Biotechnology for substainable processes and environmental protection

1) Discuss the enzymes involved in cellulose degradation

Glycoside hydrolases, NAD depending
2 Steps involved: 1 – Glycosylation, 2 - Deglycosylation:

EG - Endoglucanase

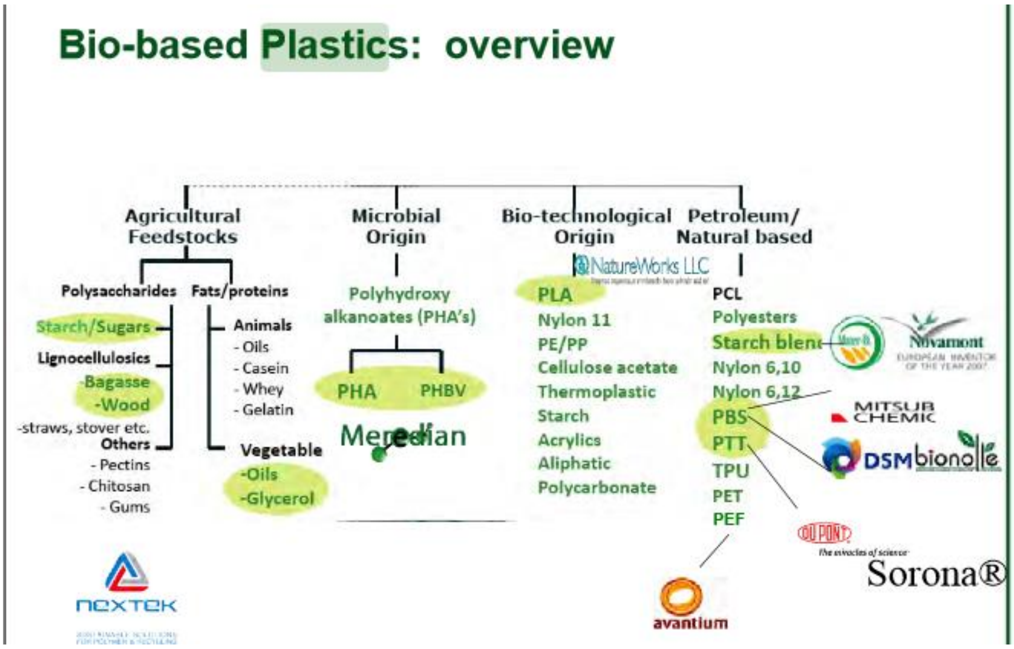
CBH - Cellobiohydrolases

βG - beta-Glucosidase

CDH - Cellobiose dehydrogenase

PMO - Polysaccharide monooxygenases

2) Plastics", sort them by origin and fate in environment + 1 example each (biological, oil, synthetic, ... ; biodegradable, undegradable)



3) Differences, advantages and disadvantages of 1st and 2nd gen biofuels:

1. Generation:

   Made from sugar, starch or vegetable oil (e.g. Ethanol). Made from compounds that could be used as food, raises prices for food especially in developing countries. Easy to extract but no significant greenhouse gas reduction compared to conventional fuels

2. Generation:
   Uses lignocellular biomass. Doesn't use food but agricultural "waste" and is therefore more sustainable, but extraction process is more difficult.

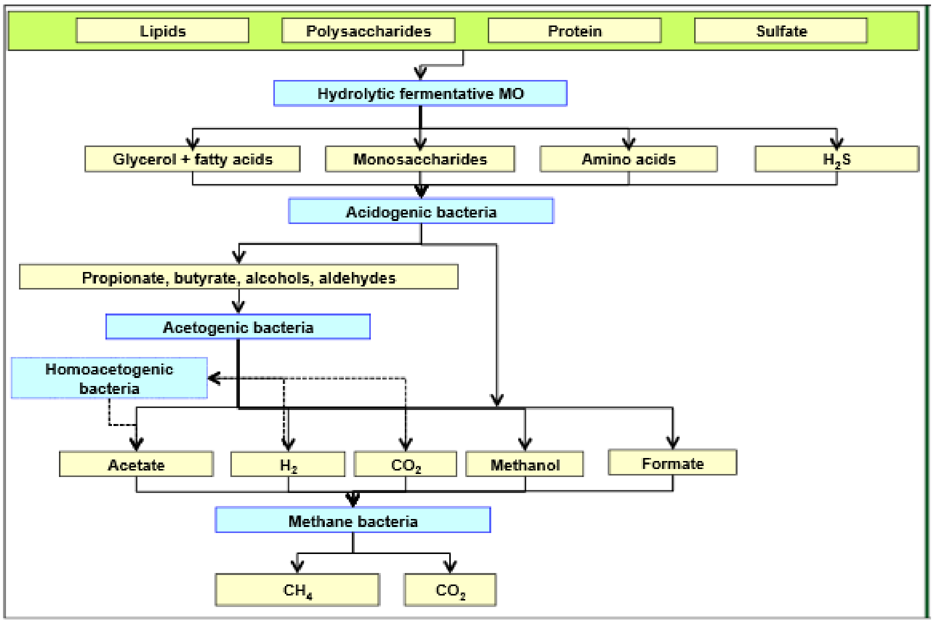
4) 2 examples of enzyme based bioremediation:

Enzymatic treatment of bleaching effluents by catalases
Azo compound degradation by azo-reductase
Hydrolases (Chitinase – Waste of Crabs, Keratinase – Degradation of chicken feathers)

5) dicuss and give an example of co-metabolization in bioremediation

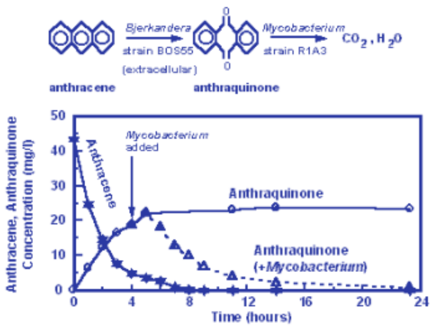
Co-metabolism is defined as the simultaneous degradation of two compounds, in which the degradation of the second compound (the secondary substrate) depends on the presence of the first compound (the primary substrate). For example, in the process of metabolizing methane, propane or simple sugars, some bacteria, such as *Pseudomonas stutzeri* OX1, can degrade hazardous chlorinated solvents, such as tetrachloroethylene and trichloroethylene, that they would otherwise be unable to attack. They do this by producing methane monooxygenase, an enzyme which is known to oxidize numerous compounds, including pollutants such as chlorinated solvents, via co-metabolism. Co-metabolism is thus used as an approach to biological degradation of hazardous solvents.

6) Microbial steps in biogas formation

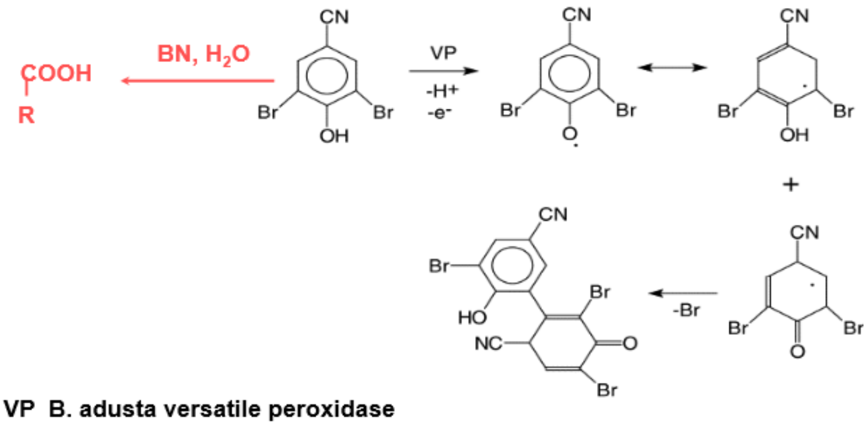


7) Describe the degradation of anthracene and bromoxynil

Enzymatic / microbial degradation of anthracene



Enzymatic elimination of bromoxynil



VP B. adusta versatile peroxidase
BN 3.5.5.6 :bromoxynil nitrilase

8) List five applications of enzymes for products of lignocellulosic material:

Biotechnology in lignocellulose processing

- **Applications of laccase in the pulp and paper industry**
  - Biopulping
  - Biobleaching
  - Treatment of pulp for enhancing paper strength properties
  - Pitch control by pulp treatment
  - Deinking
  - Mill process water and effluent treatment
- **Biografting onto lignocellulosic materials**
- **Fiber/lignin cross-linking for the production of wood composite boards**

9) Describe applications of bioremediation and the rationale for selecting the method of choice

Current applications: Water treatment, Agriculture

Also Deep Water Horizon oil spill

Microorganisms for heavy metal bioadsorption (eg MTs produced in E. coli and Metalloregulated proteins MerR and ArsR), Metal precipitation, Enzymatic Transformation of metals, Enzymatic transformation of organophosphates, Protein engineering

10) Explain In situ Processes

In situ processes:

1) Biostimulation
2) Bioaugmentation

Biofilms: FISH and In-situ PCR

- **Fluorescence in situ hybridization FISH**
  - In-situ hybridization with fluorescently labelled oligodeoxyribonucleotides (16S rRNA)
  - Stabilise cells and make permeable
  - Enumration with microscopy or flow cytometry
- **In-situ PCR**
  - FISH cannot detect functional genes (lower copies) but would be interesting to understand function
    - Limited to 20 copies of mRNA
  - Methodology
    - sample preparation: fixation and permeabilization
    - thermal cycling cellular material in solution or on glass slides
    - Detection of amplificants

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11) List Enzymatic steps in textile Processing

Biotechnology in textile processing

200 M€ Enzymes in Textile processing