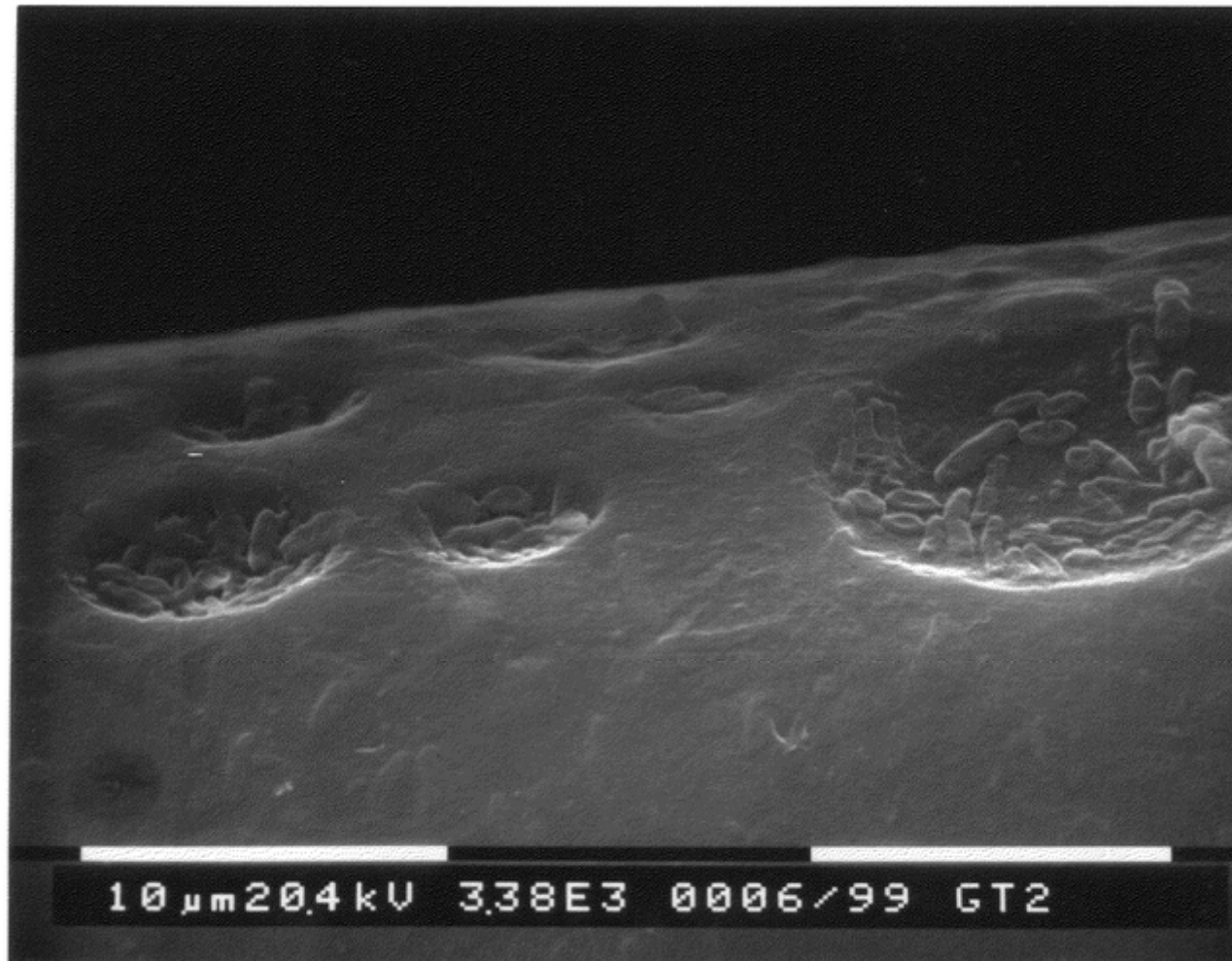


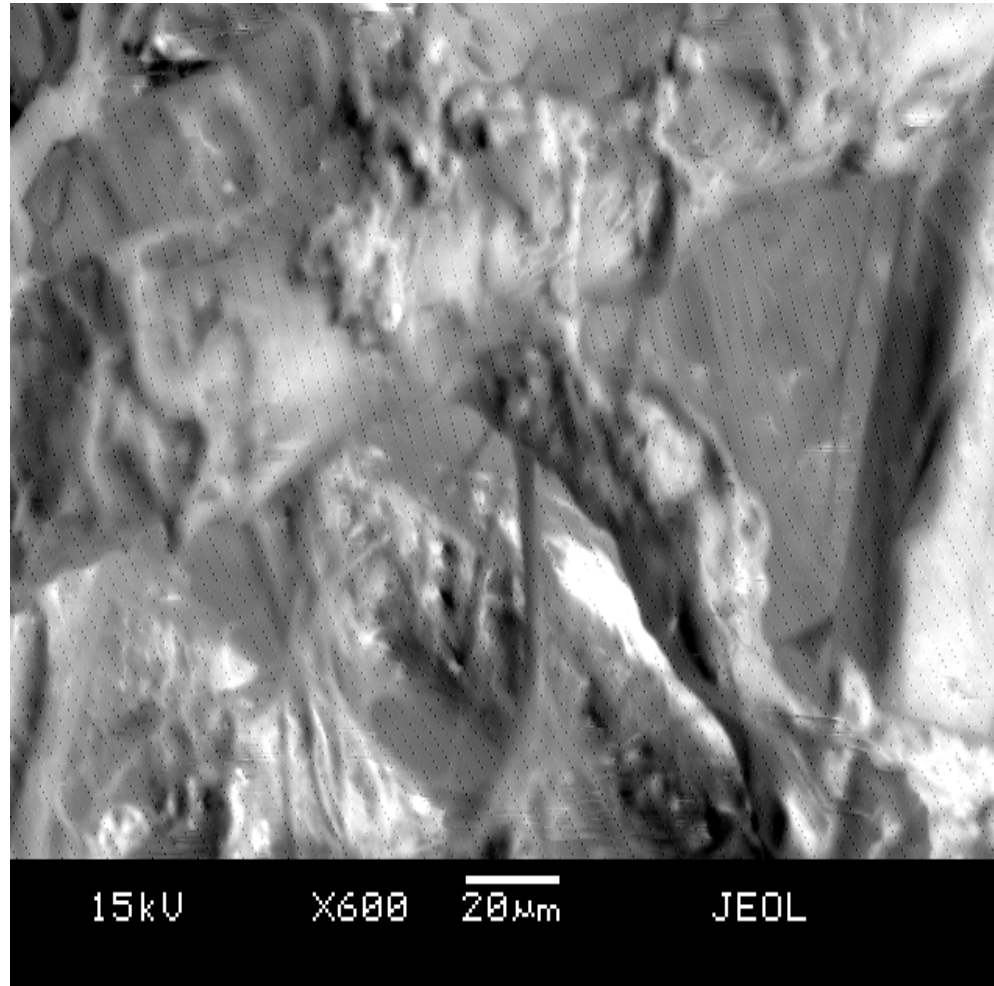
Figure 4.6. Δ Gas Measurements from Large Rig Sub-Base Probe

High-lighted days indicate the following: **Day 0** (22/05/96) = commenced “average rainfall”; **Day 19** (10/06/96) = commenced oil applications; **Day 48** (09/07/96) = Bio-Treat and Bio-Activator application; **Days 118** (17/09/96) and **183** (21/11/96) = Bio-Activator applications; **Day 237** (11/01/97) = Osmocote application; **Day 537** (10/11/97) = commenced increasing rainfall intensity; **Day 603** (15/01/98) = commenced drought; **Day 637** (18/02/98) = first-flush followed by “average rainfall”; **Day 650** (03/03/98) = Osmocote Plus application.

Strand of geotextile after 24 hours incubation



Several Months In A Test Rig



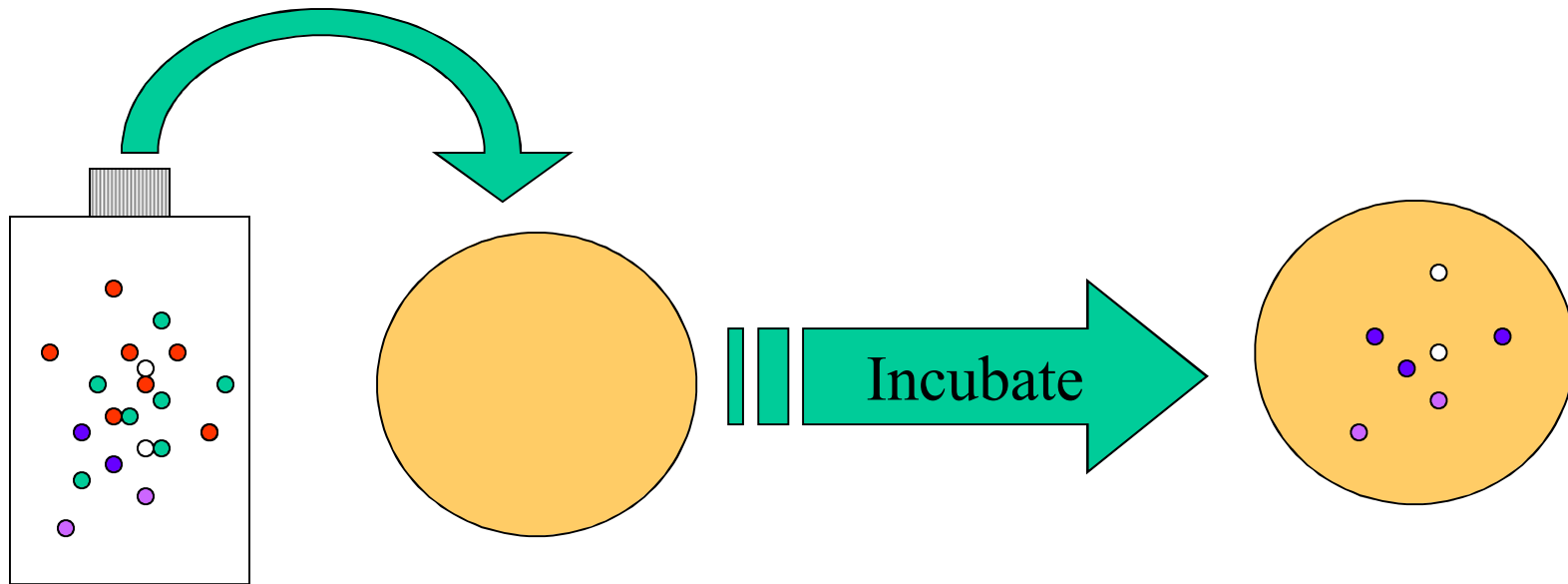
Microbial Ecology

- Identification and counting of oil degrading organisms.
- Measurement of microbial activity.

Estimation of Microbial Diversity.

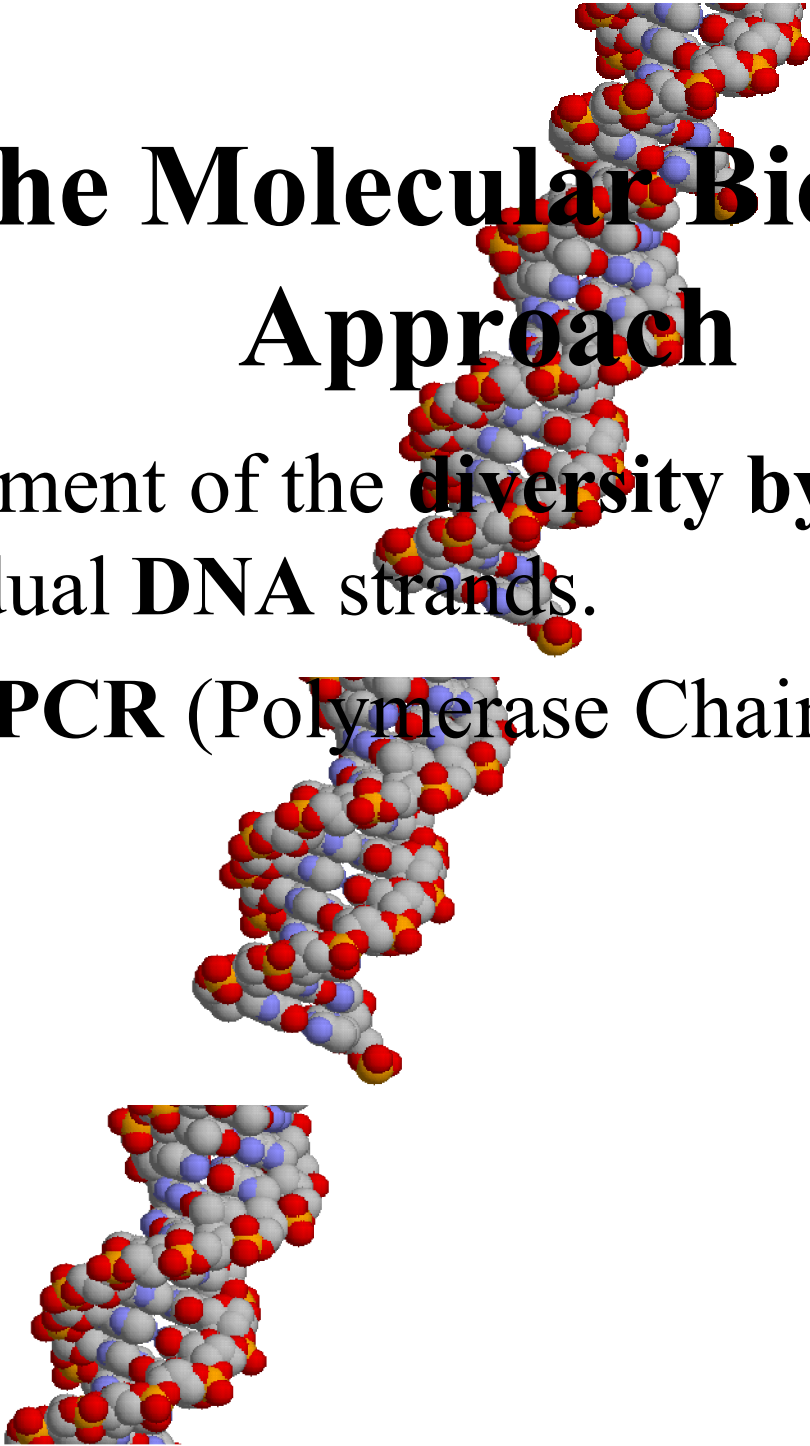
- Fatty Acid Methyl Esters
- Molecular biology
- Targetting DNA which codes for ribosomal RNA.
- Microscopic Methods

Traditional Approach



Inoculate

Only A Small Proportion Of
Bacterial Species Grow



The Molecular Biology Approach

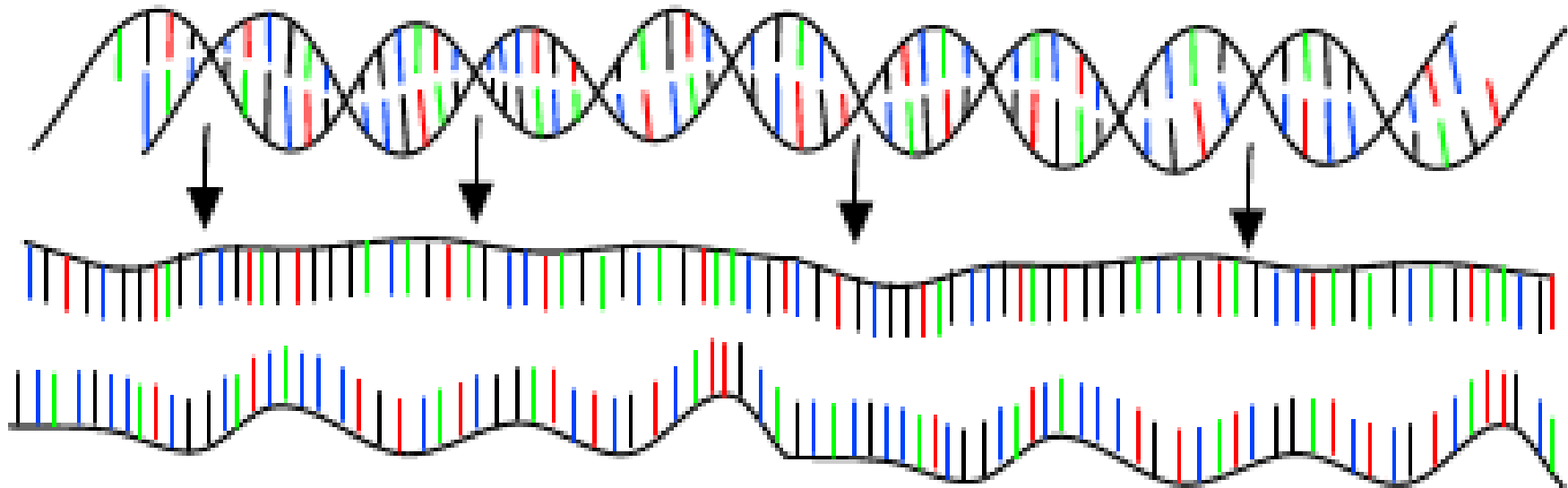
- Assessment of the **diversity** by the individual **DNA** strands.
- Using **PCR** (Polymerase Chain Reaction).

PCR

- PCR is:
 - inexpensive
 - simple method of producing relatively large numbers of copies of DNA molecules
 - sensitive
- PCR involves
 - preparation of the sample,
 - the master mix
 - and the primers, followed by
- detection and analysis of the reaction products.

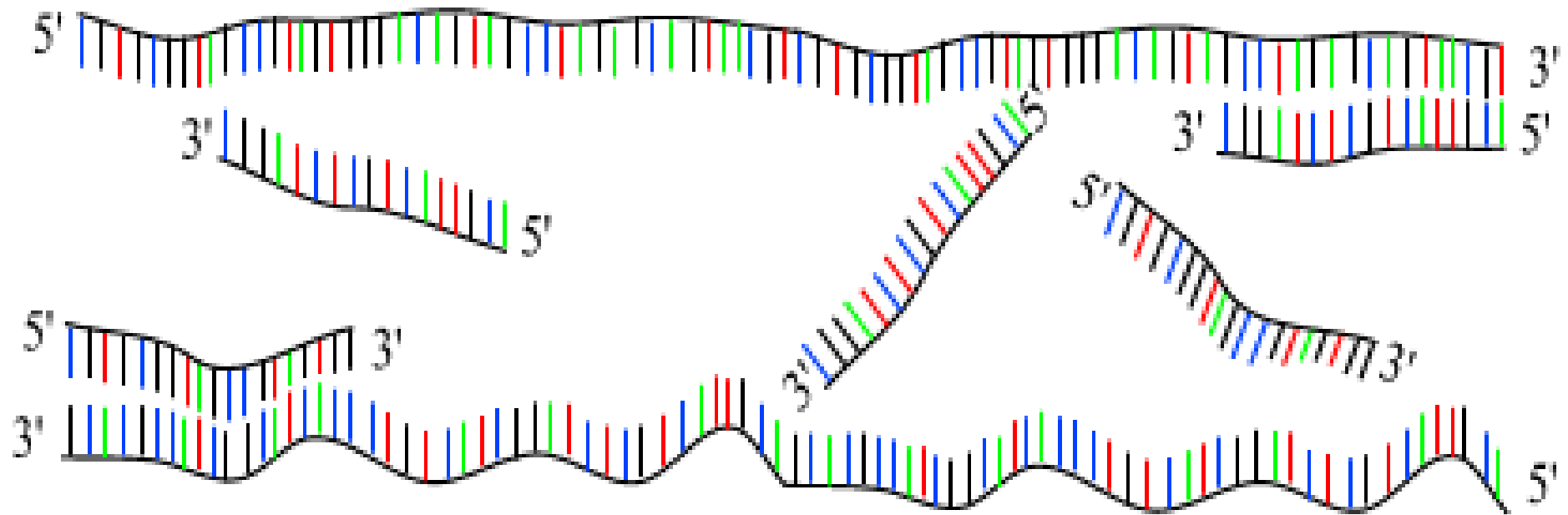
PCR

Step1: Denaturation of DNA 94°C, 1 Cycle, 15 sec



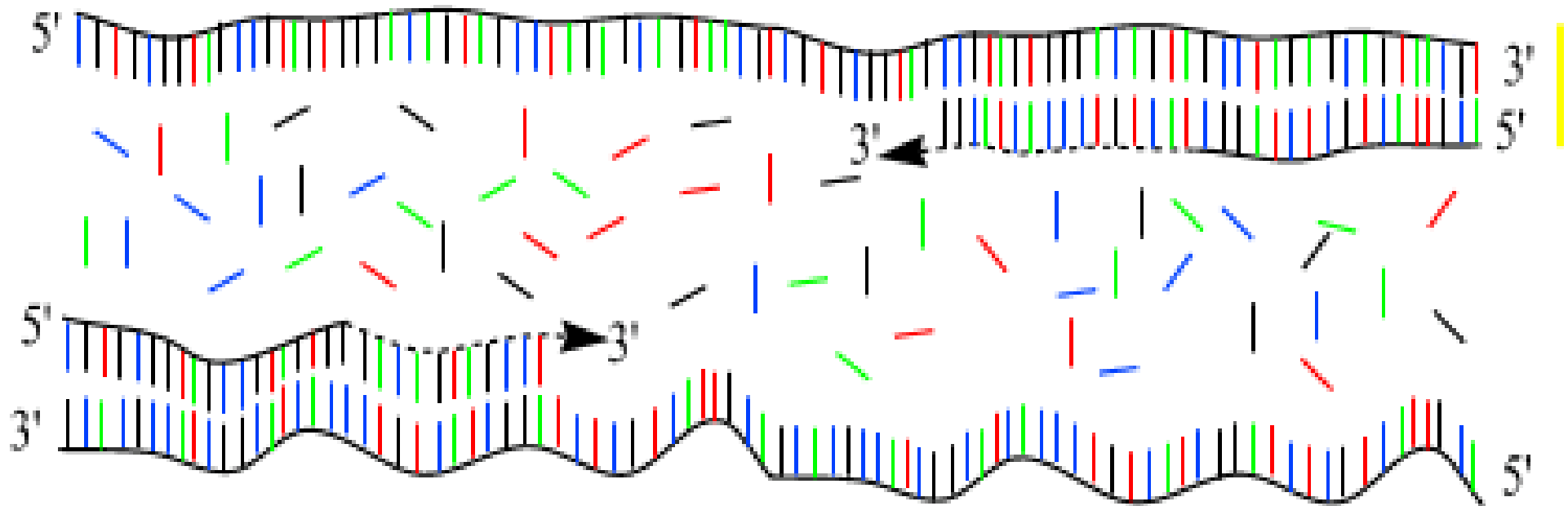
PCR

Step 2: Annealing of the primers, 57°C, 15 seconds



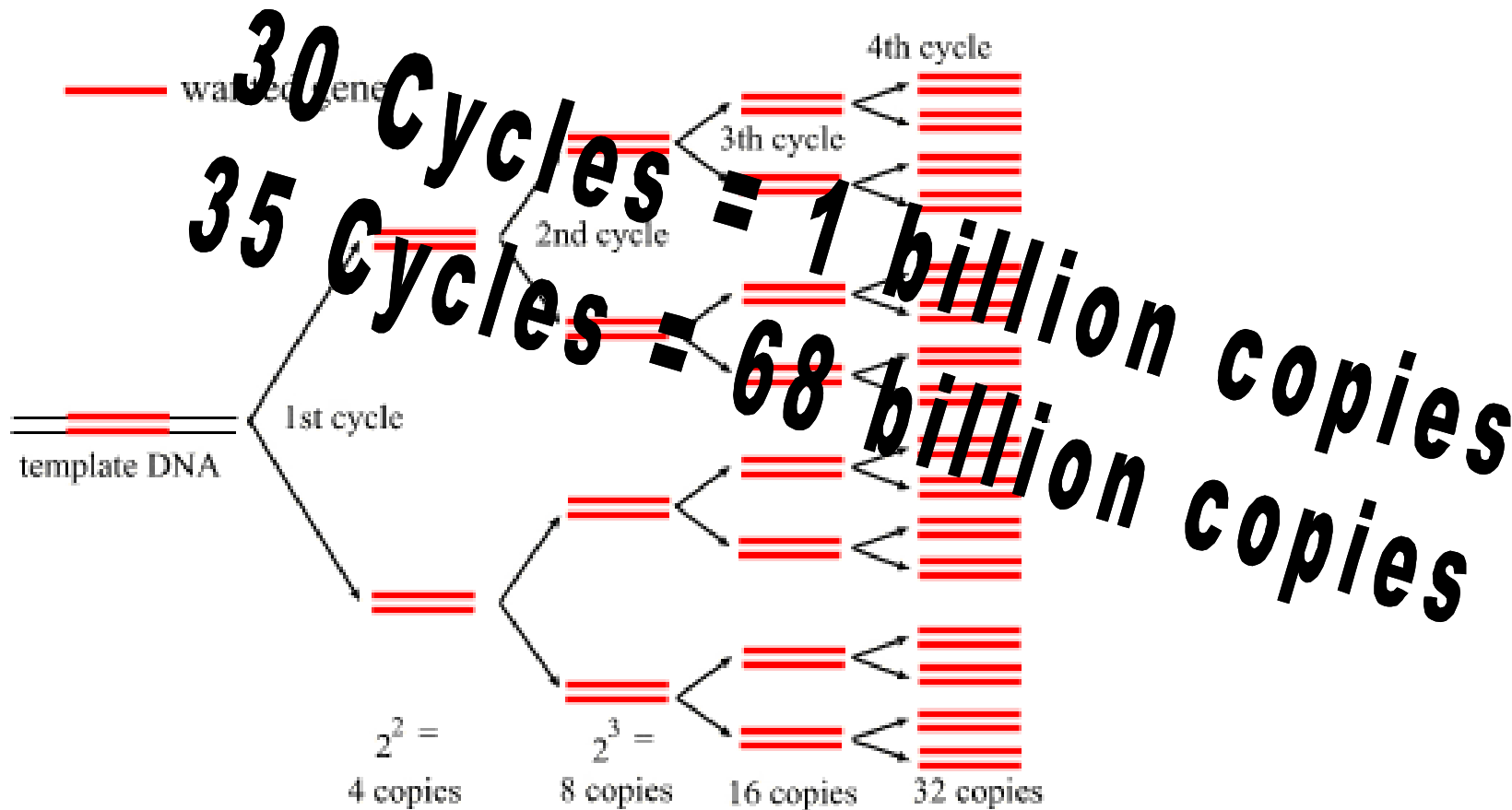
PCR

Step 3: Enzymatic Extension, 72°C , 2 minutes



PCR

Exponential increase of copies

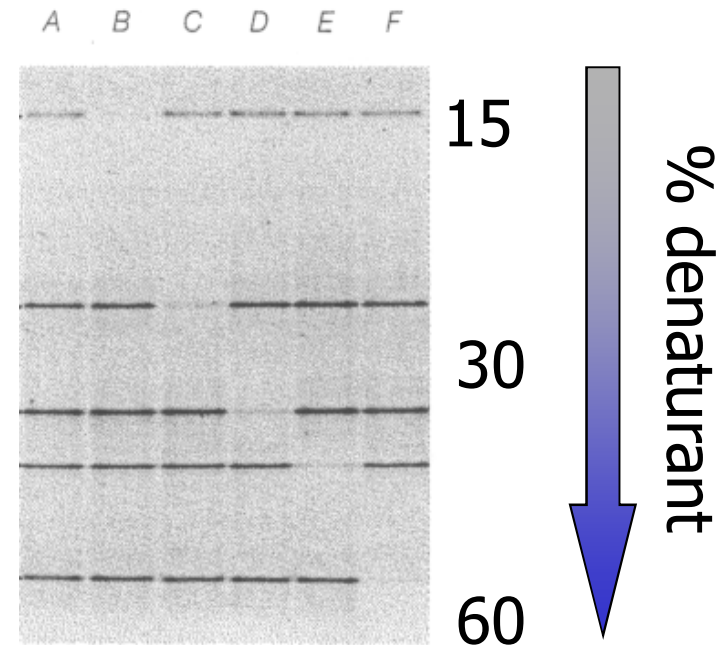


PCR - DGGE

- The theory behind DGGE is very simple, the two strands of a DNA molecule separate, or melt, when heat or a **chemical denaturant** is applied.
- Separated by electrophoresis through a gradient of increasing chemical denaturant (usually formamide and urea)

PCR - DGGE

The branched structure of the single stranded part of the molecule becomes entangled in the gel matrix and no further movement occurs.



DGGE

- Tells you how many different types
- But not what they are.
- For that we need TA Cloning

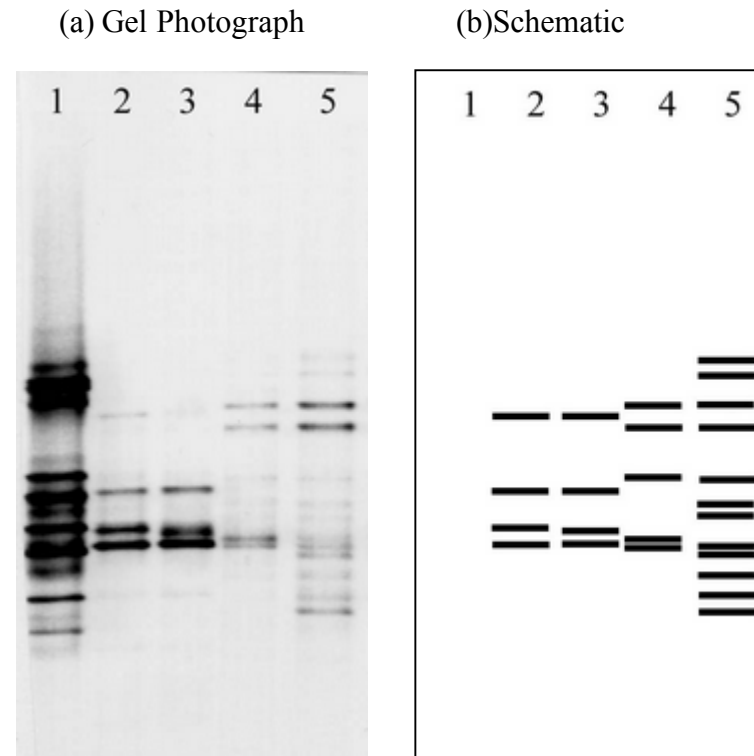
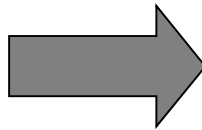
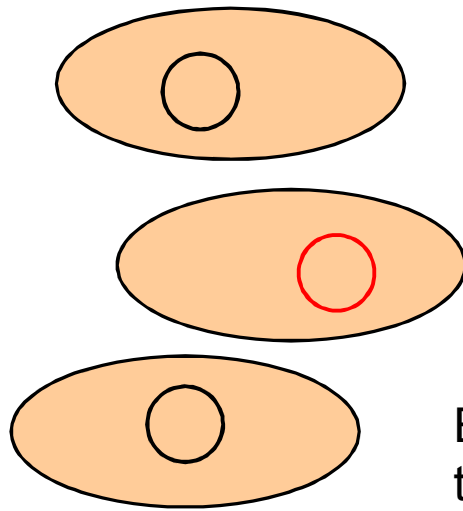
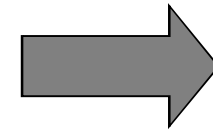


Figure 2.

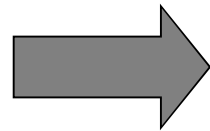
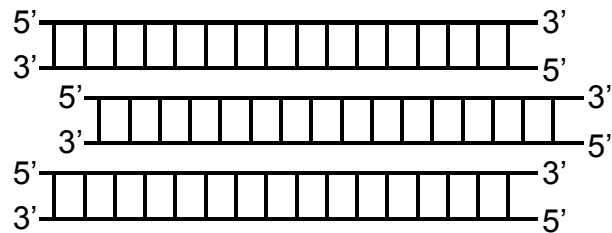
DGGE analysis of DNA samples. Lane 1, laboratory strain makers; Lane 2 initial inoculum extracted using freeze thaw; Lane 3, initial inoculum extracted using bead beating; Lane 4 long term porous pavement bacterial population extracted using freeze thaw; Lane 5 long term porous pavement bacterial population extracted using bead beating.



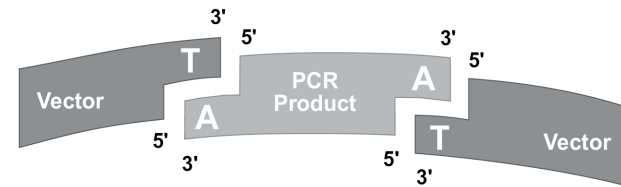
Extract DNA from
the community

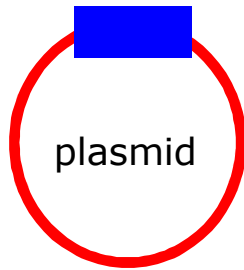


PCR with 16S
rRNA primers

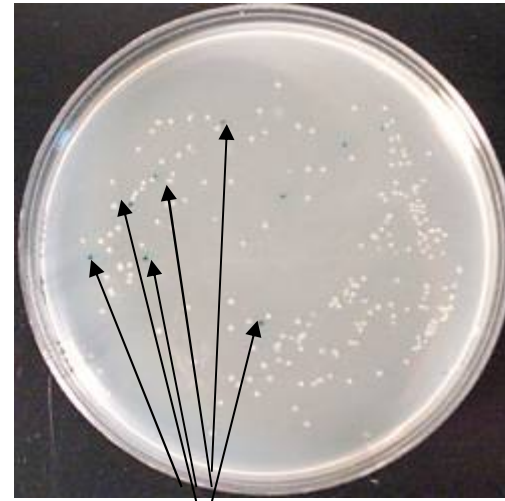


TA Ligation





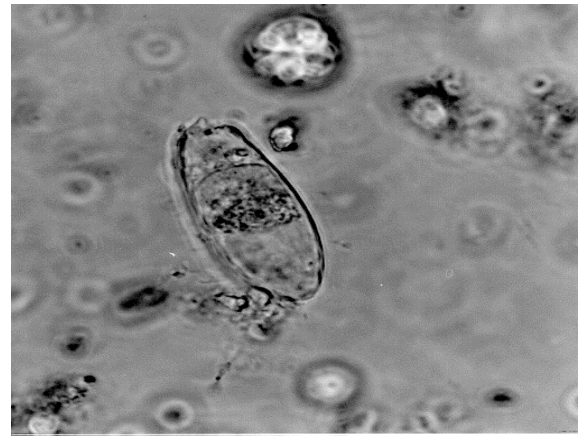
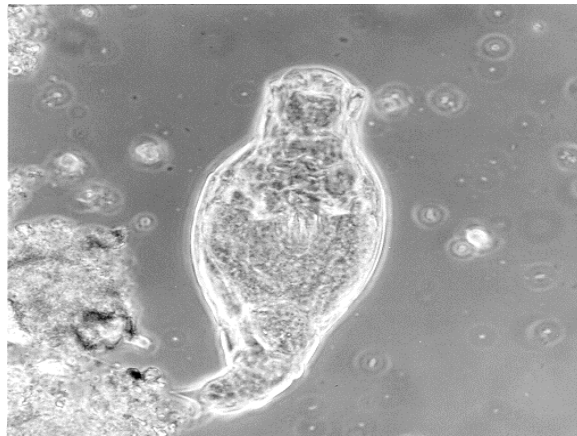
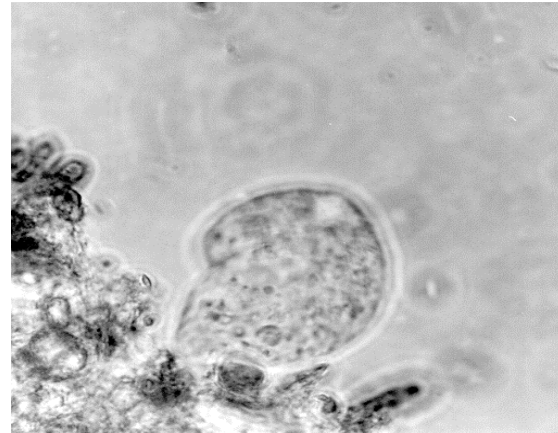
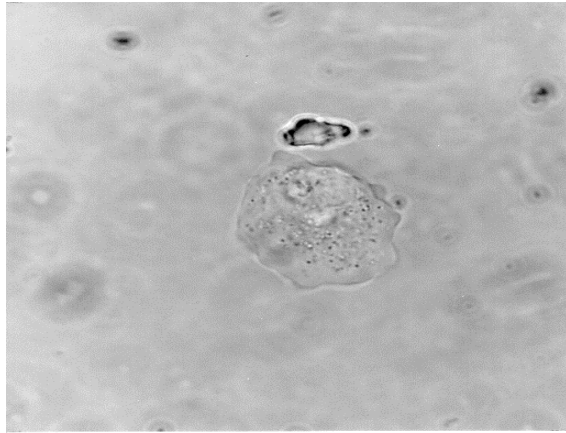
Electroporation
E. Coli



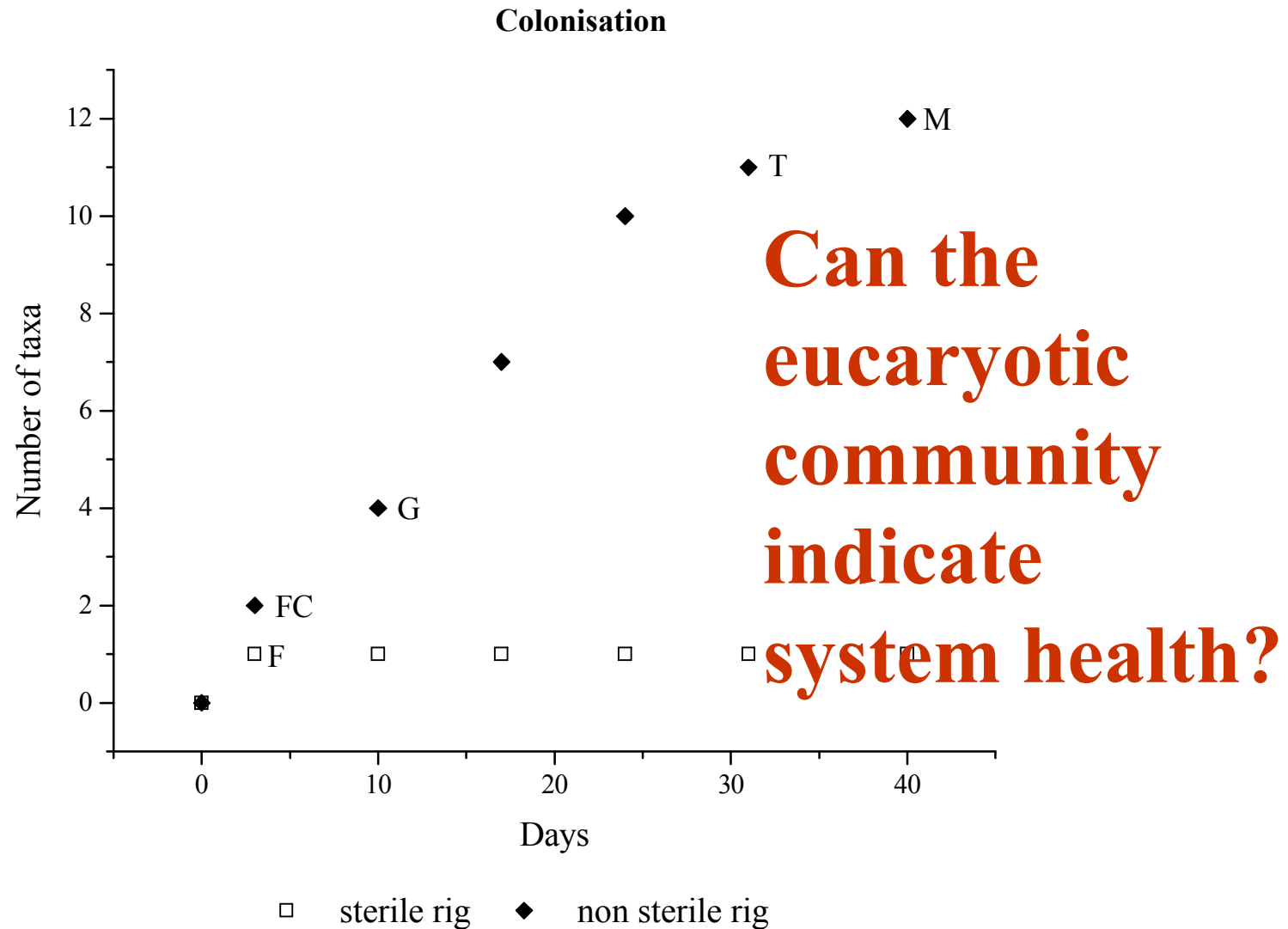
Blue colonies
presumed not to
carry an insert

- In the Permaceptor model 40% of identified clones are from oil degrading species.

Protozoan/Metazoan Colonisation

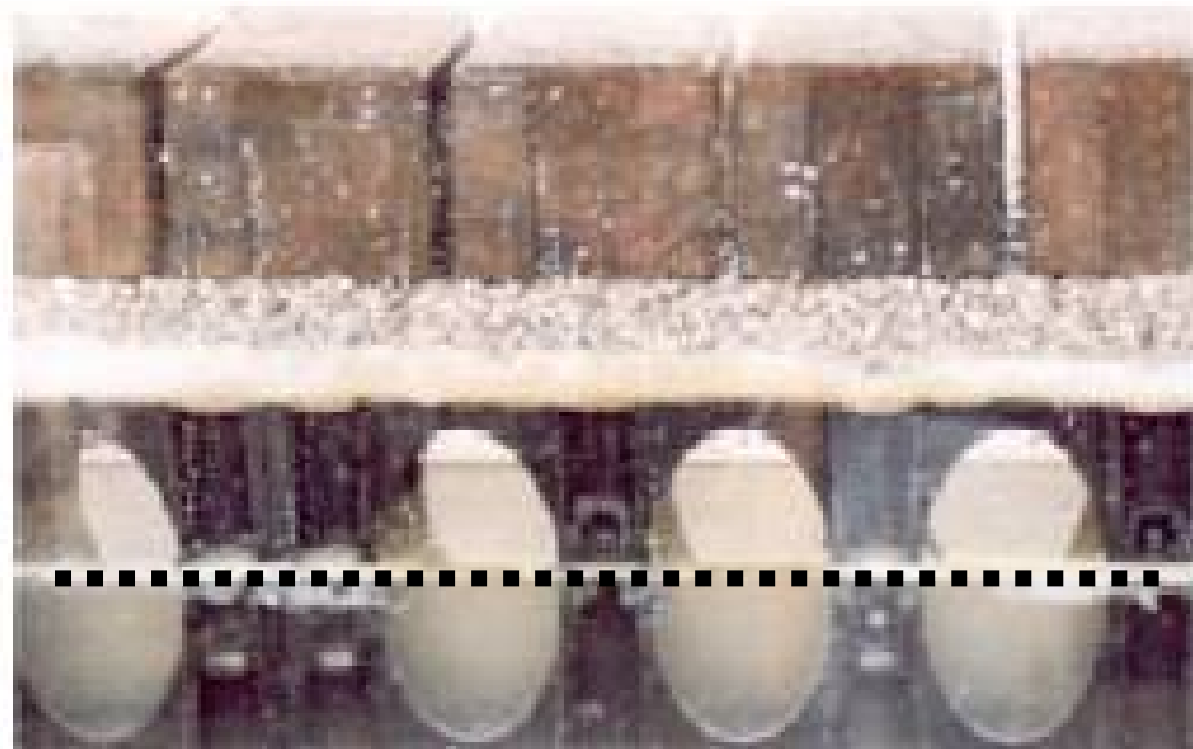


How fast does diversity develop ?



New Developments

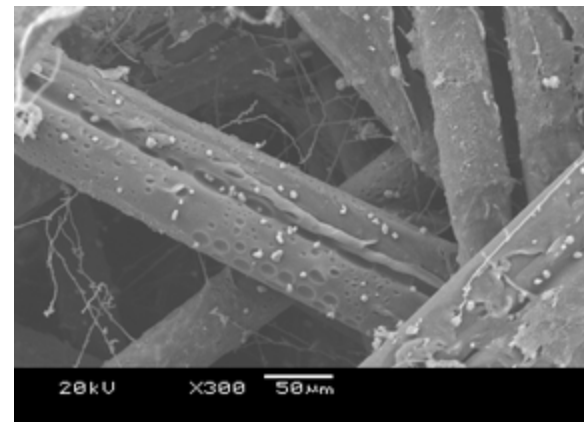
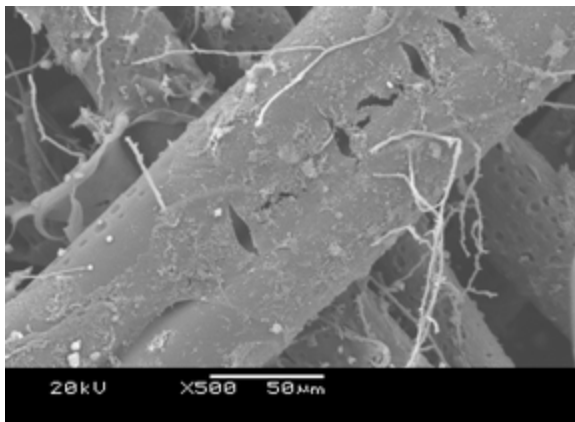
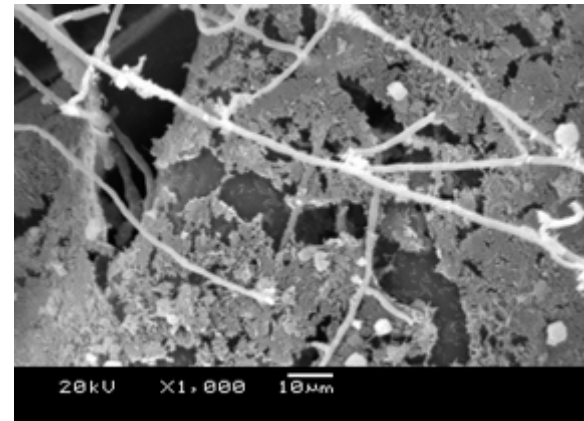
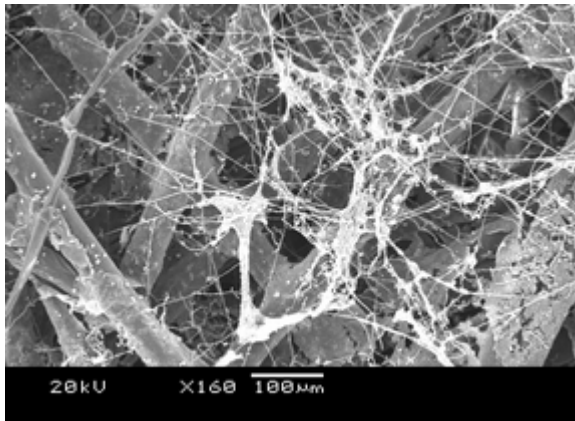
Floating Mat



Floating Mat Expt. -10g/m^2 Oil



Floating Mat



Relative Biomass Levels

- Mats extracted in chloroacetic acid
- ATP content measured using firefly luciferin.
- ATP proportional to biomass.

Nutrient sample 30 times more biomass

New Test Beds







Other Avenues



Disciplines Involved

- Engineering
- Analytical Chemistry
- Synthetic Organic Chemistry
- Bacteriology
- Protozoology
- Biochemistry
- Plant Sciences
- Electron Microscopy

Current Incumbents

- Engineering-Chris Pratt , John Davies
- Analytical Chemistry-Alan Newman
- Synthetic Organic Chemistry- Danny Lynch, Gillian Spicer
- Bacteriology – Tim Puehmeier
- Protozoology-Steve Coupe, Humphrey Smith
- Biochemistry-Jackie Wong
- Plant Sciences-Michelle Barrett, Janey Henderson
- Electron Microscopy-Neil Cresswell

Acknowledgements

- DTI
- EPG
- EPSRC
- Formpave
- SEL Environmental
- Waste Recycling Group