Accompanying notes for C9 Environmental Engineering 2.

Lecture 5 Manipulating microbes for sustainable clean-up

Slides 1-4 Despite microbial communities being the most metabolically diverse of all groups, with the ability to degrade most chemicals, contaminated sites are still prevalent. This is because microbial clean-up is not an infallible process- their beneficial effects need to be enhanced, engineered and sometimes stimulated. The example of pentachlorophenol shows in the right conditions bacteria exist that can degrade some very toxic compounds. However, as the soil contaminated with cyanide shows- even if the organisms present have the gene encoding for contaminant degradation, if the conditions are not appropriate bioremediation will not occur. In the case of the blue (**Slide 4**) the concentration of cyanide is so high it kills the microorganisms present or inhibits their activity.

Slides 5 Causes of microbial failures in terms of habitat clean-up.
The functional microorganisms or genes for degradation are present in very low numbers or absent.

The chemical conditions of the pollutant are unfavourable to microbial degradation - concentration too high or low to stimulate degradation.

The environmental conditions are biologically unfavourable for microbial growth and activity – for example pH, inadequate nutrients.

Slide 6 Manipulating microbial activity using physical and engineered approaches
Although microbial communities have huge catabolic potential in terms of bioremediation of contaminants, they are not infallible after all contamination anthropogenic origins are typically high concentration, chemical mixed and often very toxic. Microbial communities are not infallible and although they may not completely fail, biodegradation rates can be too slow for bioremediation to be a realistic option.

Slide 7 Dig and Tip
Until comparatively recently digging up the contaminated soil and burying it in landfill was the preferred option. Digging out contaminated soil and tipping it into landfill is no longer an option, not least it is expensive and generates a large carbon foot print. Consequently, in situ treatment is favoured.

Slide 8
Bioavailability- conditions for in situ bioremediation
A key issue in term of bioremediation is to optimise the bioavailability of the contaminant. As represented in the diagram, in order for the bacteria present to be effective at degrading the contaminant he has to be in close proximity. In addition, it requires other components including key nutrients such as phosphate and water. Phosphate is required as for cells to multiple and grow, and for energy transfer. All living things require water to be viable and active. The area in the diagram within the red circle represents the ideal conditions where the degradative bacteria is close to the contaminant and has all the other key components in close proximity (water, nutrients). The contaminant has to be in close proximity to the bacteria so that the degradation enzymes can act on the contaminant. Eventually the contaminant within the circled area is all degraded, so biodegradation rates fall until the bacteria can be moved to the next hot spot where the contaminant is present. Developing engineered ways to mix the soil contaminants and bacteria so they remain in close proximity as long as possible is a key engineering challenge since soil mixing is very energy demanding.

Slide 9
Options for manipulation soil contaminants
1. Physical,
2. Chemical and Biological approaches

Slide 10-11
Soil electrokinetics
With this approach an anode and cathode is placed in contaminated soil and an electric current is set off. At the anode the charge causes hydrolysis (splitting of water) generating H+ ions whilst at the cathode OH- ions are generated. This results in a pH gradient (acidic at the anode) which alters the chemical

environment of the soil contaminant. For example, the solubility of heavy metals increases in more acid condition. In addition, everything has a distinctive surface charge, this leads to electro-migration where the positively charged ions move to the cathode. Furthermore, bacteria and other organics move towards the cathode by electro-osmosis. The overall effect is that introduction of a low level of current has a big effect in terms of mixing/interaction of the contaminant and bacteria, and potential removing the metals. More details can be found: https://www.nature.com/articles/srep23833.pdf

Slide 12
Shows a combination of soil electrokinetics with bioaugmentation with bacteria able to degrade pentachloro-phenol (PCP). Electrokinetics encourages mixing of the bacteria and contaminant, warms the soil by 1-2ºC, alters the pH to optimise biodegradation in hot spots and introduces H^+ and Oxygen at the electrodes. These come together to stimulate biodegradation rates.

Slide 13
Graph shows the impact of electrokinetics on moving the PCP between two electrodes. In the field the whole PCP plume could be moved across a field site by occasionally moving the electrodes. A key requirement is that the water content of the soil is sufficiently high to enable electromigration of organic and bacteria.

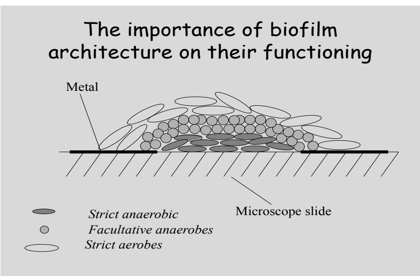
Slide 14-15
Biofilms- in terms of bioremediation treatment of water and water treatment systems, bacteria play a critical role. However, they are typically not free swimming in the water as individual cells, but form tight community clusters called biofilms. A **biofilm** comprises any group of microorganisms in which cells stick to each other and often also to a surface. These adherent cells become embedded within a slimy extracellular matrix that is composed of extracellular polymeric (snotty) substances (EPS). Because they have three-dimensional structure and represent a community lifestyle for microorganisms, they have been metaphorically described as "cities for microbes". Biofilms may form on living or non-living surfaces and can be prevalent in natural, industrial and hospital settings. The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which, by contrast, are single-cells that may float or swim in a liquid medium. For more details:

https://www.cs.montana.edu/webworks/projects/biofilmbook/contents/chapters/chapter001/section001/green/page001.html (biofilms)

Slide 14 and 15 shows biofilms growing in a laboratory bioreactor- they growing on a solid matrix and established for biotreatment of the waste water in the bioreactor. As with municipal water treatment- the waste water trickles over the biofilm- but stays anchored on the matrix of stones.

Slide 16
Biofilm development stages. Biofilms grow on surfaces, since this is often where nutrients accumulate. The process starts with a single cell attaching to a surface- may be due to charge effect, bacteria are negatively charged. The surface has a tendency to accumulate nutrients, this leads to cells multiply and proliferating. Biofilms form the basis of organic treatment in municipal water treatment plants. However, in engineered they can be problematic blocking up pipes.

Slide 17- Biofilm architecture



This shows the importance of the biofilm architecture in their functioning, such as decontamination of water. The biofilms are protective environments, the cells adhering to each other and produce extracellular polymeric material (rather like snot) which make the biofilms resistant to extremes of the environment such as toxic shock. This is what makes biofilms so difficult to kill. Cells in a middle of a biofilm can resist biocide concentration 10,000-fold higher than individual cells floating in suspension. Also, oxygen can only penetrate to a depth of 3mm. So as represented in the diagram there can be a successional change in functional group of bacteria with different oxygen requirements. On the outer surface aerobic forms proliferate. As they actively utilise oxygen, they make the centre of the biofilms deficient on O2, since it is utilised before it gets near to the lower surface communities. This enables the proliferation of anaerobic communities and activities such as reductive de-chlorination. Thus, even in an apparent aerobic environment, such as a water treatment plant, anaerobic processes can take place.

For more details see:

https://www.cs.montana.edu/webworks/projects/biofilmbook/contents/chapters/chapter001/section001/green/page001.html

Slides 18-20- Engineering Biofilm with electrokinetics
Clearly the architecture of the biofilms can have a significant impact on its functioning. This series of slides shows the basic principles of how biofilms can be engineered to have specific architectures and so function. The slide shows a modified microscope slide with a long narrow (75 micron) channel, composed of castellations. Bacterial cells are pumped into the channels. An AC current is than passed through the system. Since cells have a charge they align and controlled manner. By altering the shape of the castellations the architecture of the slides can be modified according to requirements. The challenge is to scale up the system. More details can be found here:

http://ieeexplore.ieee.org/stamp/stamp.jsp?tp=&arnumber=1266052

Slide shows the chambers with E.coli cells with the electric field off and then on. By putting the charge on the electric field holds the cells in distinctive architectures. Altering the shape of the castellations and charge conditions it is possible to alter the architecture of the biofilms

Slide 21 Stimulation of microbial activity using biological approaches (Vermiculture=worms)
Manipulating and engineering microbial communities is challenging in water systems as detailed above, however in a solid matrix such as soils the challenges are even greater. Soil mixing in order to stimulate soil activity and improve contaminant bioavailability is very energy demanding and can very disruptive to the environment. One way soil mixing can be achieved in way that does not require a big energy input is by vermiculture.

Slide 22- Engineering soil with earthworms

One way to generate soil mixing in order to enhance bioavailability is by encouraging the proliferation of earthworms. The presence of earthworms has many benefits to soil quality. Earthworms dramatically alter soil structure, water movement, nutrient dynamics, and plant growth. They are not essential to all healthy soil systems, but their presence is usually an indicator of a healthy system. Earthworms perform several beneficial functions. **Stimulate microbial activity**. Although earthworms derive their nutrition from microorganisms, many more microorganisms are present in their faeces or casts than in the organic matter that they consume. As organic matter passes through their intestines, it is fragmented and inoculated with microorganisms. Increased microbial activity facilitates the cycling of nutrients from organic matter and their conversion into forms readily taken up by plants. **Mix and aggregate soil**. As they consume organic matter and mineral particles, earthworms excrete wastes in the form of casts, a type of soil aggregate. Charles Darwin calculated that earthworms can move large amounts of soil from the lower strata to the surface and also carry organic matter down into deeper soil layers. A large proportion of soil passes through the guts of earthworms, and they can turn over the top six inches (15 cm) of soil in ten to twenty years. **Increase infiltration**. Earthworms enhance porosity as they move through the soil. Some species make permanent burrows deep into the soil. These burrows can persist long after the inhabitant has died, and can be a major conduit for soil drainage, particularly under heavy rainfall. At the same time, the burrows minimize surface water erosion. The horizontal burrowing of other species in the top several inches of soil increases overall porosity and drainage. **Improve water-holding capacity**. By fragmenting organic matter, and increasing soil porosity and aggregation, earthworms can significantly increase the water-holding capacity of soils. **Provide channels for root growth**. The channels made by deep-burrowing earthworms are lined with readily available nutrients and make it easier for roots to penetrate deep into the soil. **Bury and shred plant residue**. Plant and crop residue are gradually buried by cast material deposited on the surface and as earthworms pull surface residue into their burrows.

More can be found here:
https://www.nrcs.usda.gov/wps/portal/nrcs/detailfull/soils/health/biology/?cid=nrcs142p2_053863

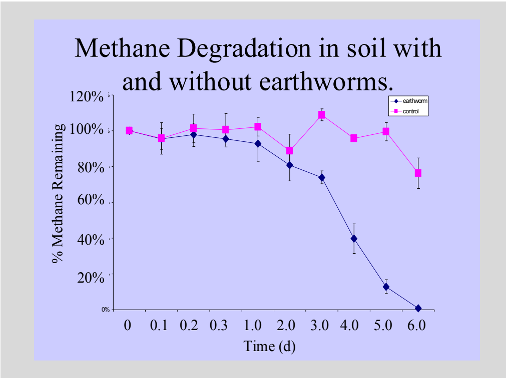
https://ac.els-cdn.com/S0929139314002091/1-s2.0-S0929139314002091-main.pdf?_tid=111569f0-d837-4d49-b989-61ac0595b18d&acdnat=1520339907_bcd320300b9885c755fe2ed5861c36e6

In addition, earthworm produce urea in its body slime which preferentially stimulates microbial communities that oxidise methane and ammonium

Slide 23 Community composition in different worm treatment
Shows a genetic profile of control soil with no worms. The soils maintain the characteristic soil horizons (layer O, A, B and C shown as depth in cm in the diagram). However, with worms added the soil layers show mixing.

Slide 24 and 25- Stable Isotope probing
This is detailed in Lecture 2 slides 25-26 and is to demonstrate the microbial community change induced by the presence of earthworms.
In this instance C_{14}-labelled methane is incubated with soils which containing active earthworms. Their activity stimulates the proliferation of methane oxidising bacteria which assimilate the CH_{14} so labelling DNA of new cell progeny. The total soil bacteria DNA is extracted, run on the gel and the C_{14}-labelled DNA sequenced. This shows the proliferation of CH_4 oxidising species.

Slide 26 Graphs shows the impact of earthworms on stimulating methane oxidation activity of bacteria.



Slide 27 Earthworm capping of landfill
The generation of methane from landfill is good example of sustainable energy. However, for older landfill generation production rates decrease making it no longer economical to collect the methane. As a consequence, the gas collection system is dismantled, leading to uncontrolled leaking of any remaining methane. This leakage is very damaging to the environment, in fact 21-fold more damaging to the ozone layer than CO_2. By placing a layer of fertile soil and inoculating with sufficiently high density of earthworms.

Slide 28 Phytoremediation
Microbes are by definition tiny so difficult to manipulate. However, soil microbial activities can be stimulated by adding plants to the system.
Phytoremediation is the direct use of living green plants for in situ, or in place, removal, degradation, or containment of contaminants in soils, sludges, sediments, surface water and groundwater. **Its** low cost, solar energy driven cleanup technique.

The approach has many advantages not least it is sustainable and as shown in the slide it can be effective at stimulating the biodegradation of soil organic contaminants, whilst also enabling co-contaminating metals to be removed by hyper-accumulation. Metal hyper-accumulating are plants that have evolved to seek-out, take-up and accumulate metals which is translocated and accumulated in the leaves. Some plants can accumulate up to 25% w/w toxic metals such as nickel. In the process of taking up and immobilising the metal, this results in the metal concentration immediately adjacent to the root decreasing as it is taken up and immobilised by the plant. The plants can be incinerated and used a bioenergy, meanwhile metals can be recovered from the ash.

The process does have its limitations- if the concentration of the contaminants is too high, the plants will not grow. The depth of clean-up is limited to the depth the roots can penetrate, and the process can be slow depending on local conditions.

Slide 29-30
Graph shows the stimulation impact actively growing plants have on microbial biodegradation of soil contaminating organics. This stimulating effect is due to a range of factors:

How does phytoremediation stimulate soil clean-up of organic contaminants?

- Plants pump nutrients into the soil.
- Up to a 100-1000-fold increase in microbial counts.
- Increased counts means increased degradative ability.
- Increased opportunities for gene exchange.
- Plants plants provide shade which helps maintain water content.
- Complex organics derived from plants stimulates community adaptation and ability to respond to complex organics.

Slide 31
Table summaries the various mechanisms and process by which plants can be exploited from remediating contaminated soil and stabilising habitats. Key advantage in terms of engineered applications is that its sustainable, can be effective over large areas, but cleanup process might take several seasons.

Overview of phytoremediation applications

Technique	Plant mechanism	Surface medium
Phytoextraction	Uptake and concentration of metal via direct uptake into the plant with subsequent removal	Soils
Phytotransformation	Plant uptake and degradation of organic compounds.	Surface water and groundwater
Phytostabilisation	Root exudates precipitate and make metal less available and toxic.	Soils, groundwater and mine tailings
Phytodegradation	Enhances microbial degradation in the rhizosphere	Soils, groundwater within the rhizosphere
Rhizofiltration	Uptake of metals into plants roots	Surface water
Phytovolatilisation	Plants evapor-transpiration of metals such as mercury and hydrocarbons	Soils and ground water
Vegetative cap	Rainwater is evapor-transpirated by plants to prevent leaching contaminants from disposal sites	

Pure Appl. Chem., Vol. 73, No. 7, pp. 1163–1172, 2001 modified from Vidali

Slide 32.
Many natural plants metabolites such as salicylic acid (the active ingredient of aspirin) are very effective at stimulating degradation of some very recalcitrant contaminants.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5000603/pdf/ijms-17-01205.pdf

Slide 33 and 34
Shows the stimulatory impact of introducing two secondary plant metabolites (cymene and Cumene) for stimulating microbial rates of herbicide (isoproturon) biodegradation. The hypothesis of how this works is that the cumene is chemically similar in some regards to the isoproturon but is more palatable to exposed microbial cells. The cumene switches on the biodegradation gene cascade and when it is completely degraded it then starts to degrade the isoproturon.

Lecture 6 Case study. Metal working fluids. End of life sustainable disposal and management of in use fluids.

Slide 1-4. Metal working fluids (MWF) are selected as a model representative of other potential waste problems generated by industry- not least 'Fracking oils'. It has been selected since it is a hazardous waste (some are carcinogenic) that has been specifically designed/formulated to be toxic. This is because MWF are oil:water (1:9) suspensions designed to lubricate metals during the working process (lathe, cutting, forming). They are toxic in large because since they are oil: water suspension so attractive an attractive nutrient source for microorganisms. Microbial colonisation and growth cause premature deterioration of the MWF whose chemical composition is specifically formulated for working specific metals/materials. In order to prevent premature biodeterioration MWF are specifically formulated to contain biocides (chemical agents design specifically to kill biological pests) and to contain other chemical constituents that known to be resist to microbial attack (such as highly branches chemicals and large polymers).

Designing/formulating products that are durable and resist to biotransformation is good for increasing the longevity of products. The problem comes when product becomes operationally exhausted and needs to be disposed. Added to this is that MWF are 90% water the rest oil and the driver now is ideally to recycle the water after decontamination, ideally on site, so reducing the cost and energy foot print associated with treatment. Furthermore, the oil has a high energy content.

Sides show
-Large quantities generated by industry, used for cutting and forming metal products in engineering workshops.
-These are specifically designed to be toxic in order to prevent bio-deterioration by bacteria when in use.
- Are synthetic so few bacteria are likely to have evolved the ability to biodegrade it.
- Its toxicity makes sustainable disposal problematic, not least it is classified as a hazardous waste so rules for their disposal are increasing stringent.
- They are 90% water in composition, the rest is oil emulsion with a range of additives for preventing metal corrosion, heat exchange. Be able to recycle their water content on-site has many advantages.

Slide 5 & 6. Various engineering workshop waste water disposal options.
Until increasing concerns regarding sustainability, the favour disposal methods have been:
•**Landfill**- increasing stringent environmental contamination rules have banned this approach- in particularly for toxic waste waters.

•**Ultrafiltration**- expensive capital cost for the equipment, not effective for removal of smaller molecules (eg synthetics), problematic sludge generated that still has to be disposed.

•**Incineration**- expensive and not sustainable, burning water based products is very energy intensive.

•**Flash Evaporation**- expensive and not sustainable. Also, importantly the water is lost.

- Best to treat on site where it is being used. Tankering waste water is costly and not good for the local environment.

http://www.ukla.org.uk/wp-content/uploads/UKLA-PERA-Best-Practice-Guide-for-the-Disposal-of-Water-mix-Metalworking-Fluids.pdf

Slide 7. Biological treatment approach
The biodegradation of the oil component scores well in terms of sustainability. With this approach the oil is broken down to CO2 and water, and the cleaned water can be recycled on site. The key issue is the detection and isolation of microbial species/strains that can survive in the extreme environment of the MWF- since they are designed specifically to be resistant to biotransformation. Exposed to chemical toxicity and high temperatures.

Slide 8. Sourcing bacteria that can biodegrade the metal working fluids (MWF)
•The best source of microbial populations able to degrade an organic contaminant is a site or substrate in which communities have had long term exposure. So, in the example of metal working fluid treatment, the

best source of an effective treatment inoculum will be engineering workshops that have had long term exposure to the target contaminant
• Microbial populations have had the time to acclimate and adapt to the toxicity of the contaminant.
• Have the potential ability to survive temporally and spatially.
• Ability to utilise contaminants as nutrient or energy source.

https://link.springer.com/article/10.1038/sj.jim.7000271

Slide 9 and 10
Detection of bacteria able to persist in the extremes of the MWF is best achieved by taking samples from engineering machines that have been exposed to MWF for many years. Genetic (DNA-based) profiling provides a measure of the temporal and spatial distribution of the most abundant microbial populations. Their consistent presences suggests' that they are well adapted to living and thriving in MWF.

Slide 11. Microbial Enrichment Procedure
Detecting temporal and spatial abundant microbial populations in widely distributed sites and machine application is the first part of the exploitation process. In order to develop a scale-able treatment process, its essential to isolate the key bacteria able to degrade the MWF. The isolation process requires an **enrichment (See Brock enrichment and isolation methods)** process whereby MWF samples (solution of biofilms growth) are collected and added to flasks containing all the nutrient required for growth, with exception of a Carbon source. All living cells require a Carbon source in order to grow and be active. In this case, this is provided in the form of waste MWF. The solution is then then placed on an orbital shaking incubator (at about 25°C) for a few days/weeks until there are signs of growth (the solution becomes opaque). The only ways there can be any microbial growth is if the microorganisms present can utilise the MWF as a carbon source which become enriched in terms of abundance (cell numbers) since the other cells present cannot grow. The process is repeated two or three times and at each step the proportion of the community that can biodegrade MWF and use it as an energy and nutrient source increases.

Slide 12 Isolating the target bacteria from the enrichment.
In order to be able to exploit the MWF- degradation bacteria in scaled up engineered application it is essential to isolate the individual colonies from the enrichment solution. This is achieved by developing an isolation growth medium containing all nutrients for growth and with MWF as the sole carbon source. It also contains agar to make a solid medium so that individual colonies can grow, and their purity checked. **See notes from lecture 2.**

Slide 13-15 – Screening for MWF degraders
Once the purity of the isolates has been checked the next stage is to check the ability of the individual selected bacteria to grow in the presence of MWF and its individual chemical constituents. It is of course important to determine if they are able to biodegrade the MWF- this is determined by measuring the decrease in Chemical Oxygen Demand when the bacteria is added growth solution.

Slide 16 Bioreactors
A consortium of 4-5 bacterial species is eventually assembled, grown up in broth and then used to inoculate a bioreactor. The MWF is then treated in batches in the experimental lab reactors. The parameters listed in the slide are then systematically manipulated to optimise rates of MWF biodegradation.

Slide 17 and 18
The bioreactors are then scaled up and performance tested. The system depicted in the graph is a 'fill and draw' approach- the bacteria cells are held anchored as biofilm on a holding matrix and the MWF treated and then the reactor emptied and replenished with waste MWF. This is shown in the peaks and troughs. When the COD is below legal consent to release levels it is discharged and a replaced with a new batch of waste MWF. The bio-degradative inoculum is retained on a holding matrix. In a continuous system the waste MWF solution is flowed over the anchored biofilm.

https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1462-2920.2004.00566.x

Slide 19

Shows functioning of a fully scaled up bioreactor treating real engineering workshop waste.

Slide 20 and 21 Treatment testing and toxicity
A key legal requirement before the treated water is deemed to be clean and can be discharged or recycled. The legal measure is to reach the consent to discharge COD level set. But another important consideration is the toxicity of the water, that is because although a waste water may have a low COD but may still have toxic components. The best way this can be achieved is employing **Bacterial biosensors**. With this the metabolic activity of cells is linked to an added gene encoding for luminescence (from a firefly). So, when the cells are metabolically active they luminescence, but in toxic waters their luminescence is suppressed. This is a semi- quantitative response and as can be seen is Slide 20 correlates well with COD.

Slide 22 Advantage of the approach
Robust & effective- cleanup normally within 3-6 days.
•Treatment can be in batches or continuous.
•Works at any scale.
•No waste by-products such as sludge.
•Can deal with a range of wastes (formulations).
•Lowers toxicity (as tested using bacterial biosensors), treating water on site.

Slide 23 and 24
Microbial biodegradation is not infallible, and some compounds are just not amenable to biotreatment or more recalcitrant. The graph shows the persistence of branched Fatty acid molecules. Bacteria are not good at degrading branched molecules.

Slide 25 Fentons Reaction
Fenton's reagent is a solution of hydrogen peroxide with ferrous iron as a catalyst that is used to oxidize contaminants or waste waters- it is a far more robust oxidising agent than bacteria. So, it is a chemical way to oxidize organics that bacterial cannot metabolise.

Slide 26- Biodegradability index
This is the ratio of the organic that can be oxidised biologically over 5 days incubation (BOD_5) period to that which can be oxidised employing more vigorous Chemical oxidation methods (COD). The graph shows that the addition of Fentons changed the chemical nature of the organic making the proportion that available for biological degradation increase.

Slide 28 – The concept of Sustainable Design
For MWF a key issue in terms of life time management and in particular end of life treatment- is to design new MWF formulations that are resistant to Biodeterioration when in operation on the machine but can be treated in a sustainable way end of life. The key challenge of current MWF formulations in terms of making amenable to end-of-life as well as containing recalcitrant components, they also contain biocides (chemical specific for killing biological cells).

Slide 29 Dealing with Formaldehyde
Currently a standard biocide employed to stop microbial growth in MWF is formaldehyde. It's very toxic and expensive to remove from water. Banned from use in MWF although it still is still widely used. The concept of 'Sustainable Design' is the formulate new products where the focus is life time management in mind. In this instance developing a biocide that is not universally toxic and recalcitrant, so it does not persist in the environment. Hence the increasing interest in natural products to make products more sustainable.

Slide 30-31 Natural products as alternative biocides
Allyl Isothiocynate and Isocyanate. Are natural biocides which selective ability to inhibit/kill microbial cells. They are also with time degradable and non-toxic to human exposure. Allicin- the active constituent of garlic has a potent biocide. It attacks the cell wall puncturing a hole in the

double membrane. The problem is many products such as allicin are unstable and breakdown very quickly. This can now be resolved by conjugation of the allicin with nano-gold.

Slide 32 – Smart delivery of biocides
What is summarised in the slide is the delivery of a volatile biocide. The biocide is encapsulated into a mesoporous silicate that is 200nm in diameter. Mesoporous silicate (MPS) are like small sponges used increasingly in medicine for intravenous deliver of drugs in patients. In this instance the MPS is loaded and capped with a sugar. It is then ready to stop microbial growth in a water body. If there are no bacteria in the water body, then the MSP stays intact. However, in the presence of bacteria- they perceive the sugar cap as a nutrient source which it is, but that causes the biocide to be released exactly where the cells are present. Thus, the biocide is only released when it is required, since it is a targeted localised release the concentration of biocide required to kill the cells is reduced and the biocide is only released into the water when it is required. In this way water is not 'contaminated' unnecessarily and can more easily recycled.

Slide 33- **Microbiological transformation of waste organics to bioenergy (methane and hydrogen) and bioplastics**

The ideal is to design a MWF product which is durable and effective for lubricating and working metals, but end of life is transformed in a sustainable manner into these instances bioenergy (methane and hydrogen) and to generate bioplastics or precursors. In this way the carbon component is transformed to useful products and the water can be recycled ideally on site.

Lecture 7 Renewable resources and bioenergy

Slide 1. The Water, energy, waste nexus
Water, energy and waste. Closely linked. For Example, extraction of oil requires a lot of water. The oil industry pumps more water than oil. Waste is increasingly seen to be a resource contain containing valuable water, metals and phosphate. Waste waters are also rich in energy by converting the organic content into methane.

Slide 2 Human ecological footprint
Society is living beyond the resources available on the plant- current consumption is not sustainable. To maintain current resource consumption rates requires 1.5 planets. Resulting in degradation of the planet. Been living beyond the Earth capacity since 1970. We need to urgent live in a more sustainable.

Slide 3. Show UK energy sources
Show change in energy sources from 1970 to 2012. Reveals little real change, the predominant energy source is still hydrocarbons. Only real change is the decrease us of coal. But there is an increase in sustainable energy generation (wind, solar).

Slide 4. Energy Consumption by sector
Nearly two-thirds energy used in the UK goes for Transport and Domestic use.

Slide 5. Development of more sustainable energy generation.
We need to mimic the efficiency and sustainability of natural systems. Photosynthesis- the combination of C02, water and sunlight, results in generation of Hydrocarbon. The waste product I water and oxygen, which are valuable resources. Photosynthesis is how all the hydrocarbons (oil, coal, gas) were generated.

Slide 6. Renewable energy sources
Wind and solar energy are clean but supplies can be unpredictable. There are also of issues with respect to storing the energy required- but storage is costly and still not efficient. Also generation of solar panels generated a lot of toxic chemical by-products from the manufacturing process.

Slide 7. Rare earth supplies for clean technologies are limited
https://www.energy.gov/sites/prod/files/DOE_CMS2011_FINAL_Full.pdf

https://www.energy.gov/sites/prod/files/2018/01/f47/EXEC-2014-000442%20-%20for%20Conrad%20Regis%202.2.17.pdf

These US Department of Environment Reports highlight that expansion of clean- energy (solar, wind turbines, batteries) technology is limited by the availability of some key earths essential for batteries, wind and solar hardware.

Slide 8 Periodic table showing key essential elements that are in increasingly limited supplies.
More details can be found here:
https://chem.as.uky.edu/datwood/sustainability-endangered-elements

Key elements such as phosphate, which are essential for healthy living, are believed to have about 50 years of reliable supplied. Phosphate is required to generate ATP and for the synthesis of DNA- so is required for all biological processes. So, nothing will grow or function without it.

Slide 9 and 10. Life cycle of metal resources

Metals differ from organic resources since they can be recycled. Organics in most cases such as oil – certainly in the case for energy generation, can only be consumed once. So, metals are now routinely recovered and recycled in the aerospace and automobile industry. However, their mining and purification is very damaging to the environment, is energy demanding.

Slide 11. Bacteria Bioleaching
https://en.wikipedia.org/wiki/Bioleaching
Mining and extracting metals is very energy demanding. Bioleaching which also can include phyto-extraction and are ways of reducing the energy footprint of mining. Extracting and crushing requires a lot of energy. Around 5-10% energy used globally is used in mining, extracting and purifying metals. Bioleaching reduces the need for physical processing of mineral containing substrates.
https://publications.csiro.au/rpr/download?pid=csiro:EP12183&dsid=DS3
Bioleaching – is the process whereby bacteria metabolise the surrounding substrate so releasing the target mineral. This reduces the amount of extraction energy required in subsequent treatments,
https://link.springer.com/content/pdf/10.1007%2Fs00253-003-1404-6.pdf

Slide 12 Phytoextraction
With this more sustainable approach plant root exudes carbon which is metabolised. This acts as a nutrient source which is degraded forming acids. In acid conditions metals become more soluble and go into solution which can be taken up by the plants. In some plants the metal is taken up and concentrated in the leaves.

Slide13 Biosensors for detecting resources (metals)
Bacteria can be genetically engineered to contain genes from a firefly that encodes for fluorescence. They can be engineered so that they fluorescence only in the presence of specific metals. The method can be semi-quantitative, very sensitive and potentially cheap,

Slide 14 Waste Hierarchy
Industry has moved on from the concept of waste- it's now referred to as a resource. The emphasis now is on reducing the loss of the resource, waste minimisation and recycling with reuse. The last resort is energy recovery and disposal, but all the previous options are preferred.

Slide 15 Plants biomass is increasingly going to be a source of bioenergy and high values organics such as those for the pharmaceuticals and bioplastics

Slide 16 Exploitation of the total plant biomass and the concept of biorefinery
A biorefinery is a facility that integrates biomass conversion processes and equipment to produce fuels, power, and chemicals from biomass. The biorefinery concept is analogous to today's petroleum refineries, which produce multiple fuels and products from petroleum. Industrial biorefineries have been identified as the most promising route to the creation of a new domestic bio-based industry.
By producing multiple products, a biorefinery can take advantage of the differences in biomass components and intermediates and maximize the value derived from the biomass feedstock. A biorefinery might, for example, produce one or several low-volume, but high-value, chemical products and a low-value, but high-volume liquid transportation fuel, while generating electricity and process heat for its own use and perhaps enough for sale of electricity. The high-value products enhance profitability, the high-volume fuel helps meet national energy needs, and the power production reduces costs and avoids greenhouse-gas emissions.

http://www.essentialchemicalindustry.org/materials-and-applications/biorefineries.html

Slide 17 Succinic acid use and production
Succinate is selected as an example of a platform chemical- that means it is a base chemical from other essential chemical can be synthesised. It is used in a lot of industries where it plays a vital role (food, polymers, precusors). The vast majority is produced from oil- however the same product can be generated using anaerobic bacteria.

Slide 18 Biomass as a biorefinery for fine chemicals
Shows the kind of products that can be from biomass using various approaches

Slide 19 Shows the chemical composition of plant tissue that microbes have to biotransform to generate high value products such as bioenergy and high value platform chemicals
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%).

These polymers are assembled into a complex, interconnected matrix within plant cell walls. Cellulose and hemicellulose are carbohydrates that can be broken down into fermentable sugars. Cellulose consists of long chains of glucose molecules (simple 6-carbon sugars) arranged into a solid, three-dimensional, crystalline structure. Hemicellulose is a branched polymer composed primarily of xylose molecules (simple 5-carbon sugars) and some other sugars. Lignin, a rigid aromatic polymer, is not a carbohydrate and cannot be converted into ethanol.

Cellulose is fairly easy to biodegrade and there are plenty of microorganisms that can degrade them. However, the lignin component of the plant tissue – which is essential equivalent to the skeleton, is much more difficult to biotransform. It is a branched molecule, and microorganisms are not good at assimilating branched chemicals. Added to that it is very effective at irreversibly immobilising cellulases rendering them ineffective.

Slide 20 and 21. Transforming plant waste to useful products
Because lignin is biologically so unpalatable it requires pre-treatment to breakdown the lignin so that it becomes to more amenable to biotransformation. Saccharification - the process of breaking a complex carbohydrate (as starch or cellulose) into its monosaccharide components. Unfortunately, this entails high concentration of acid or alkali, pressure and temperature, which reduces the sustainability credibility of the approach.

Slide 22 and 23 Anaerobic Digestion to generate biogas.

The unfavourable sustainability status of Saccharification has stimulated growing interest in Anaerobic Digestion of organics to produce biogas. But also the microbiology can be modified so instead biogas being generated from the volatile fatty acids, they can be employed to generate other high value products such as polyhydroxybutyrate which is precursor for bioplastics. This is a potentially an enormous new market

Slide 24-26 Stages of anaerobic digestion of organics by bacteria
Cows are incredible efficient at converting vegetation (grass) into meat- unfortunately the by-product is methane production which contributes to global warming. However, it's is not the cow that bio-transform the vegetation- like humans they lack the cellulase enzymes required for cellulose biotransforamtion. It's the microorganisms in the cows rumen that carry out the process.

There are a number of different functional groups of bacteria that are involved in the process of anaerobic digestion including acetic acid-forming bacteria (acetogens) and methane-forming bacteria (methanogens). These bacteria feed upon the initial feedstock, which undergoes a number of different processes converting it to intermediate molecules including sugars, hydrogen & acetic acid before finally being converted to biogas.

Different species of bacteria are able to survive at different temperature ranges. The ones living optimally at temperatures between 35-40°C are called mesophiles or mesophilic bacteria. Some of the bacteria can survive at warmer and more hostile conditions of 55-60°C, these are called thermophiles or thermophilic bacteria. Methanogens come from the primitive group of archaea. This family includes species that can grow in the hostile conditions of hydrothermal vents. These species are more resistant to heat and can therefore operate at thermophilic temperatures, a property that is unique to bacterial families.
In an anaerobic system there is an absence of gaseous oxygen. In an anaerobic digester, gaseous oxygen is prevented from entering the system through physical containment in sealed tanks. Anaerobes access oxygen from sources other than the surrounding air. The oxygen source for these microorganisms can be the organic material itself or alternatively may be supplied by inorganic oxides from within the input material. When the oxygen source in an anaerobic system is derived from the organic material itself, then the 'intermediate' end products are primarily alcohols, aldehydes, and organic acids plus carbon dioxide. In the presence of specialised methanogens, the intermediates are converted to the 'final' end products of methane, carbon dioxide with trace levels of hydrogen sulphide. In an anaerobic system the majority of the chemical energy contained within the starting material is released by methanogenic bacteria as methane.
Populations of anaerobic bacteria typically take a significant period of time to establish themselves to be fully effective. It is therefore common practice to introduce anaerobic microorganisms from materials with existing populations. This process is called 'seeding' the digesters and typically takes place with the addition of sewage sludge or cattle slurry.

There are four key biological and chemical stages of anaerobic digestion.
Hydrolysis
Acidogenesis
Acetogenesis
Methanogenesis

In most cases biomass is made up of large organic polymers. In order for the bacteria in anaerobic digesters to access the energy potential of the material, these chains must first be broken down into their smaller constituent parts. These constituent parts or monomers such as sugars are readily available by other bacteria. The process of breaking these chains and solubilising the smaller molecules into solution is called hydrolysis. Therefore, hydrolysis of these high molecular weight polymeric components is the necessary first step in anaerobic digestion. Through hydrolysis the complex organic molecules are broken down into simple sugars, amino acids, and fatty acids.
Acetate and hydrogen produced in the first stages can be used directly by methanogens. Other molecules such as volatile fatty acids (VFA's) with a chain length that is greater than acetate must first be catabolised into compounds that can be directly utilised by methanogens to generate methane.
The biological process of acidogenesis is where there is further breakdown of the remaining components by acidogenic (fermentative) bacteria. Here VFAs are created along with ammonia, carbon dioxide and hydrogen sulphide as well as other by-products. The process of acidogenesis is similar to the way that milk sours.
The third stage anaerobic digestion is acetogenesis. Here simple molecules created through the acidogenesis phase are further digested by acetogens to produce largely acetic acid as well as carbon dioxide and hydrogen.
The terminal stage of anaerobic digestion is the biological process of methanogenesis. Here methanogens utilise the intermediate products of the preceding stages and convert them into methane, carbon dioxide and water. It is these components that makes up the majority of the biogas emitted from the system. Methanogenesis is sensitive to both high and low pHs and occurs between pH 6.5 and pH 8. The remaining, non-digestible material which the microbes cannot feed upon, along with any dead bacterial remains constitutes the digestate.

https://www.cti2000.it/Bionett/BioG-2003-002%20IntroAnaerobicDigestion.pdf

Slide 27. Benefits of Anaerobic Digestion in the carbon economy
by preventing the uncontrolled emissions of CH_4
(21 times more powerful than CO_2);
• by beneficial use of the biofertiliser in agriculture, displacing mineral fertilisers;
• by reducing the transport of waste; and
• by the production of renewable energy.
•Local security of supplies, reduced dependence on the national grid.
•Resistant organics can be converted to charcoal.

Slide 28 to 30. Bio-diesel production
http://www.esru.strath.ac.uk/EandE/Web_sites/02-03/biofuels/what_biodiesel.htm

•Bio-diesel is considered to be an excellent renewable carbon-neutral fuel, but to enhance its economic viability, improved production systems must be developed.

The Table shows that with current crops used to for biofuels such as Soya and Rape seed the fuel yield is very low. Algae grown in controlled optimised conditions are much more efficient.

Slide 31 Challenges of efficient energy from algae
With genetic engineering there are opportunities to improve the efficiency of algae even further to transform light into other forms of energy and high value products. Key issues include improving tolerance in sub-optimal waters – such as salty and wastewater. What is required is development of bioreactors which enable 3-D exposure to light. There is also potential to genetically engineer to generate high value products such as pharmaceuticals. So, the strains have dual and triple functions- water cleanup, energy production and high value products.

Slide 32 Microbial fuel cells
A further way of generating bioenergy from waste water organics is employing Microbial Fuel Cells.

A microbial fuel cell is a device that converts chemical energy to electrical energy by the catalytic reaction of microorganisms (Allen and Bennetto, 1993). A typical microbial fuel cell consists of anode and cathode compartments separated by a cation specific membrane. In the anode compartment, fuel is oxidized by microorganism, generating electrons and protons. Electrons are transferred to the cathode compartment through an external electric circuit, and the protons are transferred to the cathode compartment through the membrane. Electrons and protons are consumed in the cathode compartment, combining with oxygen to form water.

When micro-organisms consume a substrate such as sugar in aerobic conditions they produce carbon dioxide and water. However, when oxygen is absent they produce carbon dioxide, protons and electrons as described below (Bennetto, 1990):
$C_{12}H22O11 + 13H2O \text{--->} 12CO2 + 48H+ + 48e-$ but could be waste water organic to energy.

https://ac.els-cdn.com/S0378775317304159/1-s2.0-S0378775317304159-main.pdf?_tid=1eb40ed3-55d8-4114-adba-0006126285c3&acdnat=1523523998_c5833f1b55676486695e6f9a4b2757af

Slide 33 CO2 reduction using electricity
This is technology which is very much in its infancy. The idea is to exploit cheap and readily available electrons from solar to use this to reduce CO_2 which is an excess. Solar energy is

getting cheaper. Electrons are exploit to reduce the CO_2 to formate, from which other chemical can be generated including bioplastics and other platform chemicals. This way cheap electricity can be used to transform CO2 to valuable products.

https://www.tandfonline.com/doi/full/10.1080/21553769.2016.1230787

Summary

- Dwindling supplies of key minerals are driving the need for more efficient use of resource/waste.
- Biomass could be an effective source of energy and resources.
- Converting waste such as CO2 to high value products.
- We need new engineering methods for obtaining resources- includes genetic engineering.
- More localised production.
- Scale-up of sustainable technologies.

Lecture 8 Nanotechnology: Solutions to environmental problems.

http://pubs.rsc.org/en/content/articlepdf/2017/en/c6en00505e (water treatment)

https://ac.els-cdn.com/S138917230670620X/1-s2.0-S138917230670620X-main.pdf?_tid=a74bb634-3702-49b5-9bb4-fb1239dbe51e&acdnat=1525435988_73edc7356722f389d027e08bf042af39
(environmental application of nanomaterials)

Slide 2 Definition
Nanomaterials is a field that studies materials with morphological features in the nano-scale, and especially those that have special properties stemming from their nano-scale dimensions. Nano-scale is usually defined as smaller than a one tenth of a micro-meter in at least one dimension (100nm), though this term is sometimes also used for materials smaller than one micro-meter.

Slide 2 Nanomaterial properties
An important aspect of nanotechnology is the vastly increased ratio of surface area to volume present in many nano-scale materials (e.g., upwards of 100 m^2 per gram), which makes possible new quantum mechanical effects. One example is the "quantum size effect" where the electronic properties of solids are altered with great reductions in particle size. The effect becomes pronounced when the nanometer size range is reached. This also includes changes in physical property.

- Such striking features such as nano-scale gold which is normally inert becomes very active/catalyst.

Slide 4 Nanoscale size effect
The key phenomenon is that decreased particle size leads to a broad range of property changes:

Physical Properties (e.g. melting point)
- Chemical Properties (e.g. reactivity)
- Electrical Properties (e.g. conductivity)
- Mechanical Properties (e.g. strength)
- Optical Properties (e.g. light emission)

Slide 5 Graphene
https://www.graphene.manchester.ac.uk/learn/applications/
Graphene is made of pure carbon, with atoms arranged in a regular hexagonal pattern similar to graphite, but in a one-atom thick sheet. It is very light, with a 1 square meter sheet weighing only .77 milligrams. The potential application of this recently discovered material are enormous. Transport, medicine, electronics, energy, defence, desalination; the range of industries where graphene research is making an impact is substantial.

Slide 6 Nanomaterial origins
There are three sources of nanomaterials. Natural (for e.g. dust), Incidental (e.g. products of combustion), and synthetic (e.g. carbon-based and metal oxides).

Slide 7 Nanotechnology applications
These are very broad ranging and likely to impact on many technological areas and products. These include inf

Slide 8-10. Environmental applications for remediation. Zero valent nanoscale iron oxide

https://link.springer.com/content/pdf/10.1023%2FA%3A1025520116015.pdf

One of the most widely used nanomaterials for remediation of both organics and metals is zero valent metal oxide. It is very effective at oxidising organics and immobilising metals. The Table shows the extend diminishing size of the material increases the rate and capacity to immobilse Chromate. It is also very effective at dechlorinating troichloroethene.

Slide 11-12. Nano-based membrane filters for removing environmental contamination
Both table shows that nanofiltration (pore size 0.001-0.0001) are very effective at remving key environmental contaminants (e.g. pesticides, divalent ions) which tradition filtering methods were ineffective at removing. This has had a significant impact in terms of removing some potential very toxic contaminants, in particular those in water.

Slide 13-16 Nanomaterials for killing pathogenic bacteria
A key challenge for society today is the issue of antibiotics resistance. With 70 years of, in many cases, over use bacteria have developed resistance to conventional anti-biotics. An alternative approach is to use nanomaterials. Silver for instance is has been known for centuries to have strong antimicrobial properties:

Silver nanoparticles act primarily in several ways against:
(1) nanoparticles attach to the surface of the cell membrane and drastically disturb its permeability and respiration activity;
(2) they are able to penetrate inside the bacteria and cause further damage by possibly interacting with sulphur- and phosphorus-containing compounds such as DNA;
(3) Nanoparticles release silver ions, which have an additional contribution to the bacterial killing effect of the nano-particles.

At nano-scale silver is significantly more toxic than at macro-scale, and even the shape has a differential kill of cells

https://particleandfibretoxicology.biomedcentral.com/track/pdf/10.1186/1743-8977-8-36?site=particleandfibretoxicology.biomedcentral.com

https://link.springer.com/article/10.1007/s12257-016-0641-3

Slide 17 Nanoscale Titiania as a means of killing bacteria
It is not only intrinsic toxicity of materials that can kill bacteria. Titania is inert and not toxic towards bacteria. This can be seen in the graph. However, when the titania is exposed to ultra-violet light, it releases free oxygen radicals from the titania killing the bacteria. The advantage is that the killing agent is potent but is short lasting, so there is no opportunity for the exposed bacteria to adapt and develop resistance to it.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2644973/pdf/1423-0127-16-7.pdf

Slide 18. Smart nanomaterials for killing microbial cells

https://pubs.acs.org/doi/pdf/10.1021/nn700191f

http://rsif.royalsocietypublishing.org/content/royinterface/14/126/20160650.full.pdf

Another way of exploiting nanomaterials for killing bacteria is as a delivery system. Mesoporous silicates are essential nano-scale sponges. In the papers detailed above they have been exploited as biocide delivery systems. Compounds that may not be suitable as killing agents because they are too volatile, can be immobilised with the mesoporous silicates which are then capped, so preventing leakage. So the killing agents is kept locked in the silicates, which vary in size from 40 to 200 nm in diameter. However, when bacteria are present the cap can be removed, for instance by the application of ultrasound. Alternatively, the cap can be made of a sugar- when the bacteria detect the particle it assimilates the sugar so releasing the killing agent.

Slide 19. Nanomaterial manipulation of cells

The potential to manipulate microbial cells with nanomaterials is enormous from killing, isolating and removing. One way this can be done is to alter the surface chemistry of the nanomaterial, making it with a positive surface charge. Bacteria are typically negatively charged, so the nanomaterial is attracted to the cells by electrostatic attraction. This can be used to make the cells magnetic in the case of

Slide 20. Combined methods of killing cells with nanomaterials

Another more sophisticated way of targeting bacterial cells is employing a combination of nanomaterials and antibodies. Antibodies are proteins produced by the body as a defence against foreign bodies. When a foreign body enters our system, our cellular system response by producing antibodies that attach to it and killing for stance a bacterial cell. Antibodies are generated in response to specific foreign bodies. In the case of the example give in the Figure, the antibody is amalgamated to nano-scale gold. In this case when the antibody attaches to its target, it is then to exposed to laser irradiation. This causes the gold to heat up eventually burning hole in the cell wall.

Slide 21-22 Detection of cells and chemicals in the environment

As well as killing cells nanomaterials are being developed that can sense chemicals and chemical changes in the environment. The presence of a chemical cause a reaction (for instance a change in charge differential which can give out a signal.

Slide 23-25 Nanomaterial manipulation of cells

With this approach bacterial cells are made to ne magnetic by coting then with nano-scale iron. The iron sticks to the surface of the cells. Cells can then be manipulated by applying a magnet.

Slide 26 Mechanism of toxic response to nanomaterial exposure

Many factors influence the toxicity of nanomaterials. Particle size and shape for instance. Rod shaped nanoparticles are more toxic that spherical shaped forms. Its is uncertain why this might be but is attributed to the release of electron and ions from the end of the rods damaging the cells. A key influence on toxicity is the surface chemistry of the material- for instance hydrophobicity or the presence of functional chemical groups. Once significant way nanomaterials can be toxic towards cells is by releasing free oxygen radicals. Free radicals are very toxic to biological molecules.

Slide 27-28 Fate of nanomaterials in the environment

A major issue of any technology is potential negative impact on the environment. Nanomaterials are part of a very new technology and with all new technologies it is essential we are look out of unanticipated consequences.

Slide 29- REDOX potential

As detailed in Slides 8-9, the addition of zero-valent iron for remediating TCE is enormous. However, it's a very reactive material and that is why its employed and this reactivity may have unwanted consequences on the environment. As can be seen on the slide addition of zero-valent caused a rapid and significant drop in the REDOx potential of the sampled river water. This caused a devastating decrease in bacterial counts, which would be a concern if it were not for the observation that bacterial numbers showed a significant increase. This increase in bacterial counts is difficult to explain, but could be due a sudden increase nutrients availability as consequence of the drastic alteration of the chemical environment.

Slide 30- Impact of zero valent nano-scale iron on exposed river microbial communities.
Shows the impact of zero-valent iron addition to the exposed river water microbial community. Genetic profiling of the river microbial community as a consequence of zero iron addition revealed that despite a significant impact on bacterial counts, the composition of the microbial community revealed very little long terms detectable alteration.

Slide 31-32 Microbial synthesis of nanomaterials
Some microbial cells are excellent chemists and can generate uniform nanomaterials. Growing cells in a solution of gold, causes it to be taken up by the cells. As a defence against high concentration toxicity the cells precipitate out the gold as uniform nano-spheres. Making uniform nano-materials is not easy and often needs significant effort of separating the particles according to their size. The ability of microorganisms to generate uniform particle represents a cheaper and more sustainable option.

Slide 33. Summary of Nanomaterials and the Environment
Nanomterials have enormous potential in terms of environmental engineering including

- Remediation
- Nano-filtration of contaminants
- Killing microbial pathogens
- Sensing of pathogens and other contaminants.