Accompanying notes for C9 Environmental Engineering 2.

Lecture 1

Slide 1
Introduction to Environmental Engineering.

Environmental engineering 'is the application of science and engineering principles to improve the natural environment (air, water, and/or land resources), to provide a healthy environment and remediate historically polluted sites'.
The focus of these 8 lectures is the potential of bioengineering in developing sustainable ways of maintaining and improving the environment, cleaning up pollution and preventing end-of-pipe contamination.

Slide 2. We urgently need to move from a linear economy where things are used once and thrown away to one in which materials are preserved and recycled. One of the primary focus of academics and industry is sustainable energy and resources.

Slide 3
Course is broken down into 8 lectures
1.What is Environmental Bioengineering?
2.Detecting and counting microbial life.
3.Types of Environmental Bioremediations.
4.Engineering biological systems.
5.Clean-up of chlorinated compounds.
6.Case study- bacterial treatment of waste.
7.Energy from waste, resource recovery.
8.Environmental engineering and nanotechnology- the future.

Slide 4
The key environmental challenges we face globally. The key one is the current global population is about 7.3 billion and estimated to increase to 9.7 billion by 2050, even though the rate of increase is anticipated to slow. This leads to the requirement of large scale delivery of:
1. Food
2. Clean energy
3. Water
4. Resources to make things(metals) and grow crops (phosphate and fertilizers).

The key issue is delivering of all these in a sustainable way. That is, it does not destroy the environment (does not deteriorate water and soil quality), has low energy demand and is focused on a circular economy (resources are recycled and reduced, clean manufacturing synthesis).

Slide 5
Current ways we manufacture and generate energy have historically caused enormous environmental damage/contamination.

https://www.worldwildlife.org/threats/pollution

http://www.bbc.co.uk/news/health-41678533

https://www.bbc.co.uk/news/science-environment-45652149

This means we have to clean up the contamination which has already occurred and find sustainable ways to prevent new contamination and reduce carbon dioxide emissions.

Slide 6
Humans have had an impact on their environment from killing species (remember the Dodo), deforestation in favour of agricultural land and synthesis of materials such as plastics that are not naturally degradable in the environment. This has led to wide distribution and in some cases accumulation of some very toxic chemical contaminants such as Asbestos, Mercury, Dioxins, Hexachlorobenzene (HCB), Polychlorinated Biphenyls (PCB). The problem is that contamination is widespread and diffuse. Also, contaminants are toxic and carcinogenic, some such as Trichloroethene have been correlated to increase incidence of Parkinson Disease. The driver now is to install engineered approaches that prevent contamination discharge rather than trying to treat it once it gets into the environment.

Slide 7
However, we still have significant environmental issues that require new thinking in engineering. This includes what are called Emerging Contaminants such as hormones (and their analogues such as phthalate) and plastics (such as micro-plastics). Hormones in particular are present in very low concentrations but have a disproportionately large impact- such as impacting on fertility. which has decreased 50% in the past 50 years

https://www.nhs.uk/news/pregnancy-and-child/western-sperm-counts-halved-in-last-40-years/

https://www.scientificamerican.com/article/sperm-count-dropping-in-western-world/

Slide 8
Although engineering activities over the centuries have caused a lot of historical contamination, they have also positively impact on the way we live and improving human health and quality of life. Up to the late 1800's the Thames was an open sewer, water related disease was rife.

In 1865 Joseph Bazalgette, a civil engineer designed and built the London sewer/ clean water network. Key deliver it separated clean from dirty water.

Slide 9/10
The Environmental Engineering of the future will be focused on development of sustainable systems and mimicking ecological systems where there is no accumulation of waste, resources are recycled, energy generation is clean and resources such as water are recycled employing low energy systems. Ecosystems work very efficiently, energy is conserved, water is recycled and there is not accumulation of recalcitrant waste.

A key opportunity in terms of enabling the development of sustainable engineered systems for the environment is exploitation of microorganisms and plants. That is in terms of environmental clean-up, prevention of contamination and clean synthesis of products.

Slide 11 Reading list

Brock Biology of Microorganisms. MT Madigan, JM Martinko, J Parker. Prentice Hall, Upper Saddle River, NJ.

2) Microbial Ecology: Fundamental and Applications. RM Atlas & R Bartha. Addison Wesley Longman, Menlon Park.

3) Microbiology. LM Prescott, JP Harley, DA Klein. WCB McGraw-Hill, Boston

Slide 12
In terms of traits microorganisms are very amenable to exploitation in engineered systems. They are the most diverse of any group of organisms contain aerobic, anaerobic, photosynthetic and heterotrophic (organisms that require food originating from other organisms- fixed carbon) can also genetically engineered to optimise specific traits.

Slide 13/14
A very valuable feature is that microorganisms are asexual and multiple by binary fission- reproduce by dividing in two. Thus, microbial counts for bacteria potentially double every 20 minutes, so cell counts increase at exponential rates. They are small, ranging from 1-4 μm x 0.5- 1 μm. But numerous- a gram of soil typically contains between 10^6 and 10^9 bacteria, with up to 10^6 different genetic types.

Slide 15
Highlights the fantastic diversity of microbial life. On the tree of life, there are three branches one consists entirely of bacteria, the second consists of Archaea (very similar to bacteria but distinctive), the third tree consists, of fungi, and all animals and plants.
http://www.wikilovesmonuments.org.uk/?pk_campaign=Centralnotice

Slide 16
•Metabolically they are by far the most diverse of any group- treat toxic xenobiotics as nutritional opportunities.
•Very resistant to extreme conditions such a pH, temperature, UV and desiccation.
•They are asexual and reproduce exponentially by division.
•They generate very useful products (antibiotics, alcohol, bioplastics)
Microns in size so are easily distributed and indeed universally distributed.

Summarises the features the key features that make them of so much interest in terms of exploitation in engineered systems

Slide 17-20
Summarises the ability of bacteria in particularly to survive and thrive in extremes condition of pH (Acidophiles and Alkaliphiles), temperature (Thermophiles), salt conditions Halophiles and osmotic conditions extremes. They can also survive high ultra- violet and others forms of radiation. Philes- means 'loving'. Eg Francophiles

Slide 21
Summarises the vital central role microorganisms play in terms of agriculture (fixing nitrogen, solubilising phosphate, industrial applications such as biocatalysis of processing such as producing bioplastics, biosensors, bioremediations.

Slide 22
The primary feature that makes microorganism so important in terms of developing more sustainable processing in engineering is their metabolism. Metabolism is the 'sum total of all chemical reactions and physical workings occurring in a cell.

Slide 23

Summarises the metabolic diversity of bacteria, which includes Chemotrophs (which use chemical energy) and phototrophs which use light as energy sources. Many are like humans in that they are Chemo-heterotrophs, they use Oxygen as the final electron acceptor and utilise fixed carbon as a nutrient source. However, other forms of bacteria fix C02 as their primary source of nutrient. Alternatively, photoautotrophs fix C02 and use H2O rather than fixed substrate for carbon assimilation- some of these are aerobic and others are anaerobic.

Slide 24
Shows as test tube method of demonstrating the different metabolic forms of bacteria.

- Aerobic forms grow on top on the agar, where O2 concentrations are highest.
- Strict anaerobes grow away from the top surface of the agar where O2 levels are lowest.
- Facultative forms can survive both aerobically and anaerobically.

Slide 25
Summarises cells metabolism. It's a combination of catabolism which results in breakdown of complex molecules to their components parts i.e. break down of protein to amino acids, with the release of energy. Can result in waste products with high value such as alcohol. The opposite process is anabolism. Energy is used to build up molecules such as proteins from amino acids. The energy for this synthesis process comes from chemical or light energy.

Slide 26-27
In the process of Catabolism complex molecular such as carbohydrates, lipids and proteins are broken down to sugars, lipid and amino acid. In the process energy and electrons are released which goes to generating Adenosine triphosphate (ATP)- which acts as an energy deliver molecule. ATP is used in the process of anabolism whereby macromolecules (carbohydrates, proteins and lipids) are synthesised from their building blocks (sugar, lipid and amino acids).

Slide 28
A central aspect of the process of anabolism is electron transfer. This is the process whereby an electron is transferred from one atom/molecule to another. A compound which loses an electron in the form of hydrogen becomes oxidised. The molecule which gains the electron becomes reduced. The two reactions (reduction and oxidation) are opposite to each other and always occur together and referred to as REDOX reaction. In the process of electron transfer energy is transferred. This is central to all biological processes involving electron transfer including both respiration and photosynthesis.

Slide 29
Energy cascade in cells- during respiration**.** With this glucose is oxidised as it passes down a sequential metabolic degradation pathway resulting in the removal of hydrogen and their accompanying electrons. The final step in the process of glucose oxidation it is degradation to CO2. The electron and hydrogen ions generated by the respiratory pathway combine with oxygen (the terminal electron acceptor), to produce water. The energy in the form of the electrons and hydrogen is captured and transferred to ATP, which is delivered to the cells, as required, to drive their functional roles.

Slide 30
ATP – the currency of energy. Is the energy delivery unit, Adenosine triphosphate. Is a nucleotide with three phosphates linked as a short chain. The last phosphate is the chain is removed by hydrolysis, losing the last phosphate molecule results in the generation of

adenosine diphosphate. In this process the phosphate bond is broken releasing 7.5 kcal/mol

ATP + H2O=ADP + phosphate+ energy

Slide 31
Summary of aerobic and anaerobic processes involved in biodegradation of organics. In the process of Aerobic (oxidation) degradation of organics, electrons are transferred to oxygen which is reduced to CO2 and water. The energy goes to power cells or the synthesis of new biomass.

With anaerobic biodegradation, the electron is transferred from the organic to the nitrate (or sulphate or others) which acts as the electron acceptor.

The source of carbon varies depending on the metabolism of the organisms.
Autotrophic- such as photosynthesis, the carbon also referred to as the reducing equivalents are provided by CO2

Heterotrophic organisms- obtain their reducing equivalents from fixed carbon sources, such as plant biomass (as we do).

How the organism obtains energy for living and growing? **Chemotrophic** (are heterotrophs) they get their energy from fixed carbon. So, humans are heterotrophic and chemotrophic.
Phototrophic- energy is obtained from light. So plants are autotrophic and phototrophic.

Slide 32
Table summaries oxidation and reduction

Slide 33
Summarises the energy difference in generation from acetate aerobically (with oxygen) and anaerobically. Production aerobically generates -844 kJ/mol and anaerobically -792 kJ/mol.

Slide 34
Although microorganisms hold enormous potential in terms of metabolic versatility, growing aerobically and anaerobically, growing photo-tropically and heterotrophically, they also generate forms that can cause disease and kill you. Therefore, it's important to be able to detect and count them.

Slides 35.
However, some microbes are effective pathogens and will kill other organisms. A key requirement for control, kill and exploitation of microorganisms in the environment is detection and counting the number of individuals present.

Lecture 2
Detecting and counting microorganisms in samples

Slide 1
For exploitation (in terms of engineered and biotechnology) and safety (avoiding harmful micro-organisms) it is essential to be able to count the number of microbial cells in a sample- ideally quickly and accurately.

Microbial cell counts also tell us a lot about the samples/habitat, if it is contaminated with something very toxic for instance, cells will be killed and microbial counts will be low.

Slide 2
The challenge of environmental microbiology study

By definition small- 0.5-1.0 x 1.5-5.0 μm.
•Numerous- g^{-1} of soil contains > 10^8 . The same in a mL of water.
•Few distinguishable morphological features.
•Fantastically diverse- g^{-1} of soil contains > 2.5 X 10^6 genome equivalents (species). Most only detected once in any sample.
•Reproduce very rapidly so highly adaptable.
•Plastic- with no firm definition of species.
•Prior to introduction of molecular biology only understood <1% of the microbial community.
•Even if you can identify in situ if you can't culture you have no physiological understanding.

Slide 3
Summary of microbial count methods

Direct microscopic count, live and dead
•Viable cells count (colony counts), counts just living
•Turbidity measurements- total measure of biomass of cells present based on light absorption
•Measurement of total nitrogen
•Measurement of biochemical activity e.g. O_2 uptake of CO_2 production during respiration
•Measurement of dry or wet weight of cells
•Stable isotope probing- enables selective counting
Raman Microscopy- differentiates individual cells

Slide 4
Enumeration of bacteria by microscope and graticule
With this, a suspension of cells or maybe river water, is placed on a microscope slide containing chamber of precisely known volume and divided into a grid of squares. A cover slip (a transparent slide of glass) is placed over the chamber. The whole slide is placed under a microscope and the number of cells counted per small square is determined. For accuracy up to 100 squares are counted. Since the volume of water is known, the number of cells per ml of suspension can be determined and then expressed per millilitre (ml). The method provides an estimate of total counts, but does not differentiate between living and dead cells. Can be slow process to count- the cells.

Slide 5
Spectrophotometer for measuring the turbidity of the cultures
The basic principle of this approach is light is beamed through the culture suspension. As light hits the cells it is absorbed. The higher the number of cells, the greater the amount of light absorbed. The amount of light is described as the Optical Density. The graph shows that the approach is a very good and quick way to determine where the cells are in the growth cycle.

Slide 6
Standard Agar Plate Count
The plate count method or spread plate relies on bacteria growing a colony on a solid nutrient medium. The colony becomes visible to the naked eye and the number of colonies on a plate can be counted- one colony is assumed to have been derived from one microbial cell. To be effective, the dilution of the original sample must be arranged so that on average between 30 and 300 colonies of the target bacterium grow on the dilution plated selected for enumeration. Fewer than 30 colonies makes' the interpretation statistically unsound and greater than 300 colonies often results in overlapping colonies and imprecision in the count- its also laborious counting so many small colonies. To ensure that an appropriate number of colonies will be generated several dilutions are normally cultured. The laboratory procedure involves making serial dilutions of the sample (1:10, 1:100, 1:1000 etc.) in sterile water and cultivating these on nutrient agar in a Petri-dish that is sealed and incubated.

For detailed procedure see:
http://users.aber.ac.uk/hlr/mpbb/index_files/Page299.html

Slide 7
Shows when bacterial cells are in microscopic for and their transformation into a colony which is visible on an agar plate. In solution bacterial cells are too small to see, however as soon as they are dispensed onto an agar plate the cells grow by multiplying to form a colony which is visible. A colony is a cluster of cells derived from a single cell in the original suspension.

Slide 8
Media: Providing Nutrients in the Laboratory
Table detailing some of the principles equipment and materials for growing bacteria.

Slide 9
Agar plate count method with dilution series for determining the number of viable bacteria in soil
https://www.youtube.com/watch?v=_1KP9zOtjXk

https://www.youtube.com/watch?v=JtYtqpBLC14

Slide 10
Slide highlights the fact that some nutritional agars are selective for specific for certain types of bacteria which may also be differentiated by their distinctive colony colouration. Other agars are made selective by the addition of inhibitors (such as antibiotics) which only allow certain types of bacteria resistant to the antibiotic to grow. They are referred to selective agars. The opposite forms are able to grow a broad spectrum of bacterial types.

Slide 11
Streak Plate Method
This method is used to check the purity of a bacterial culture that has been isolated. It's basically a method of spreading (diluting) out the bacteria across the agar surface. By separating the colonies, it is possible to determine if there are more than one colony type- therefore assess the purity of the culture. More than one colony morphologies suggests' that the culture is mixed.

Slide 12
Anaerobic culture methods
Oxygen has to be removed from the media and culture atmosphere. O2 can be drive off by heating or adding a chemical/catalysis such as thioglycolate (a reducing agent) which combines with O2.

Slide 13
Anaerobic culture methods
An alternative approach to making the culture anaerobic is to place inoculated cultures into anaerobic jar which can be made air tight and the gaseous condition modified according to culture requirements. This can be done by introducing appropriate gas mixtures. Or by placing two sachets containing catalysts into the containing, one which combines with O2 the other generates H2. Cultures are then incubated under appropriate anaerobic conditions.

Slide 14
Anaerobic culture methods
Shows an anaerobic chamber, which is basically an enclosed sealed air tight Perspex box which is gases out with nitrogen. Sealed gloves insertion enables anaerobic culture work to take place on the bench in the appropriately adjusted gas environment.

Slide 15
Capnophiles Require High CO_2
Some bacteria thrive in high CO2 atmospheres, these can be grown by placing a lighted candle with the culture in sealed jar.

Slide 16
Determining low counts of bacteria
It is essential to be effective at detecting low counts of bacteria in samples, in particularly to check the purity of drinking water. If a litre of water only contains 100 bacteria they are unlikely to be detected if only 0.1 mL is analysed using culture plate counts. Since some bacteria can kill you (pathogenic) it is important we can detect them. This can be achieved by filtering a litre of water through a 0.2 mm pore diameter filter. Bacteria are about 2-4µm in size, so this filters them out and concentrates them. The filter is then placed on an agar plate, which is incubated. The number of bacterial colonies is then determined and expressed as the number per mL.

Slide 17
Measuring biomass on the basis of CO2 production from cells in culture.
The rational of this approach is that bacteria metabolising aerobically, assimilate carbon and break it down to CO2. The generation of CO2 in a closed flask is shown in the diagram - detection by the CO2 reacting with the NaOH (sodium hydroxide), confirms cells are present and active. The NaOH is titrated and the amount of CO2 calculated per mL/hr. The greater the number of active cells, the greater CO2 production.

Slide 18
Direct measurement of cell weight. With this, cultures are filtered through a 0.2 pore filter, which collects all the cells on the filter. Filters are then dried to constant weight (80C) and weighed-this enables as comparative assessment of biomass of cells in the water.

Slide 19
Table shows the relative merits of microbial biomass determination of cells by various methods.

Slide 20-24

Details more advanced technologies for detecting key function types of bacteria, such as those that degrade specific contaminants, based on DNA methods. Slides and web page below summarises the basis of DNA replication.
https://www.yourgenome.org/facts/what-is-dna-replication

Slide 25-26
Table isotope probing. Details how to detect the actually cells within a mixed community able to degrade the contaminant. The example given in the slide is carbon(13)- labelled phenol. With this approach bacteria are fed C-13-labelled phenol. Bacteria able to degrade phenol grow on it, the carbon in their DNA is labelled with C13, which makes it heavier than cells growing on the non-labelled (which consist of the C12 isotope) substrate. This enable separation on a sucrose density gradient since C13 DNA is heavier that C12 labelled DNA. More details can be found in the link here:

https://www.nature.com/articles/35001054.pdf

Slide 27-31
Raman spectrometry for detecting bacteria able to degrade a target compound. In this example cells actively degrading a compound are labelled with H3O- Deuterium-labelled water. Labelled cells cause a Raman shift shown in Slide 30. These cells can then be separated from non-active cells by Fluorescent Activated cells sorting (FACS).
http://pubs.rsc.org/en/content/articlepdf/2015/an/c5an01074h

Lecture 3
Bioengineering for environmental clean-up (Bioremediation).

Slide 1
Engineering environmental clean-up

Slide 2 Defining pollution
Pollution is the introduction of contaminants that cause adverse changes. Can be chemical, or energy such as noise heat or light.

Slide 3
Categories of the different classes of pollutants/contaminants. Two forms: 1) Naturally occurring compounds that are present in unnaturally high concentrations, for example crude oil accidentally released into the sea. 2) Xenobiotics- foreign man-made synthetic compounds that have never occurred in the natural world, so no microorganisms can degrade it.

Slide 4
Definition of Bioremediation- the use of bacteria and fungi and plants to break down or degrade toxic chemical compounds that have accumulated in the environment.

Slide 5
Categories the degradability of contaminants
Biodegradable (petroleum), Partially biodegradable (chlorinated compounds) and not bio-degradable (mercury, uranium)

Slide 6
List below summarises the combination of key features required for bioremediation to occur.

-Need nutrient balance including nitrogen, phosphorus, sulphur, and a variety of trace nutrients together with carbon.
• Key nutrients are commonly limiting for microbial growth in natural systems.
• Acclimatisation period - a period during which no degradation of chemical is evident; also known as
adaptation or lag period when the.
• Length of acclimatisation period varies from less than 1 h to many months.
• Acclimatisation of a microbial population to one substrate frequently results in the simultaneous
acclimatization to the ability to degrade some structurally related molecules (co-metabolism).

Slide 7
Biodegradation profile- the combination of components required. Summaries the combination of environmental, contaminant and microbial factors that interact to determine rates of biodegradation of contaminants.

Slide 8
Biodegradation of contaminants in soil- diagrammatical representation of bioavailability of contaminants and biodegradative action of bacteria. In order to achieve optimal rates of biodegradation the combination of factors summarised in the diagram in the red circle are required. This includes sufficient numbers of bacteria with the genes that encode for degradation, other nutrients such as phosphate essential for growth and activity and water. The cells also have to be in close enough proximity (Bio-accessible and bioavailable) so their enzymes

(biological catalysts) are close to the target substrate. With this combination the contaminant degrades. However, the action of the bacteria lowers the concentration in the immediate vicinity of the contaminant, so rates of biodegradation fall. In order to degrade contaminant molecules not in close proximity of the cells must be made accessible, the soils have to be agitated to get cells and contaminant close together. This is a key challenge of bioremediation- the continuous mixing of biodegradative cells with the contaminated portions of the soil in sustainable way- since soil is very heavy and mixing is energy intensive.

Slide 9

Bioremediation approaches- In terms of cleaning up contamination in the environment a key decision to be made is - should the treatment be *in situ* (treat it where it is) or *ex situ* (remove and treat off site). For *in situ* the other issue to be addressed is to determine if the indigenous microbial community has the ability to biodegrade the contaminants (intrinsic bioremediation). However, if intrinsic rates are slow then engineered interventions are required to stimulate rates. The other consideration is the chemical state of the contaminant- vapour, liquid or solid.

Slide 10

Intrinsic remediation

There is increasing interest in intrinsic bioremediation for control of all or some of the contamination in exposed sites. Intrinsic bioremediation relies on the inherent capacity of microorganisms to metabolise the contaminants - should be tested at laboratory and field levels before use of intrinsic bioremediation.

The site conditions that favour intrinsic bioremediation are:

The right catabolic genes are present and they are being expressed.

Ground water flow throughout the year- the contaminant should not be very mobile. If they are they constitute a risk in particularly to supplied of drinking water.

Supply of electron acceptors and nutrients for microbial growth/activity must be readily available.

There must be an absence of toxic compounds.

Bioremediation of waste mixtures that contain toxic levels of heavy metals such as mercury, lead, arsenic, and cyanide can create a problem.

Advantages

1. Ease
2. Low cost

Disadvantages

1. Can be Slow.
2. Public perception- looks like you are not taking the problem seriously.
3. Long term monitoring required- which can be expensive, but is a legal requirement.

Slide 11

Engineered remediation *Ex-situ*

Another type of bioremediation is *ex situ* bioremediation, which is when spills are taken out of the area to be cleaned. This type of bioremediation is generally only used when the site is threatened for some reason, usually by the spill is invading a sensitive area, the land is urgently required or it is demonstrated to be exceptionally toxic. *Ex situ* bioremediation is only used when necessary because since it's expensive and damaging to the area, since the contaminated land is physically removed.

Important Parameter in deciding remedial options

•Accessibility- can you get to the contamination? Can it be excavated?
•Biodegradability-
•Rate of Biodegradation- Is introduced contaminant degrading
•Is a microbial consortium required?
•Will microbial end products cause problems?

Advantages
1. Controlled environment- nutrients can be added and pH adjusted
2. Ease of monitoring-

Disadvantages
1. Not applicable to large contamination plumes
2. Not applicable to low level concentration
3. Cost- expensive,
4. Complexity- need good infrastructure and logistics,

Slide 12
Bio-stimulation- Amendment with a substrate such as a limiting nutrient or electron acceptor or donor to promote the growth of the indigenous microbial community. Most commonly this is by the addition of key element such as phosphate which is limited so addition stimulates activity. It might be necessary to test a whole bunch of parameters in soil labs tests to determine which nutrient is limiting in order initiate community stimulation.

Bioaugmentation-Addition of naturally occurring or genetically modified populations, or genes, which endow the community with a valuable trait, such a degradation of a toxic recalcitrant, which enable bioremediation to occur at an accelerated rate or in a site where the biodegradation genes are absent. Would be applied as a strategy, if soil/genetic analysis revealed the biomass of potential bio-degraders was low and was completely undetectable.

Slide 13

In situ Engineering- shows a sunken shaft/well into a contaminated sit requiring bio-stimulation or bioaugmentation.

Slide 14

In situ Engineering- Bioventing

With this approach an injection well is sunk into the contaminated aquifer and air pumped into the anaerobic zone.

Slide 15
Typical bioventing system- the migration of the contaminant plume is monitored by sinking monitoring wells. In parallel air is injected into the plume to stimulate aerobic biodegradation rates.

https://www.navfac.navy.mil/navfac_worldwide/specialty_centers/exwc/products_and_services/ev/erb/tech/rem/biovent.html

https://conservancy.umn.edu/bitstream/handle/11299/59453/1/5.2.Hellekson.pdf

Slides 16
Aeration amendments. Shows the application of nutrient additions to stimulate rates of biodegradation, including the addition of oxygen release compounds for aerobic degradation of petrol spills.

Slide 17
Oxygen release compound to treat BETEX- petrol

Slide 18
Amendments to create anaerobic conditions. Some contaminants such as highly chlorinated compounds require reducing conditions in order to be degraded. This includes displacing their Cl^- substitutions with H^+. This is achieved by adding nutrients which are degraded aerobically so utilising all the O2 and in the process generating H2. The anaerobic conditions favour degradation of chlorinated compounds.

Slide 19
Hydrogen Release Compound Case Study- shows the addition of hydrogen release compound in the dichlorination of PCE. The generation of the breakdown product DCE is proof that PCE is biodegrading.
https://regenesis.com/eur/remediation-products/hydrogen-release-compound-hrc-2/

Slide 20
Bioslurping
http://www.cpeo.org/techtree/ttdescript/bislurp.htm
Bioslurping combines elements of bioventing and vacuum-enhanced pumping of free-product to recover free-product from the groundwater and soil, and to bioremediate soils. The bioslurper system uses a "slurp" tube that extends into the free-product layer. Much like a straw in a glass draws liquid, the pump draws liquid (including free-product) and soil gas up the tube in the same process stream. Pumping lifts light non-aqueous phase liquids (LNAPLs), such as oil, off the top of the water table and from the capillary fringe (i.e., an area just above the saturated zone, where water is held in place by capillary forces). The LNAPL is brought to the surface, where it is separated from water and air. The biological processes in the term "bioslurping" refer to aerobic biological degradation of the hydrogen when air is introduced into the unsaturated zone. This is akin to bioventing, a technology described separately. When free-product removal activities are completed, the bioslurping system is easily converted to a conventional bioventing system to complete the remediation.

Slide 21
Combining *ex-situ* and *in-situ* bioremediation. This is a belt and braces approach. Contaminant is sucked up and treated above ground *ex-situ* in a bioreactor, some of the contaminant is pumped up and recovered. The reduction of contaminant concentration by pumping out and recovery, reduces inhibition of indigenous communities biodegradation activity. In parallel oxygen and nutrients are pumped to stimulate in situ biodegradation even further.

Slides 22 and 23

***Ex-situ* Bioremediation**- Bioreactors, composting, landfarming and biopiling are ways on biodegrading the contaminants ex situ in more optimised conditions. The contaminated material (soil) is excavated first and then treated off site, or on the ground level.

Slide 24
Landfarming
Land Farming is an approach whereby contaminated soils are mixed with soil amendments such as soil bulking agents and nutrients, and then they are tilled into the earth. The material is periodically tilled for aeration. Contaminants are degraded, transformed, and immobilized by microbiological processes and by oxidation. Soil conditions are controlled to optimise the rate of contaminant degradation. Moisture content, frequency of aeration, and pH are all conditions that may be controlled. Land Farming differs from composting because it actually incorporates contaminated soil into soil that is uncontaminated. Composting also generally takes place in aboveground piles.

Slide 25
Composting
Composting is a process by which organic wastes are degraded by microorganisms, typically at elevated temperatures. Typical compost temperatures are in the range of 55° to 65° Celsius. The increased temperatures result from heat produced by microorganisms during the degradation of the organic material in the waste. Windrow composting has been demonstrated using the following basic steps. First, contaminated soils are excavated and screened to remove large rocks and debris. The soil is transported to a composting pad with a temporary structure to provide containment and protection from weather extremes. Amendments (straw, alfalfa, manure, agricultural wastes and wood chips) are used for bulking agents and as a supplemental carbon source. Soil and amendments are layered into long piles, known as windrows. The windrow is thoroughly mixed by turning with a commercially available windrow turning machine. Moisture, pH, temperature, and explosives concentration are monitored. At the completion of the composting period the windrows would be disassembled and the compost is taken to the final disposal area.

Slide 26
Biopiling- Biopile remediation is an *ex situ* version of soil bioventing, where contaminated soil is constructed into engineered piles or cells with the aim of enhancing conditions required for biodegradation through greater control of oxygen, nutrient such as phosphorus and nitrogen and water. The microbial activity of these microorganisms degrades hydrocarbon contaminants such as diesel that are adsorbed to the soil particles. Perforated pipes aerate the biopiles through blowing air into the soil or drawing air from the ambient atmosphere and in addition enable greater control of VOCs. Where considerable moisture may be present, a leachate collection can be installed, with the piles being constructed on impermeable geotextile membranes or clay layers in order to prevent the contamination of the surrounding land by such leachate. The piles, which may be covered with a man made cover to conserve heat and optimal conditions for the microorganisms, are also turned periodically in order to homogenise the soil and ensure complete aeration.

http://learnbioremediation.weebly.com/biopiles.html
http://www.cpeo.org/techtree/ttdescript/biopil.htm

Slide 27
Slurry Bioreactor
A slurry bioreactor may be defined as a containment vessel and apparatus used to create a three phase (solid, liquid, and gas) mixing condition to hasten the biodegradation of soil-bound and water-soluble contamination as a water slurry of the contaminated soil, sediment,

or sludge and biomass (usually indigenous bacteria) capable of degrading targeted contaminants.
http://www.cpeo.org/techtree/ttdescript/bislurr.htm

Slide 28
Optimal Environmental Factor Parameters- Summary of the optimal condition for biodegradation of contamination in aerobic habitats such as soil.
•Available soil moisture 25-85% water holding capacity.
•Oxygen >0.2 mg/L DO, >10% air-filled pore space for aerobic degradation.
•Redox (Reduction-Oxidation) potential Eh > 50 millivolts.
•Nutrients C:N:P = 120:10:1 molar ratio
•pH 5.5 to 8.5.
-Temperature 15-45C.

Slide 29
Chart shows the Composition of clean air

Slide 30
Sources of pollution- these can be point sources, sources such as a chimney, vent or end of pipe.

Slide 31
Criteria pollutants
There are six common air pollutants of particular concern.

- These are carbon monoxide, lead, nitrogen dioxide, ozone, particulate matter, and sulphur dioxide.
- They are the only pollutants with national air quality standards that define allowable concentrations in ambient air.
- Exposure to these substances can cause health and environmental effects, and property damage.
- Pollution interaction- on warm days hydrocarbons can react with oxides to create secondary oxides nitrogen to form ozone which can cause permanent lung disease
- Health effects include heart or lung disease, respiratory damage, or premature death. Environmental effects include smog, acid rain, radiation, and ozone depletion.

Slide 32
Natural sources of pollution

- It includes wild land fires, dust storms, and volcanic activity also contribute gases and particulates to our atmosphere.
- Unlike the above sources of air pollution, natural "air pollution" is not caused by people or their activities.
- An erupting volcano emits particulate matter and gases; forest and prairie fires can emit large quantities of "pollutants”
- Plants and trees naturally emit VOCs which are oxidized and form aerosols that can cause a natural blue haze
- Dust storms can create large amounts of particulate

Slide 33
Particulate matter

- Particulate matter (PM) is the term for small particles found in the air including dust, dirt, soot, smoke, and liquid droplets.
- Particles can be suspended in the air for long periods of time.

- Sources of PM include cars, trucks, buses, factories, construction sites, tilled fields, unpaved roads, construction, wood burning, agricultural burning, wildfires, prescribed fires, and natural windblown dust.
- Particles less than 10 μm in diameter tend to pose the greatest health concern because they can be inhaled into and accumulate in the respiratory system

Slide 34
Air Cyclone
- Based on the general principle of inertia separation- particle laden gas is forced to change direction
- As gas changes direction the inertia of the particles causes them to continue in the original direction and be separated from the gas stream.
- Good for large particles.

Slide 35
Electrostatic precipitators (ESPs)
- Based on the general principle of inertia separation- particle laden gas is forced to change direction
- As gas changes direction the inertia of the particles causes them to continue in the original direction and be separated from the gas stream.
- Good for large particles.

Slide 36
Some other air treatment options for air particulates and fumes
- Absorption – gaseous pollutant dissolved in liquid, also referred to as scrubbers include spray towers.
- Adsorption- gas or vapour is brought into contact with a solid surface (activated carbon, silica).
- Condensation- gas comes in contact with a cold surface.
- Combustion- by incineration, converts pollutant to CO2 and water.
- Catalytic incineration.
-

Lecture 4.
Slide 1. Bioremediation of chlorinated aliphatic and aromatic hydrocarbon (CAH) contaminants.

Slide 2. Contaminated sites in the UK. There are two challenges in terms of application of engineered approaches for maintaining a clean healthy environment. 1) Development of clean-technologies for preventing contamination as a consequence of manufacturing practices. Secondly 2) dealing with sites that are already contaminated. Many sites (in total 300,000 ha in the UK) are contaminated with both organic (petroleum products, chlorinated compounds, pesticides), and toxic heavy metals (mercury, cadmium, zinc widely used in industry).

Slide 3. Legislative drivers
Summarises the key legislative Act of law which places the onus of responsibility, including heavy fines and even imprisonment. Also, companies are also increasing concerned with their public image in terms of environmental responsibility. When contamination has been identified it can no longer be neglected it becomes the responsibility of the owner to undertake remedial action. The Act aims to control and reduce pollution, including reducing/stopping emissions.

Slide 4. Chlorinated contaminants
Are the most widely and amongst the most toxic of environmental contaminants. All habitats tested have been found to be contaminated- distribution is ubiquitous. Are toxic, carcinogenic and difficult to treat or biodegrade. That is because they are synthetic and few bacteria have had enough time to adapt and evolve a degradative pathway.
Examples, uses, chemical and physical characteristics
•These include tetrachloroethylene (perchloroethylene, PCE), trichloroethylene (TCE Trichloroethene), cis-1,2-dichloroethylene (cis-1,2-DCE), trans-1,2-dichloroethylene (trans-1,2-DCE), vinyl chloride (VC), 1,1,1-trichloroethane (1,1,1-TCA), 1,1-dichlorethylene (1,1-DCE), 1,2-dichloroethane (1,2-DCA), carbon tetrachloride (CTET), chloroform (trichloromethane, TCM) and methylene chloride (dichloromethane, DCM).
•Extensively used as industrial solvents, dry cleaning and degreasing, pharmaceutical, paints.
•Aromatic (ring molecule) and aliphatics (chain chemicals).
•Toxicity limits in UK for PCE is 10 µg/L (Water Quality Regulation).
•The physical and chemical nature of contaminants dictate their fate and transport.

Slide 5. Chlorinated contaminants have been correlated with diseases such as Parkinsons. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3621032/

Slide 6 The Properties determine fate in the environment. Demonstrates that the physical fate of contaminants is dictated very much by the physical and chemical nature of the contaminant. A chlorine is chemically denser than water, so in an aquifer it sinks and collected on impermeable layers. In contrast an oil plume floats on water.

Slide 7. Relating properties to chemical structure
Shows pentachlorophenol structure. The number of chlorine groups significantly impacts on the chemical/physical properties. The Chlorine groups are known as substituted chlorines since they substitute Hydrogen groups

Slide 8. Properties of chlorinated aliphatic hydrocarbons that control their fate and determine their properties.
•The number of substituted chlorine atoms affects their behaviour.

•As the number substituted chlorine atoms increases, molecular weight and density generally increases.
•Similarly, as chlorination increases, so vapour pressure and solubility decreases (high vapour pressure=volatile).
•A CAH (chlorinated aromatic hydrocarbons) released to the surface as a pure liquid (non-aqueous phase liquid-NAPL) will seek phase equilibrium.
•Will remain a NAPL, absorb to soil (sorbed phase), dissolve in water or volatilise (vapour phase)- determined by properties of the contaminant. The division is determined by the partition co-efficient.

Slide 9 and 10. Partition (P) coefficient
Is the ratio of concentrations of a unknown compound in a mixture of two immiscible phases (water and octanol) at equilibrium. It's a very good way to predict the fate of a compound in the environment since it determines how hydrophobic (water hating) and hydrophilic (water loving so is soluble in water). The risk of the hydrophilic contaminant is that they can move quickly through the soil and contaminate water resources.

•Is defined as the ratio of concentrations of a compound in the two phases of a mixture of two immiscible solvents, at equilibrium. Hence these coefficients are a measure of differential solubility of the compound between these two solvents.
•Normally one of the solvents chosen is water while the second is hydrophobic such as octanol. Hence, it's a measure of how hydrophilic ("water loving") or hydrophobic ("water fearing") a chemical substance is. Partition coefficients are useful for example in estimating distribution of drugs within the body.
•As an example, hydrophobic drugs with high partition coefficients are preferentially distributed to hydrophobic compartments such as lipid layers of cells, whilst hydrophilic drugs (low partition coefficients) preferentially are found in hydrophilic compartments such as blood serum.

The diagram in Slide 12 shows a separation funnel used calculate partition coefficient- the tow immiscible layers can be seen.

Slide 11
Fate of chlorinated aliphatic/aromatic hydrocarbons

- In attempt to reach equilibrium, CAH can migrate and spread by diffusion. Diffusion rates are determined by concentration gradients which cause the CAH to seek phase and concentration equilibria.
- The extent of subsurface migration is a function of the volume, area of the release, duration, and physical and chemical characteristics.
- Most have densities greater than water (1,100 to 1,600 kg/m3) and are less viscous than water, results in rapid migration.

Slide 12
Remediation challenges with respect to dealing with chlorinated contaminants
Their density, hydrophobicity, both of which are high. So, they stick to soil which makes remediation challenging.
•Surface heterogeneity makes it difficult. Not evenly distributed, which accumulation of hot spots with high concentrations.
•Phase variation- is it in the water or liquid phase, or immobilised on a solid surface.
•Detection can still be a problem, in particularly in remote locations. Analytical methods for detecting and charactering chlorinated contaminants are expensive and technically challenging.

Slide 13-14

The microbiology of biodegradation of chlorinated aromatic/aliphatic hydrocarbons (CAH)

•Unlike many contaminants such as oils and tars, comparatively few microbes will biodegrade CAH.

•In order to decide to go for a natural attenuation of the contaminant you need to know if:

1.The right microbial populations (genetic code) are present and in sufficient numbers.

2.In the case of a consortium, all members are present.
For example, methanogens provide the hydrogen necessary for respiratory dehalogenation by providing electron donors (see later slide).

3. Must avoid generation and accumulation toxic breakdown products such as Vinyl Chloride (VC).

4. Thus complete declorination is required to prevent accumulation of VC.

Dehaloccoides- Is a unique bacteria species since it can completely degrade the trichloroethene leaving no toxic by-products such as Vinyl Chloride.

Slide 15

The biodegradation of chlorinated solvents can occur via two processes

•Its use as a primary growth substrate. This is probably the most important mechanism in the environment.

•The chlorinated substrate is employed by the microbial populations as an energy source, it is used as an electron donor or acceptor.

• When used as an electron donor the contaminant is oxidised. Conversely when used as an electron acceptor, it is reduced via a reductive dechlorination process called halorespiration.

Slide 16 Co-metabolism

•Chlorinated solvents can also be degraded via co-metabolic pathways. During cometabolism, microorganisms gain energy for growth from metabolism of a primary substrate (for example methane), and chlorinated solvents are degraded fortuitously by enzymes present in the metabolic pathways. The organism obtains no known benefit from the biodegradation of the chlorinated solvent, which in some cases yields products that are toxic to the microorganism. Co-metabolism reactions can be either oxidation or reduction reactions.
•Eg methane stimulates degradation of TCE (methane mono-oxygenase). So, when methane oxiders degrade methane, their enzymes fortuitously degrade TCE- the enzymes I this case are non-specific and degrade a broad range of contaminants.

Slide 17 Reductive dechlorination

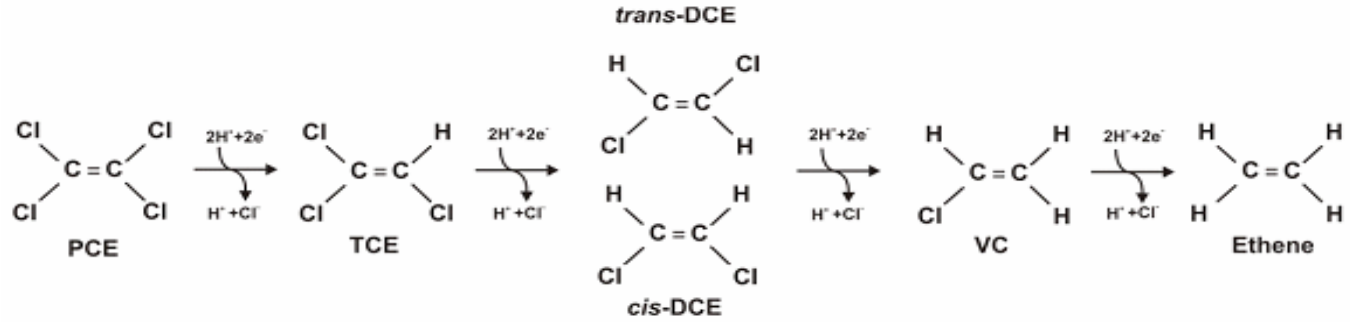
Reductive dechlorination is the most important process for the natural biodegradation of chlorinated solvents. The chlorinated solvent is utilised as an electron acceptor and a chlorine atom is removed and replaced with a hydrogen atom, resulting in the reduction of the chlorinated solvent. When this reaction is biological, and the bacteria utilises the substrate for energy, the reaction is termed halo-respiration. Due to the fact that halo-respiration leads to the growth of microorganisms, this process is robust and provides a foundation for potential intrinsic bioremediation of sites contaminated with chlorinated solvents.

•During halorespiration, hydrogen is used directly as an electron donor. The hydrogen is produced in the terrestrial subsurface by the fermentation of a wide variety of organic compounds, including petroleum hydrocarbons and natural organic carbon. Due to its importance in the microbial metabolism of the halorespirators, the relative supply of hydrogen precursors compared to the amount of chlorinated solvent that must be degraded is an important consideration when evaluating natural attenuation.

So, we can potentially mix wastes to stimulate microbial clean- if it were allowed.

Slide 18-20 Reductive dechlorination of PCE
•When tetrachloroethene (PCE) represents the primary contaminant, sequential reductive dechlorination may yield trichloroethene (TCE), cis-1, 2-dichloroethene (cis-DCE), minor quantities of trans-1,2-dichlorethene, vinyl chloride, and ultimately non-toxic, chlorine-free end products in the form of ethene and ethane. The dechlorination of PCE to cis-DCE is a relatively fast process, whereas the further dechlorination of cis-DCE to VC and ethene is significantly slower.

●



The breakdown products tell you a lot about the conditions in the habitat, the microbial communities present and potential “pinch points”- also helps to predict rates of recovery.

Slide 21Abiotic degradation mechanisms of chlorinated solvents
•Abiotic degradation mechanisms involve chemical reactions to transform chlorinated solvents without biological processes. These mechanisms include hydrolysis, elimination and abiotic reductive dechlorination. In general, the rates of abiotic degradation may be slow relative to biological mechanisms. However, the abiotic mechanisms may play a significant role in the overall remediation of a site at which CAH contamination is present, depending on the specific site conditions. Hydrolysis and elimination reactions are generally independent of redox conditions, while abiotic reductive dechlorination is highly dependent on redox reactions.
•Hydrolysis is a substitution reaction in which a CAH may react with water to substitute a chlorine atom with a hydroxyl group, producing organic alcohols or acids. Generally, less chlorinated CAHs are more susceptible to degradation by hydrolysis. Hydrolysis is a common transformation mechanism for 1,1,1-TCA, chloroethane and chloromethane, producing acetate, ethanol, and methanol, respectively.
•UV- degradation at the surface of the ground/water, exposing the contaminant.

Slide 22 Bioremediation of CAH- and limitations
The vadose zone, also termed the unsaturated zone, is the portion of Earth surface between the land surface and zone of saturation ("vadose" is Latin for "shallow"). It extends from the top of the ground surface to the water table. This is where a lot of industrial and urban contamination accumulates.

The diagram shows the sequence of REDOX condition from close proximity to the leak to the more aerobic zone away from the leak. The process and sequence here is that in close proximity to the leak the readily degradable contaminants are degraded, and in the process reducing the oxygen level, so it is entirely anaerobic. This enables strict anaerobic classes of bacteria such as the methanogens to be proliferate and be active. Moving away from the source of the plume, the REDOX environment becomes increasingly aerobic results in a succession (sulphate, iron, nitrate/manganese) eventually moving to the plume margin (furthest from the contamination source) which is fully aerobic
Although PCE and TCE degradation may occur in most anaerobic environments, including manganese and iron reducing conditions, DCE and VC dechlorination occurs only under sulphate reducing and methanogenic conditions.
In addition, microorganisms are sensitive temperature, pH, contaminant toxicity, nutrient conditions.
If economical, conditions can be engineered to alter the condition to stimulate rates of biodegradation.

Slide 23 Stimulating bioremediation- ways of stimulating intrinsic rates if they are too slow
Bioaugmentation- process of stimulating bioremediation by the addition of microbial cultures with the proven ability to catabolise chlorinated contaminants.
Stimulates intrinsic rates.
•Usually a consortium (2 or 3 strains) of bacteria is employed.
•Still need suitable in situ conditions for growth and activity.

Slide 24 Biostimulation
An insufficient availability of electron donors is commonly cited as a primary reason for incomplete reductive dechlorination of solvents.
• With biostimulation electron donors stimulate dechlorination (most commonly acetate, but also formate and methanol, but also used complex sources such as molasses and whey, municipal waste and veg oil).
•The stimulant is injected into the source area. However, can lead to competitive inhibition, preferential growth near the injection well (biofouling) and limited ability to stimulate the "right" bacteria. Biofouling can reduce permeability and prevent further substrate delivery. Biofouling is a problem if the injection is continuous.

Slide25 Picking the right stimulant for bioremediation
The effectiveness of each stimulant is site specific. In one site ethene formation from PCE was stimulated by acetate, methanol, and formate. Whilst is other these did not work.
•Due to the variable responses to substrates, selection of the "right" stimulant" can only be assured by microcosm studies.
•However, H_2 is the ultimate electron donor for reductive dechlorination. This can be achieved by H_2 sparging, H_2 generating electrodes or addition of fermentable substrates such as ethanol, lactate, propionate or complex molasses.
•Problem is there may be competition from other microbial populations for the electron donors (eg methanogenic and sulphate reducers). However, dechlorinating bacteria exhibit H_2 consumption threshold concentrations that are at least an order of magnitude below methanogenesis. There dechlorinators outcompete methanogens at low H_2 partial pressures.

Slide 26 Enhanced bio-attenuation
Summarises the kind electron acceptors (for petroleum spills) and electron donors (for chlorinated solvents) available. Also shows the delivery technologies available including Liquid, bio-sparging and slow release compounds.

Slide 27 Indirect formation of electron donors

With this approach HRC (Hydrogen release compound) is hydrolysed by indigenous microorganisms present to release lactate. This is degraded by fermentative (anaerobic) bacteria which break it down to propionate, acetate and H2. The latter is utilised to dechlorinate the contaminant. The only issue is that they may be completion for the H2 with the methanogens, that produce methane from the H2.

Slide 28 Surfactant
One of the reasons for recalcitrance of many contaminants is their hydrophobocity and therefore poor bioavailability. Some bacteria produce biosurfactants that help them access hydrophobic compounds. (e.g. Fairy liquid on dry soil).

Slide 29 Iron corrosion chemistry
Fe is a reducing agent, accepts electrons from the chlorine contaminant- so reducing it. Metallic or zero valent iron (Fe^0) is a moderate reducing agent, which can react with dissolved oxygen (DO) and to some extent with water.

$$2Fe^0 + 4H^+ + O^2 = 2Fe^{2+} + 2H_2O \quad (1)$$

$$Fe^0 + 2H_2O = Fe^{2+} + H_2 + 2OH^- \quad (2)$$

The above equations are the classical electrochemical/corrosion reactions by which iron is oxidized from exposure to oxygen and water. According to equations (1) and (2), iron mediated reactions produces a characteristic increase in pH and decline in solution redox potential (Eh). A highly reducing environment (Eh <0) is created through the rapid consumption of oxygen and other potential oxidants and production of hydrogen.

Slide 30-31 **Fe corrosion chemistry**
Laboratory research in the 1990s demonstrated that through this classic corrosion chemistry, iron can effectively reduce a broad array of organic compounds, such as chlorinated aliphatics, nitro aromatics, PCBs, pesticides and related compounds. In this reaction elemental iron acts as the electron donor while relatively oxidized compounds serve as the electron acceptor (e.g. are reduced), resulting in the contaminants being transformed into less toxic or even benign end products. For example, TCE can readily accept the electrons from iron oxidation and be reduced to ethane in accordance with the following stoichiometry:

$$C_2HCl_3 + 4Fe^0 + 5H^+ = C_2H_6 + 4Fe^{2+} + 3Cl^-$$

Trichloroethene degrades to ethane