# Engineering microbial consortia by synthetic biology

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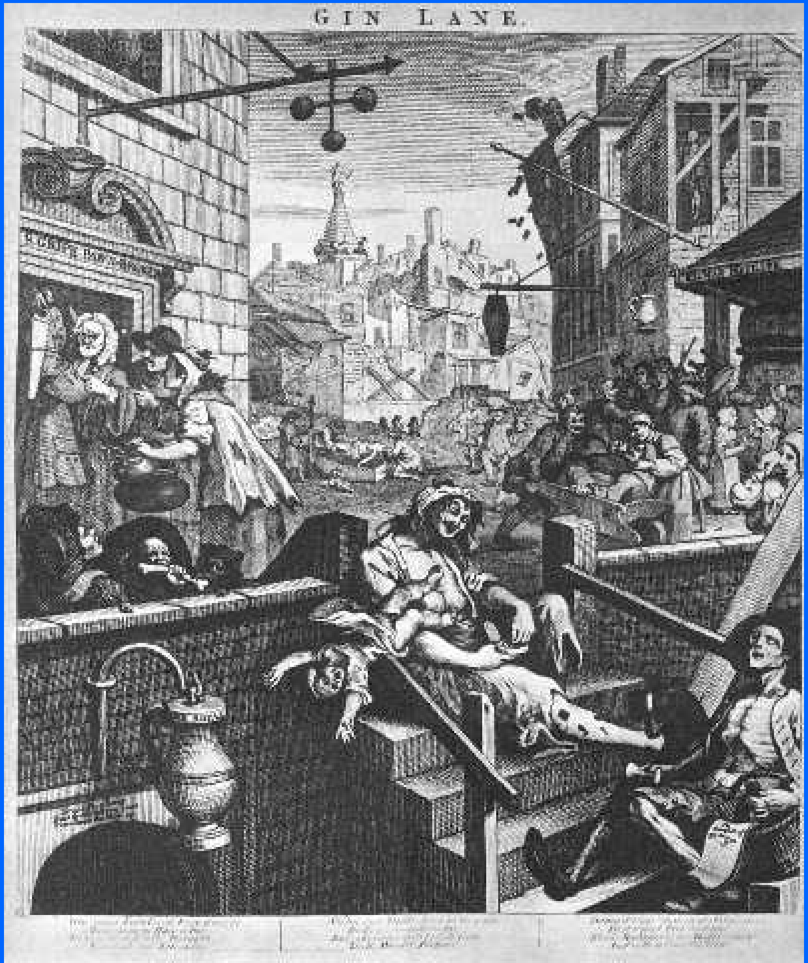
# Global challenges

- Water
- Energy
- Food
- Resources
- Pollution/Resources/Waste

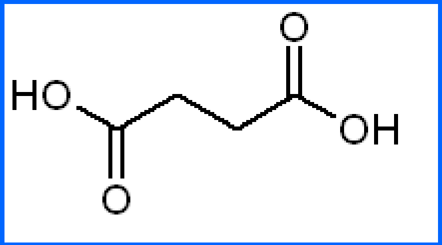
# We need a step change to solve some key challenges.



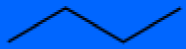

Joseph Bazalgette 1819-1891

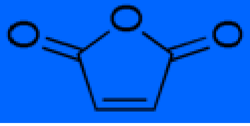
# Converting green waste into energy and high value products

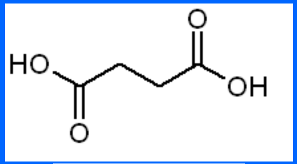
# Succinic acid- primary platform chemical




- Butanedioic acid, $C_4H_6O_4$
- Applications
  - Food additive
  - Disinfectant
  - Corrosion inhibitor in Coolant systems
  - Intermediate for preparation of pharmaceuticals and others








Petroleum → n-butane C4 → maleic anhydride C4 → succinic acid C4

$0.977/kg

$5.9~9/kg, 15,000t/y

Glucose → succinic acid

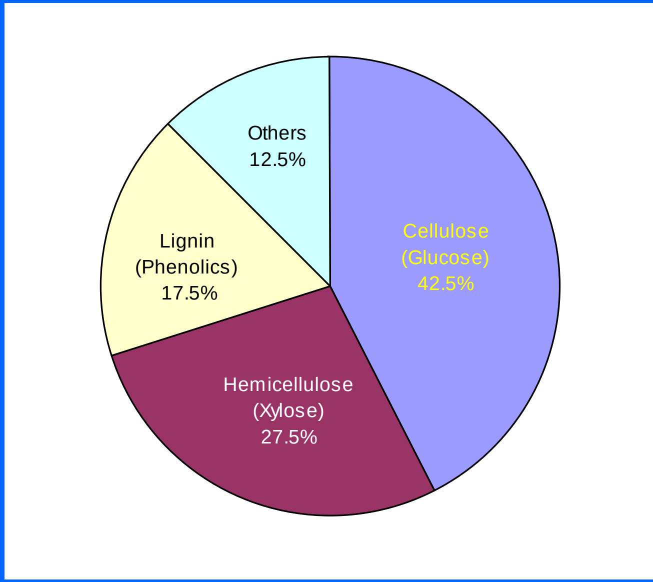
$0.39/kg

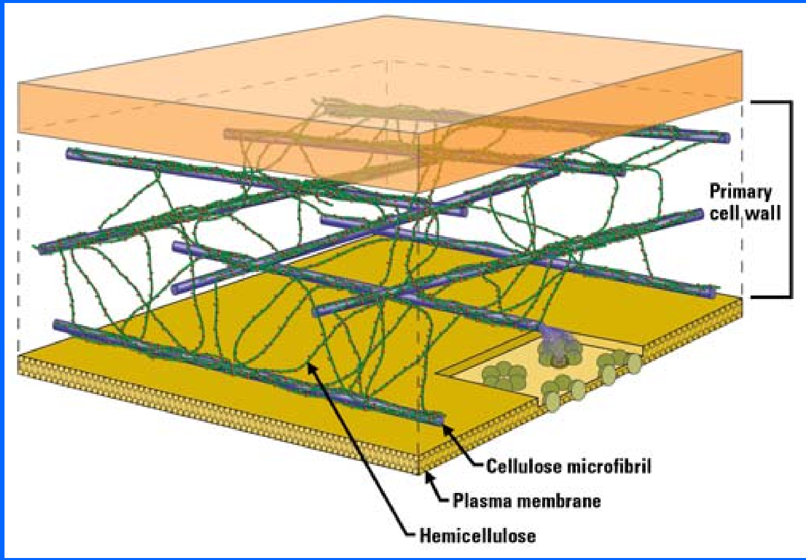
30,000,000 t/y

# Microbial generation of ethanol and other high value products from plants

Components of cellulosic biomass

- Most plant matter consists of three key polymers: Cellulose (35 to 50%), hemicellulose (20 to 35%), and lignin (10 to 25%).



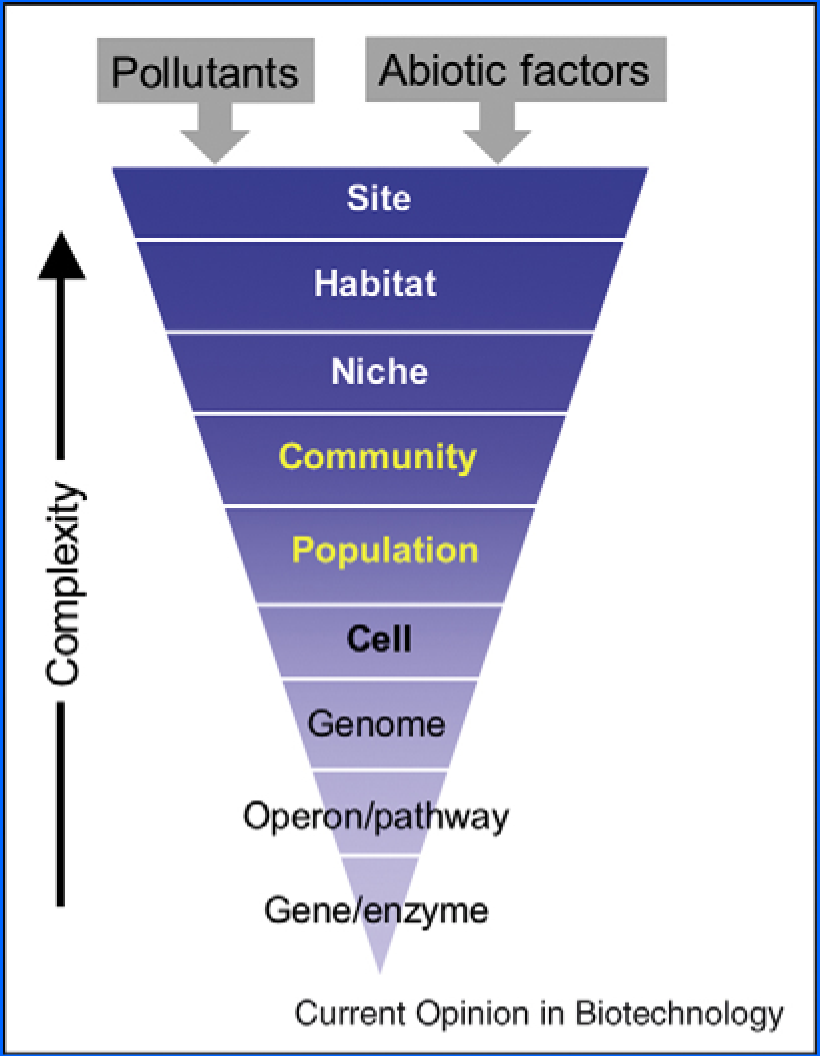

We current have few microbial strains that at efficient at converting plant carbon to useful products- strains typically selective utilise the pentose or hexose components, not both.

# Isolation of hemicellulolytic bacteria from bovine rumen. Fishing for the right microbial strains.

Dealing with environmental pollution is often a key example of how synthetic biology will help solve our problems.

But how?

# Application of synthetic biology in microbial contaminant treatment



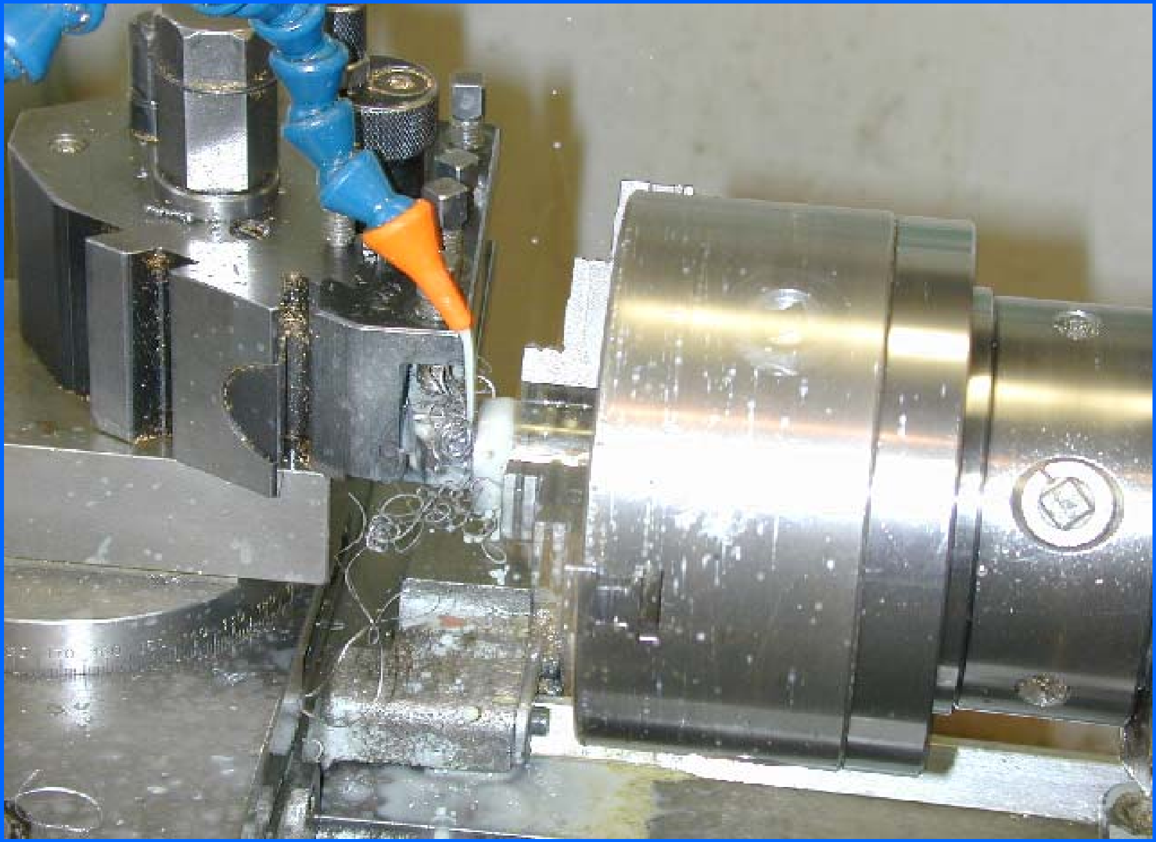
# Microbial consortia offer an important frontier for synthetic biology

- They can perform complicated biochemical tasks- conversion of cellulose to ethanol.
- Can endure more extreme conditions than mono-cultures.
- Offer delivery systems for introducing synthetic genes.

# Case study

# Metal working fluids (MWF): Case study

- 400 million $l^{-1}$ waste produced pa in UK
- 22.4 X $10^9$ $l^{-1}$ pa worldwide



- Its packed with biocides and specifically designed to be biologically resistant.

# Large scale biological treatment of waste metal working fluids

# Disposal of waste MWF

- **Landfill**- banned.
- **Ultrafiltration**- expensive capital, not effective for removal of smaller molecules (eg synthetics), problematic sludge.
- **Incineration**- expensive and not sustainable.
- **Flash Evaporation**- expensive and not sustainable.
- **Biological Treatment-** variable success to date.

# Source of microbial inoculants for bioreactors

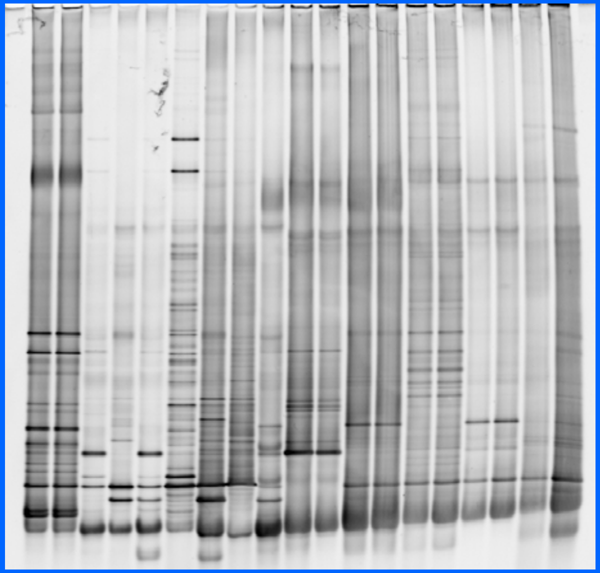
1. Activated sludge.
2. Commercial concoction of dubious origin.
3. Systematically assembled based on a sound ecological understanding of the microbial community in source substrates.

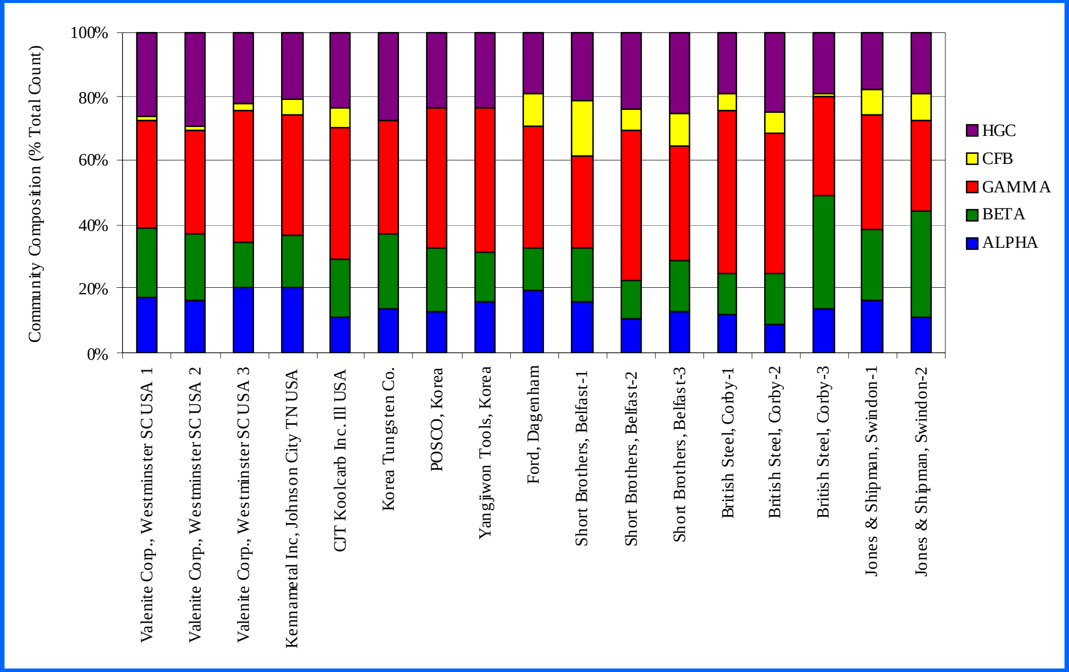
Three selection criteria:

- Survival temporally and spatially.
- Ability to utilise contaminants as nutrients.
- Tolerance to co-contaminants.

# Bacterial treatment of MWF

# Bacterial community analysis of spent MWF worldwide

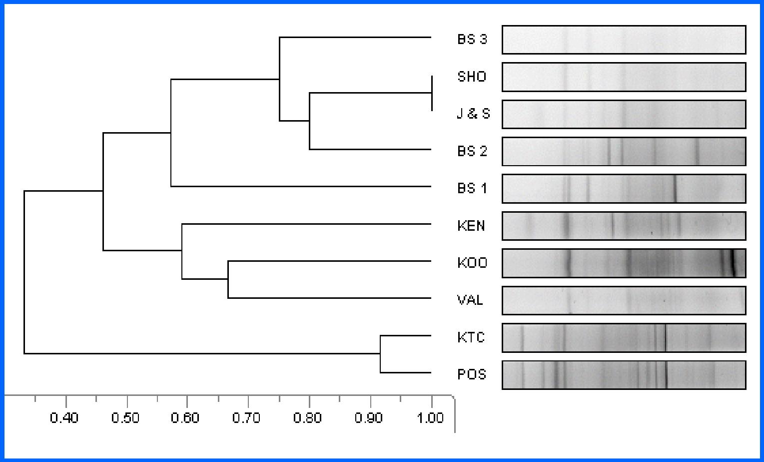




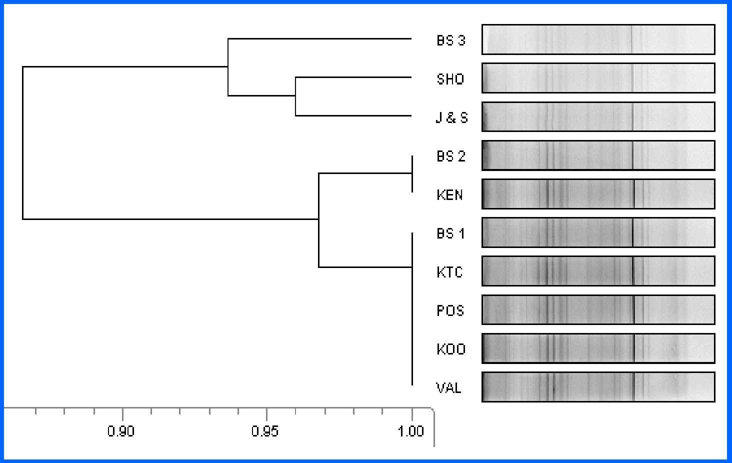

Denaturing Gradient Gel Electrophoresis    Fluorescent in situ hybridisation (FISH)

# Genetic finger print of bacteria that grow preferentially (enriched) in MWFs.

Before Enrichment

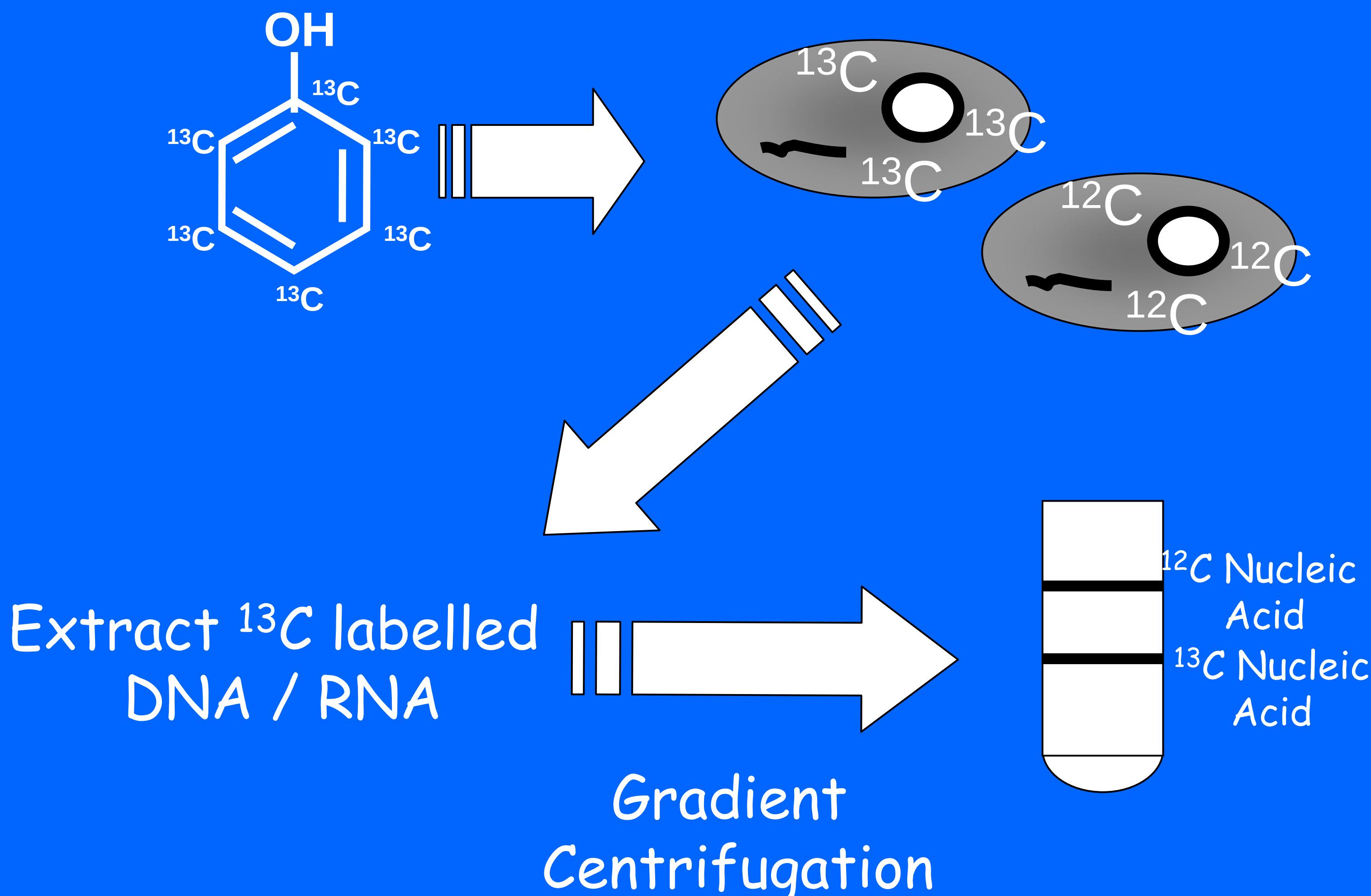



After Enrichment




A very selective habitat with limited array of specialists

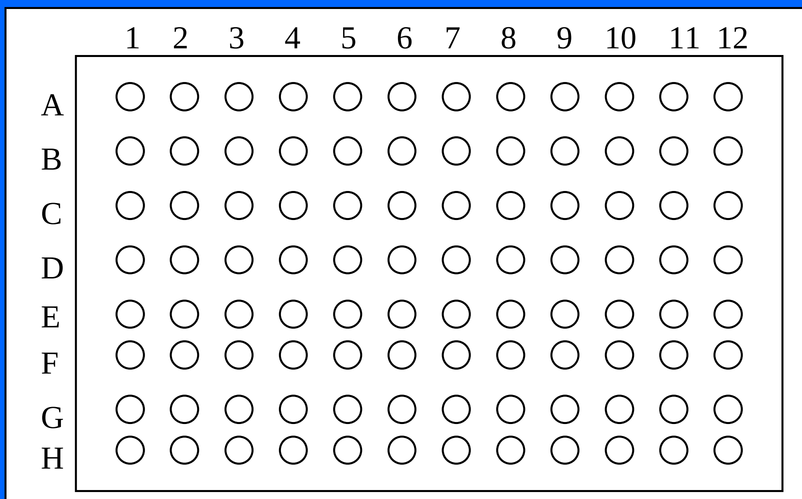
# Detecting the right cells?
# (Stable isotope probing)



# Rapid Component Screening

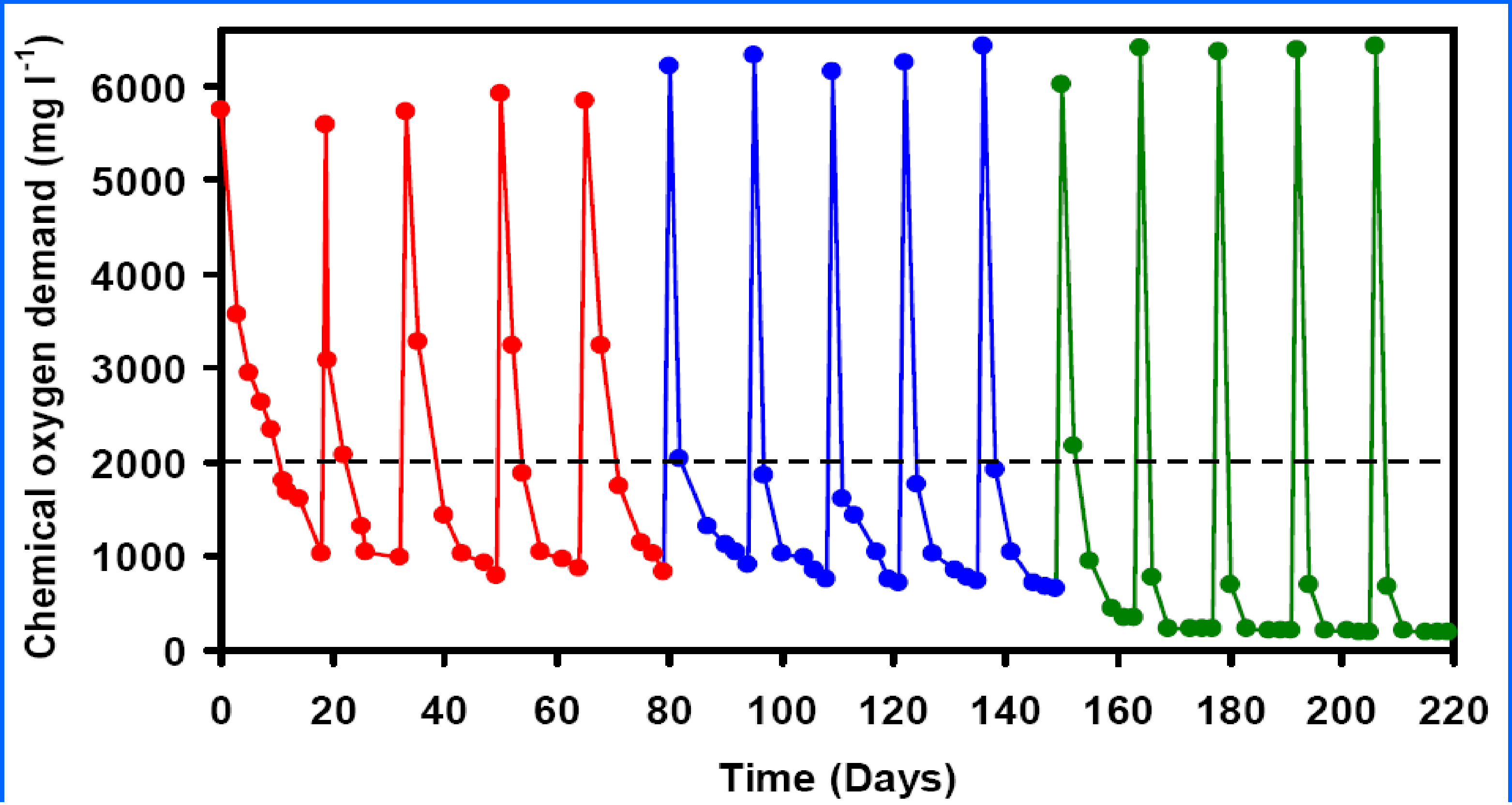
Bacteria were selected on 3 selection criteria:

1) natural abundance.
2) tolerance to co-contaminants.
3) ability to biodegrade




# Bench top bioreactors

# Large scale performance of our bacterial consortium



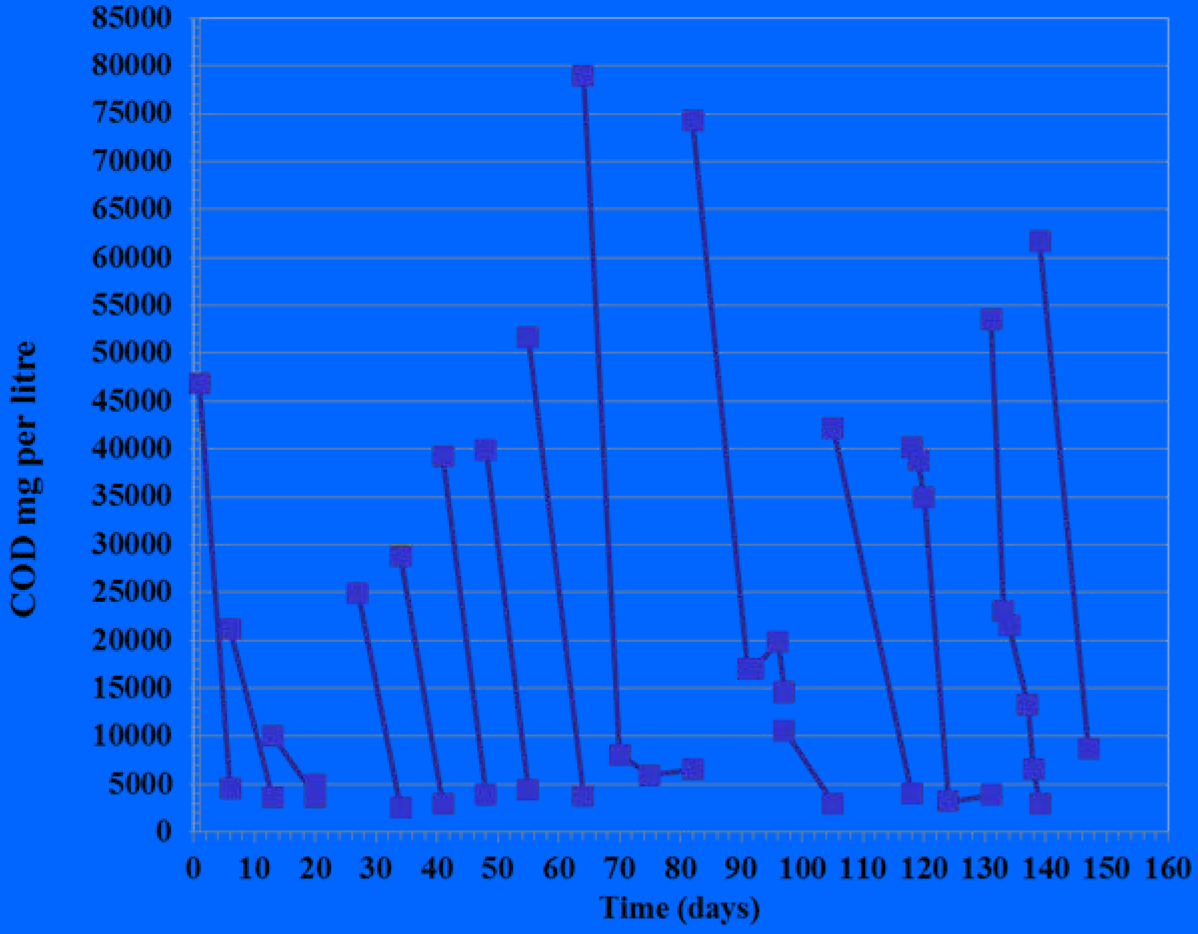

COD equates to the contaminant load

# Field scale performance

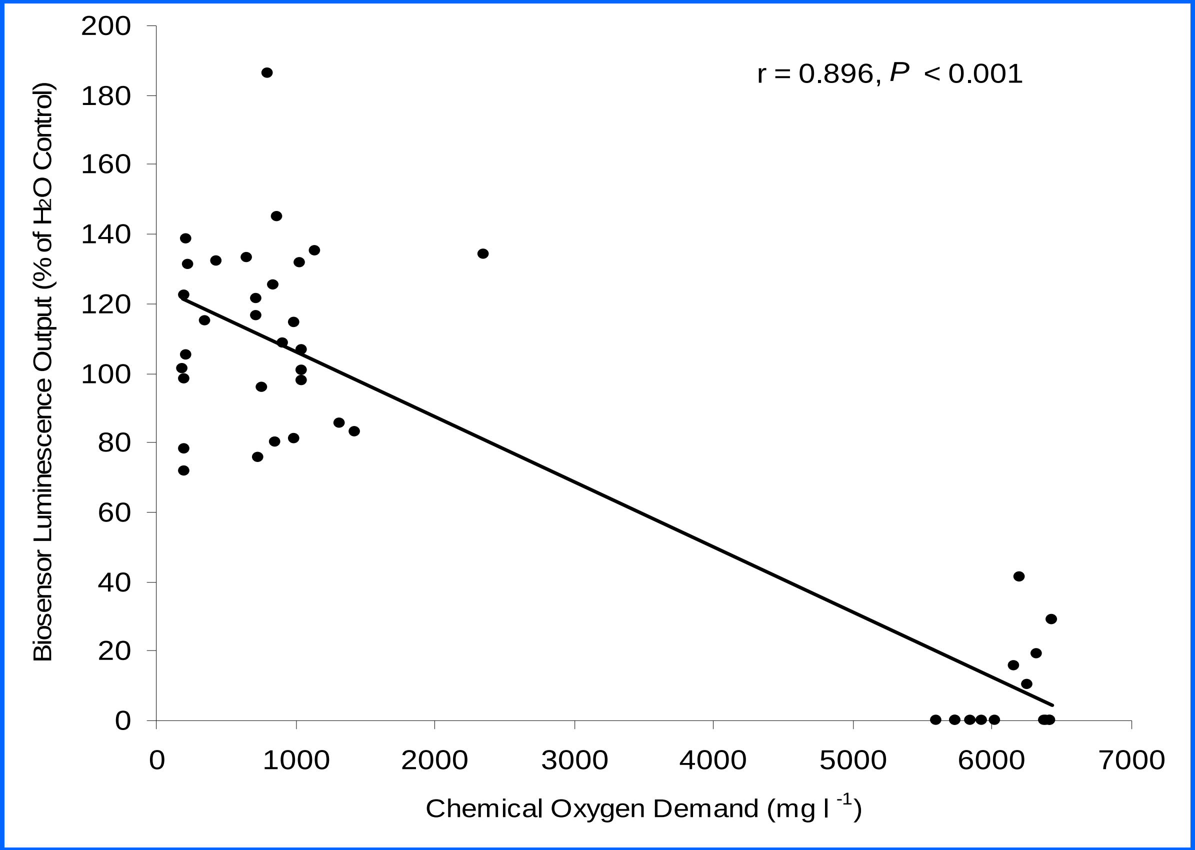
microbial SOLUTIONS

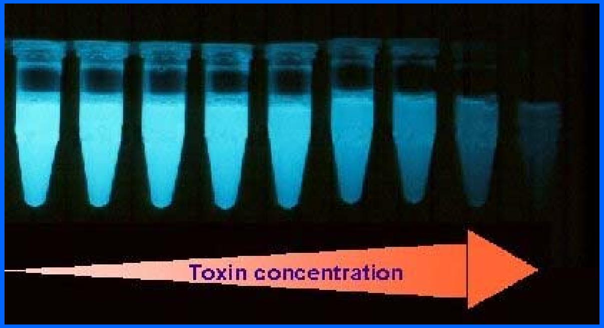
## Pollutant load reduction- chemical oxygen demand

BAE SYSTEMS



# Toxicity of MWF effluent before and after treatment




Bacterial biosensors

Toxicity goes down and importantly so does pH from around 9 to 7.

# No biological system is infallible- reasons for failure

- Operator incompetence.
- Community biomass collapse because of fluctuating inputs.
- Accumulation of recalcitrant components to toxic concentration such as amines and chemical complexes.
- Presence of toxic heavy metals.

# Challenge of engineering stable consortia and synthetic biology

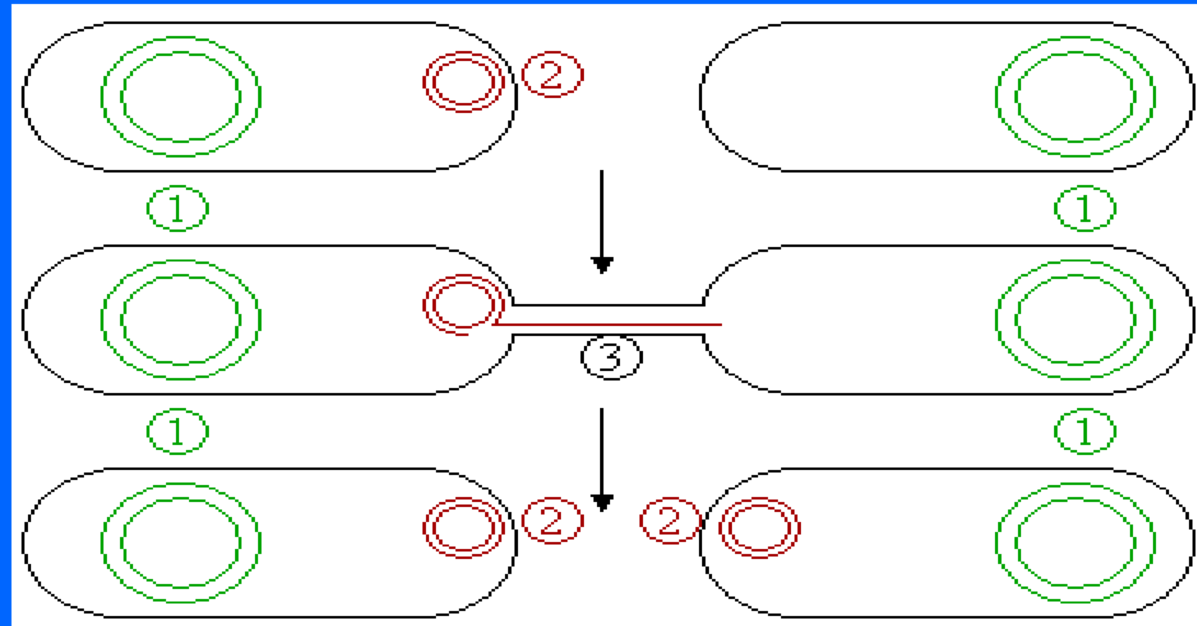
- Controlling the multiple interactions to maintain community stability- this may require re-introduction.
- Introduction and incorporation of complementary genes.
- Some of the most useful strains are difficult to modify- Gram positives.
- Directed evolution for fine tuning optimal behaviour.

# Physical and engineered approaches for forcing artificial consortia.

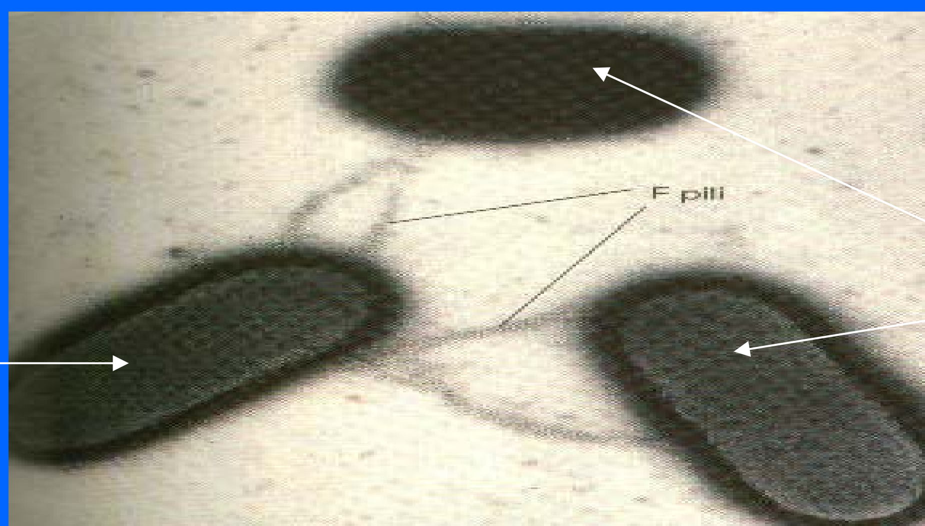

Ultrasound manipulation of soils

# Forced evolution using ultrasound

Accelerating rates of gene transfers




Plasmid donor: naphthalene degraders isolated from Exeter site




Recipient: *Pseudomonas putida* KT2440

1: Chromosome, 2: plasmid, 3: Pilus

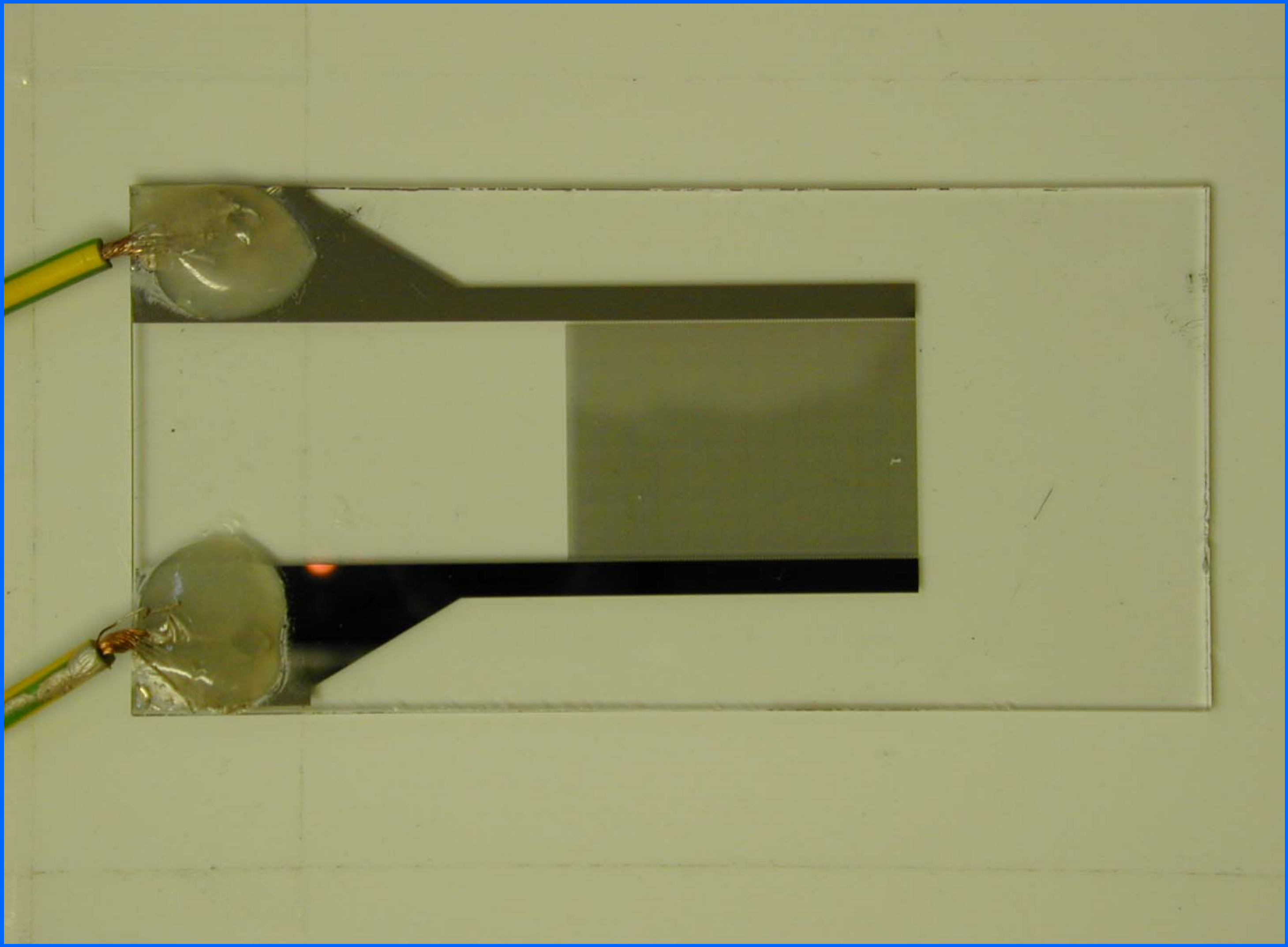
Song et al Nucleic Acid Research 35, (2007) and patented.

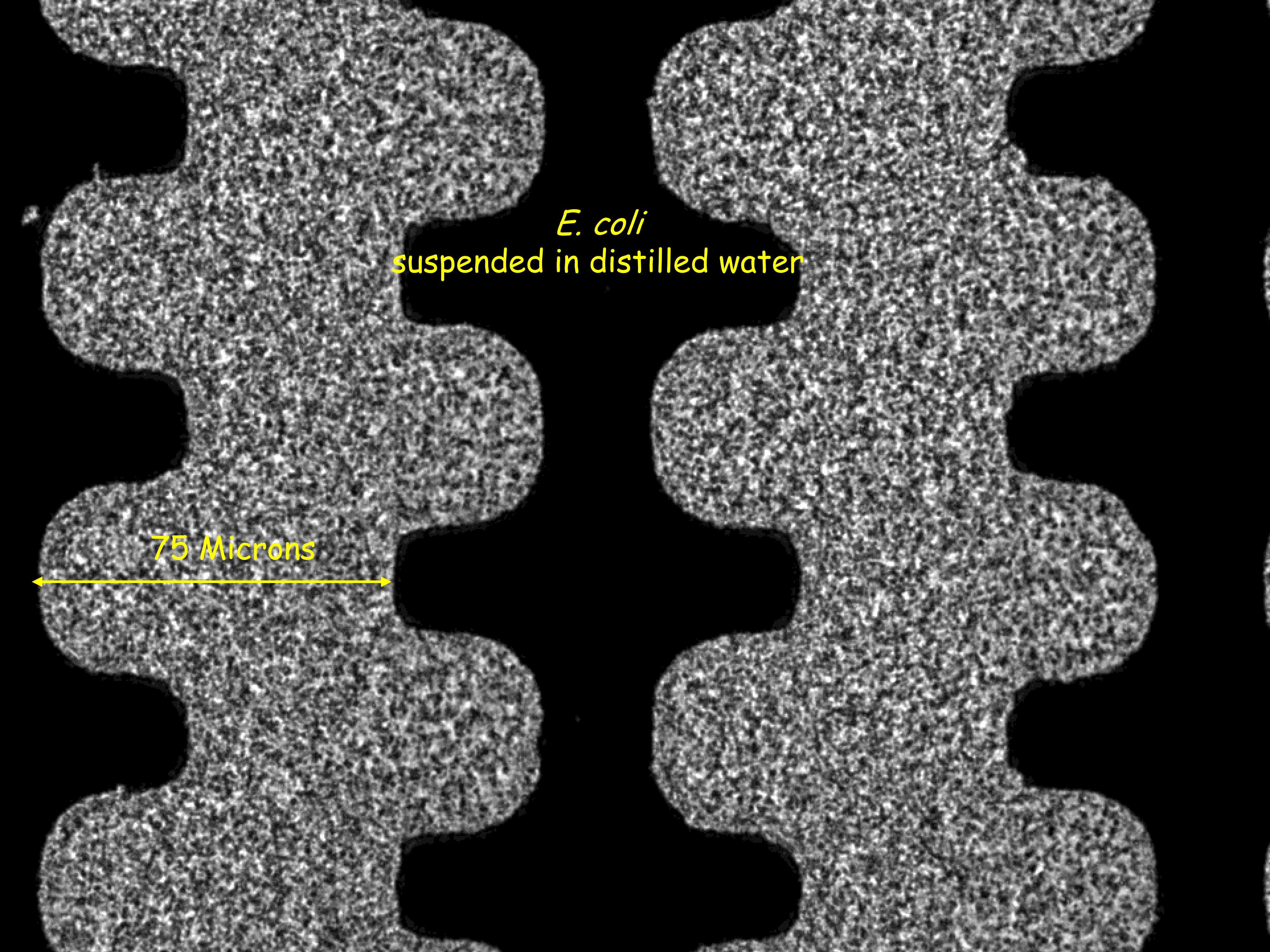
# Ultrasound DNA Delivery

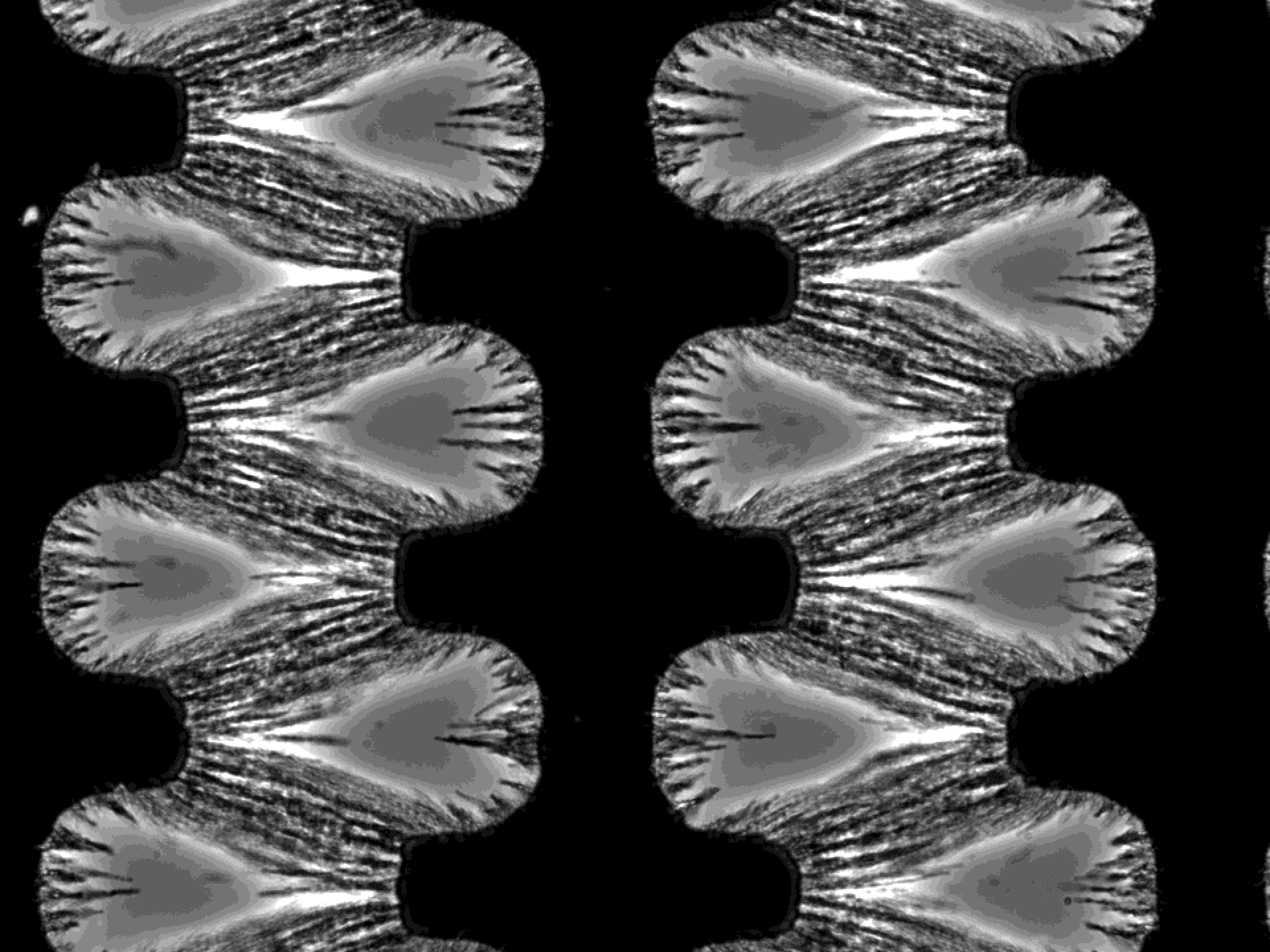
- At least 9 times more efficient than conjugation.
- The low frequency (20-40kHz) makes it scale-able.
- Most conventional methods such as electroporation requires very specific conditions such as low-ionic strength media.
- Does not require direct contact- so can be done remotely and *in situ*.

- Ultrasound also stimulates chemical transfer rates and increases bioavailability.

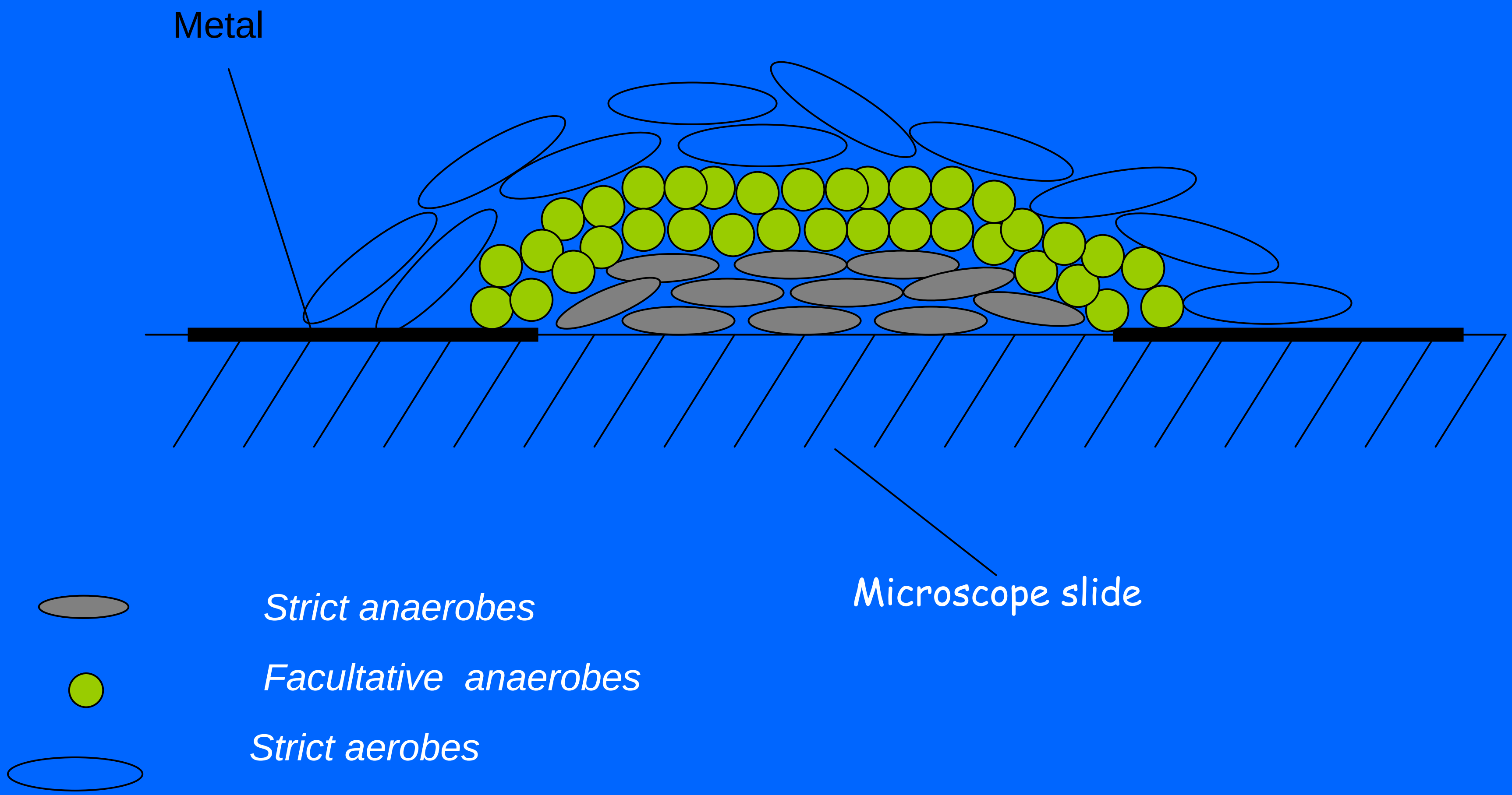
# Electrokinetic manipulation of cells



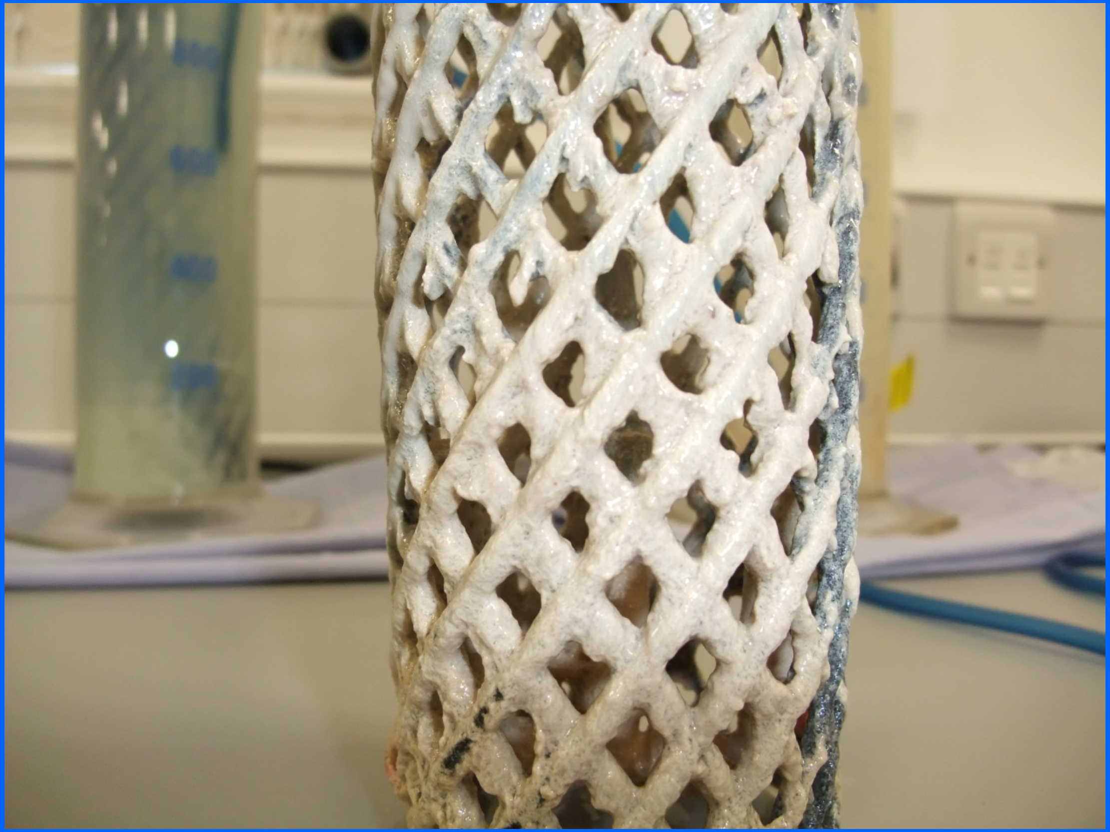

*E. coli*
suspended in distilled water
75 Microns



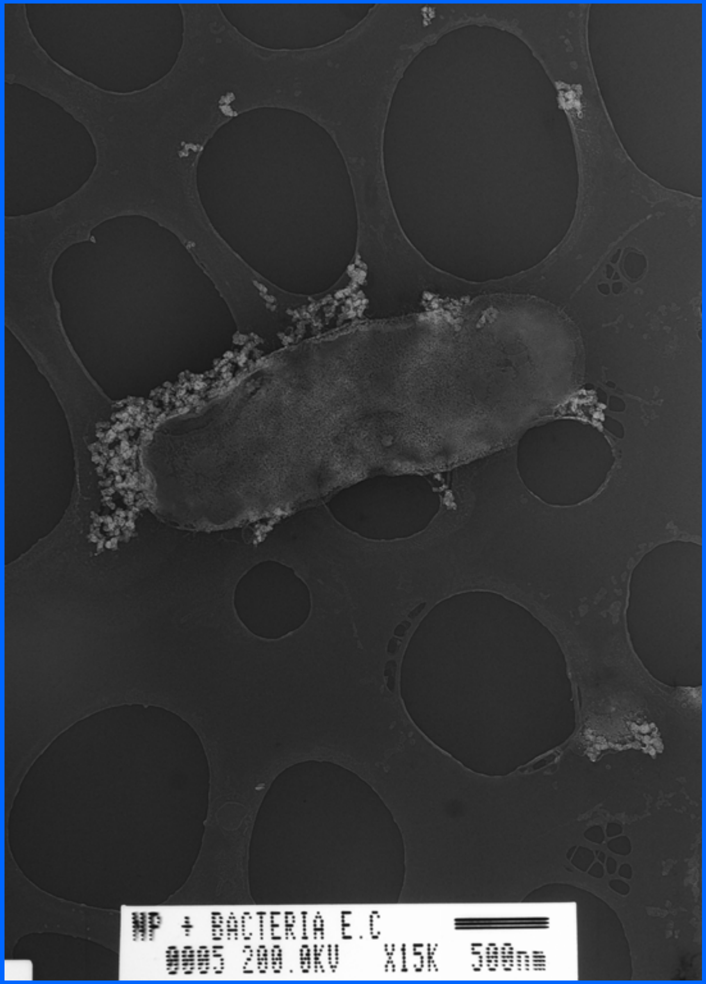
# Building layered consortia

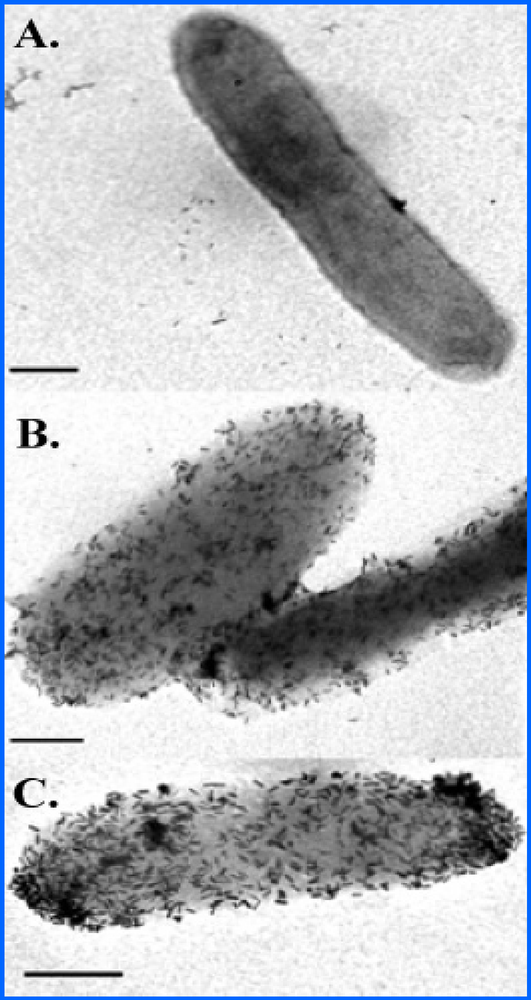


# Artificially constructed biofilms

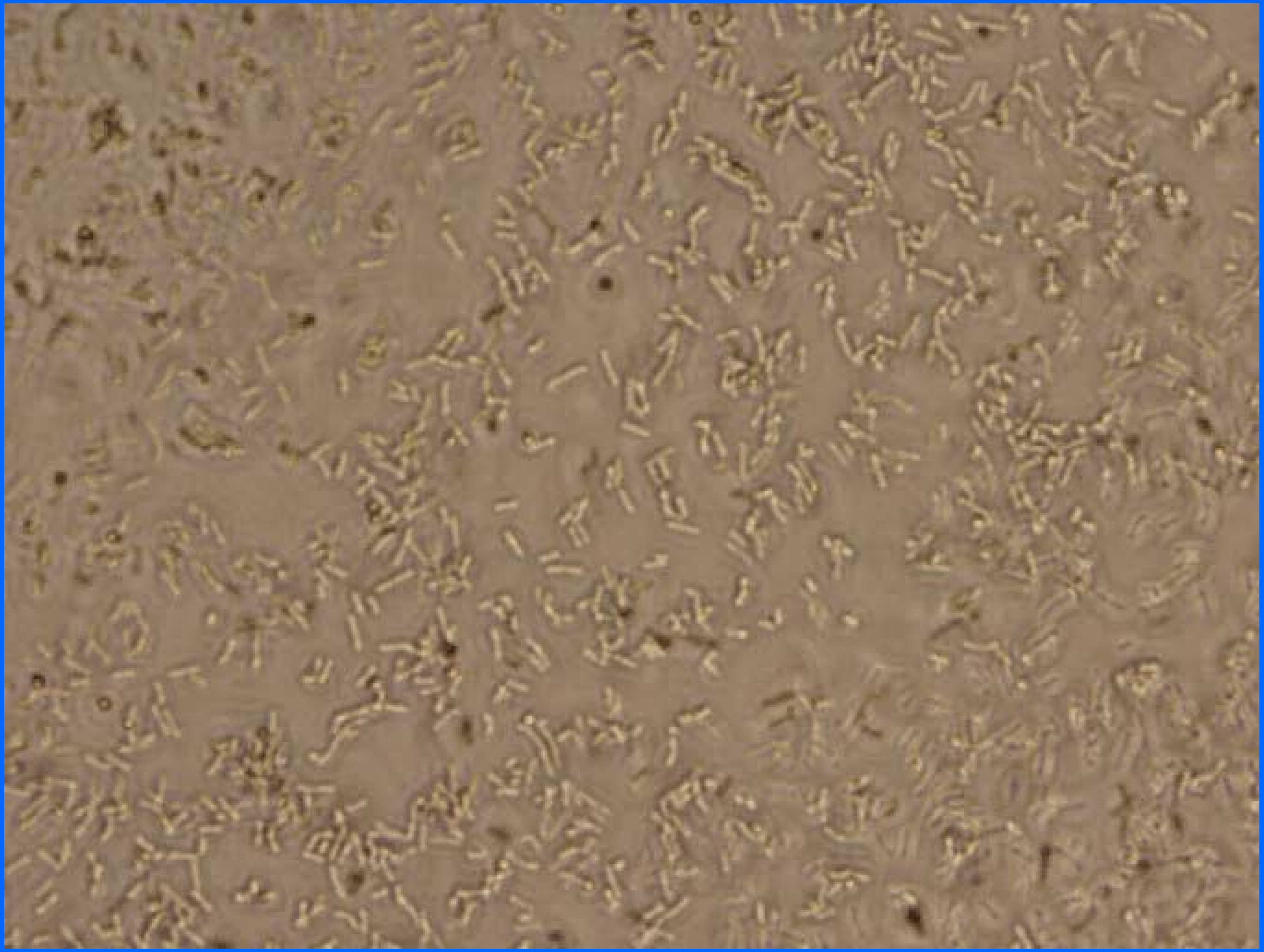


# Manipulating microbial cells with nanomaterials



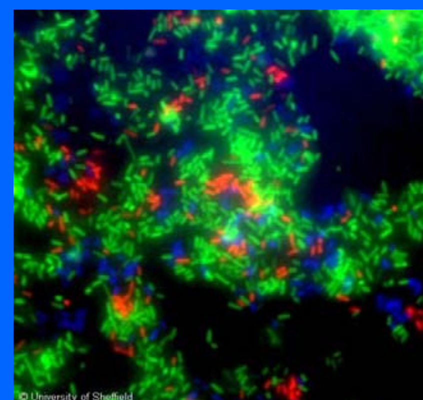
# Manipulation of bacterial cells with magnetic nanomaterials



Wei Huang University of Sheffield

# A synthetic biology approach to biotreatment of recalcitrant components of metal working fluids.

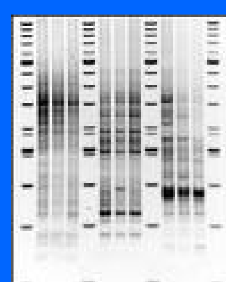
Ultrasound




Microbial community

Bioreactor + Xenobiotic

What survives?
Novel biology?
Community Function?
Enhanced Diversity?



Artificially encoded proteome
Encoding $10^8$ *de novo* enyzmes

# Conclusions

- Synthetic strains has great potential but could hold even greater promise when working with natural strains and consortia.
- Not least it is a good way to understand what makes good strains for exploitation.
- Scale up and control are key issues.
- Safety, containment and the right opportunity.

# Acknowledgement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