Environmental enzymology past, present and future perspectives

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Woods, A. F. (1899) The destruction of chlorophyll by oxidising enzymes. *Centralbl. Bakteriol. Parasitenk.*, Abt. II, 5, 745.

'I have also determined by experiment that oxidizing enzymes of decay especially the peroxidase, *may occur in soil and as a rule are not destroyed by the ordinary bacteria'*

ISI Web of Knowledge - soil + enzyme 2000-2010
SBB papers >500
All papers and patents >15,000

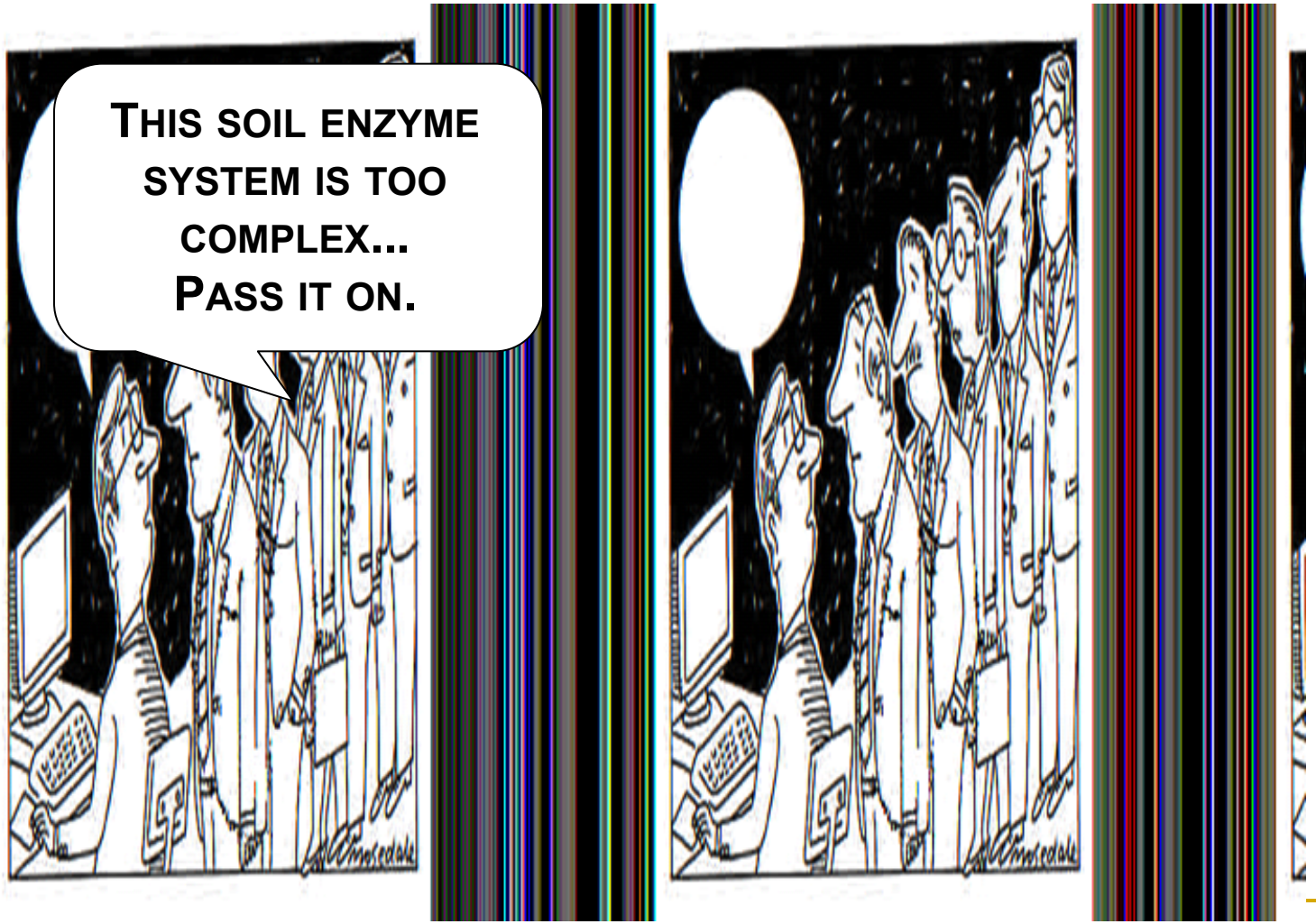
'Producing activated charcoal from lignocellulose containing residual solids and enzymes - useful to treat gout'

Since then:110 years of soil enzyme research

- What?
- Where?
- Why?
- How?

Is it getting any clearer and can we hope to ever apply our knowledge and manipulate the processes?

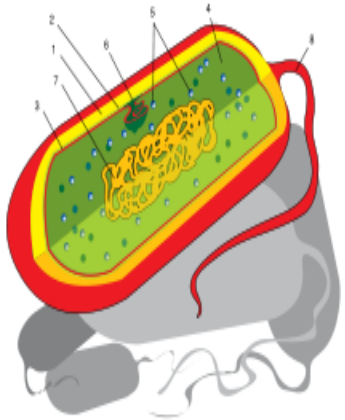
Are the problems too difficult for even the Enzymes in the Environment RCN to solve?

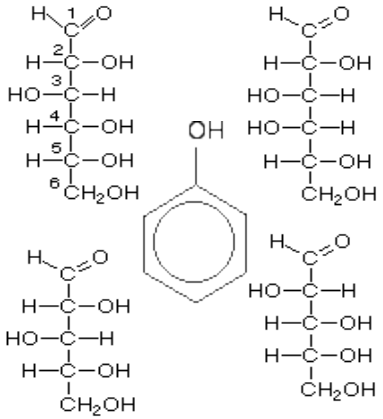


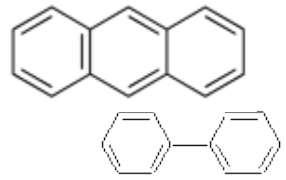
Simple and complex (and changing) substrates

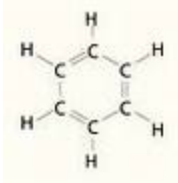
- **Urea hydrolysis to ammonia and carbon dioxide by the most 'popular' soil enzyme urease and cellobiose cleavage to by cellobiase to glucose are not comparable with ligno-cellulose breakdown.**
- **In other words, the complex multi-enzyme, multi-step and, necessarily, extracellular processes involved in lignocellulose degradation have kinetic properties that are worlds apart from simple hydrolysis of soluble low molecular mass substrates**

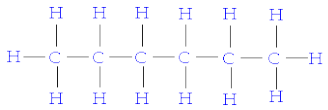
The substrate, the microbe, the enzyme: a complicated story











Some of the problems faced by the soil and sediment microbes

Detect, move towards, and convert organics to soluble structures that can then be transported into the cell and metabolised

Employ the simultaneous and/or sequential activities of a large number of extracellular enzymes and many bacterial and fungal genotypes

More than 50 different extracellular enzymes are involved in the decay of a plant leaf before the soluble products even enter the microbial cell!

And then there are all the enzymes of glycolysis

Environmental enzymology: why does it matter and what questions should we ask?

1. Why conduct environmental enzyme research
2. How are macromolecular, insoluble biopolymers degraded in soil?
3. How are the necessary extracellular enzymes regulated?
4. Where are extracellular enzymes located once they have left the cell?
5. Are there strategies for the effective functioning of these extracellular enzymes?
6. Can microbial and enzymatic transformation processes be manipulated for the 'benefit of the humans and the environment'?

Environmental enzymology research: looking for a purpose

soil ‘fertility’ soil ‘health’ and soil ‘sustainability’

soil organic matter and climate change

C and N cycle modelling

reclamation and remediation

biocontrol (pre- and post-harvest)

matching plants to soils and climates

manipulating the rhizosphere

targets for transgenics

relevance to many other applied research fields

Extracellular enzyme research is high on many R and D lists

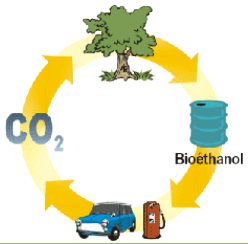
- Phytopathogens
- Mycoparasites
- Ruminants
- Earthworms
- Ants and termites
- Protozoa
- Mycorrhizal fungi
- Legumes
- Bioethanol
- Food science
- Human health

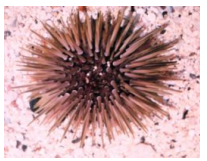
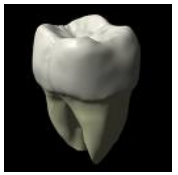








Environmental enzyme research: fundamental (curiosity driven?) studies

substrate detection and enzyme regulation

spatial and temporal location of enzymes and substrates

plant-microbe and microbe-microbe communication

applying exciting new methods: microscopical, analytical, metatranscriptomics, metaproteomics and lots of other other 'omics'

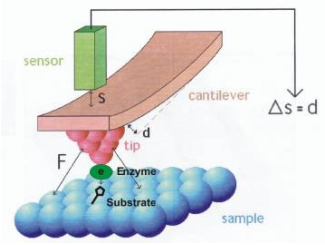
The lure of microbial ecology – a blessing or a curse?

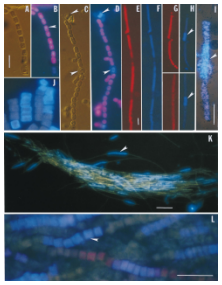
Be aware of (but beware of): communities, biofilms, mobile DNA, quorum sensing, chaos theory

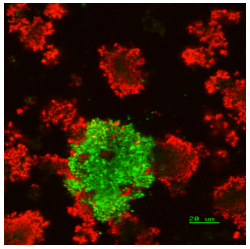
and keep a sharpened Occam's razor nearby

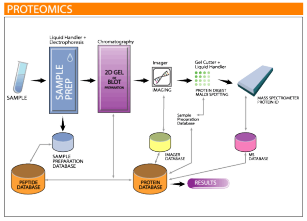
Exciting new ways to reveal the origins, location and activities of enzymes in soil

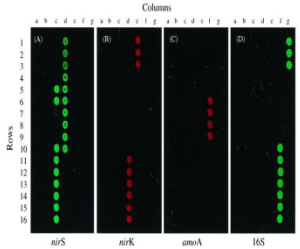
- Atomic force microscopy
- Immunofluorescence
- Autofluorescence
- ‘Omics galore
- Functional gene probes
- Nano-sensors

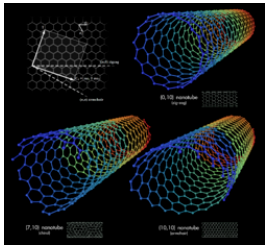








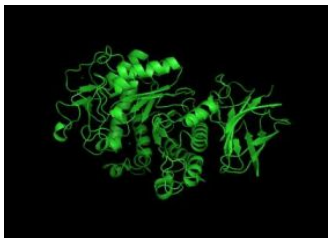




Complex substrates - Ligno-cellulose

(i) the easy bit - cellulose to glucose

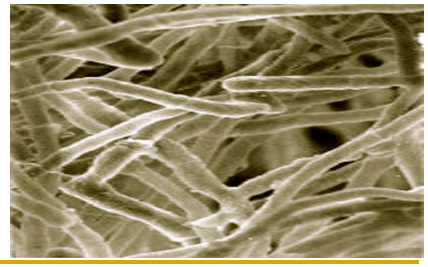
- 1,4-β-D-glucan-4-glucanohydrolases (EC 3.2.1.4)
- 1,4-β-D-glucan cellobiohydrolases (EC 3.2.1.91)
- 1,4-β-D-glucosidases (EC 3.2.1.21)



But there are dozens of enzymes and isoenzymes in each group based on: reaction kinetics, substrate specificity, pH and temperature optima, molecular mass, stability, active site topology, catalytic domains, etc.

Why? the substrate and the microbial microenvironment is ever changing…

The classic cellulose degrader, *Trichoderma reesei* has >30 cellulases and a secretome of >100 proteins



Ligno-cellulose the impossible substrate
(ii) the difficult bit - lignins to quinones, phenols, aldehydes etc

- **manganese peroxidases (EC 1.11.1.13)**
- **lignin peroxidases (EC 1.11.1.14)**
- **laccases (EC 1.10.3.2)**
- **(and lots of other 1.10.3s and 1.11.1s)**
- **cellobiose dehydrogenases (EC 1.1.99.18)**
- **pyranose-2-oxidases (EC 1.1.3.10)**
- **glyoxal oxidases (EC 1.1.3. -)**
- **superoxide dismutases (EC 1.15.1.1)**
- **aryl alcohol oxidases (EC 1.1.3.7)**

ad infinitum

***Coprinopsis cinerea* has a secretome of 1769 proteins of which at least 100 are enzymes**

Phanerochaete chrysosporium a white rot fungus that may have solved the problems

P. chrysosporium **degrades cellulose, hemicellulose and lignin and has 87+ genes for extracellular glycosyl hydrolases, 103+ for 'ligninases' and a total predicted secretome of 790 proteins!**

P c **also has bioremediation potential: PAHs, PCBs, PCPs, organo-chlorine and phenoxyacid pesticides, phthalates, dioxins, TNT and so on**

Cellulases and 'ligninases' regulation: two very different stories

- ***Cellulase*** **inducers and derepressors? Dimers sophorose, laminaribiose and xylobiose.** ***Not*** **glucose**
- **Direct or indirect regulation? Sophorose increases disaccharide permease activity**
- **Inducers may arise from autohydrolysis of fungal cell wall**

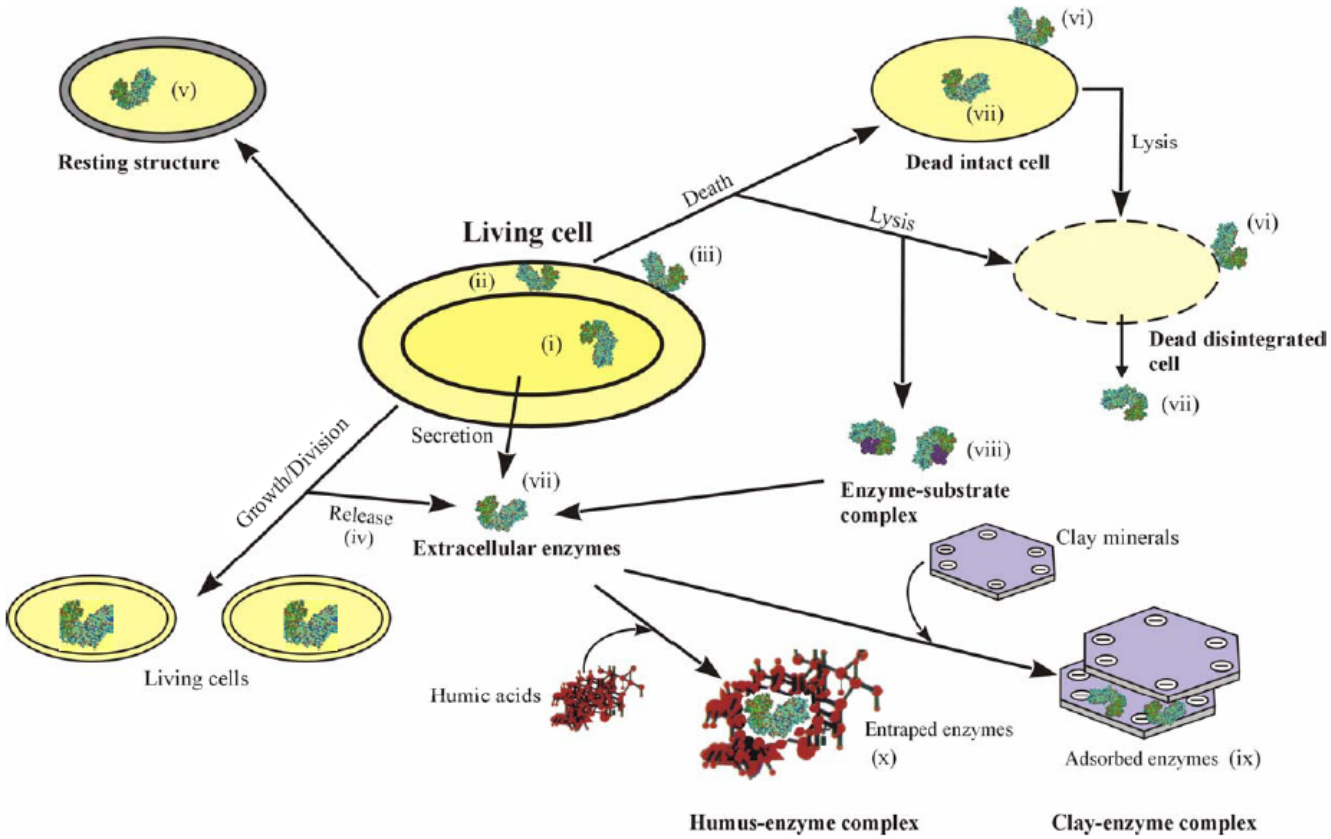
- ***'Ligninase'*** **expression regulation is a 'stress response'**
- **Sulphide and sulphate, Fe^{2+}, N, redox potential, matric potential, CO_2, ionic strength, temperature, oxalic acid,**

The rate limiting steps in converting ligno-cellulose to soluble monomers for cell uptake and metabolism must number in the dozens.

How do microbes and their enzymes locate and degrade natural polymers in soil?

- How are the necessary extracellular enzymes regulated?
- Where are extracellular enzymes located once they have left the cell?
- What contribution does each location make to the overall process?
- Are there various strategies for the effective functioning of extracellular enzymes?

Where are microbial enzymes located in soil?



Wallenstein MD & Burns RG (2010)

Contribution of each enzyme compartment to the total substrate catalysis will change in time (and space)

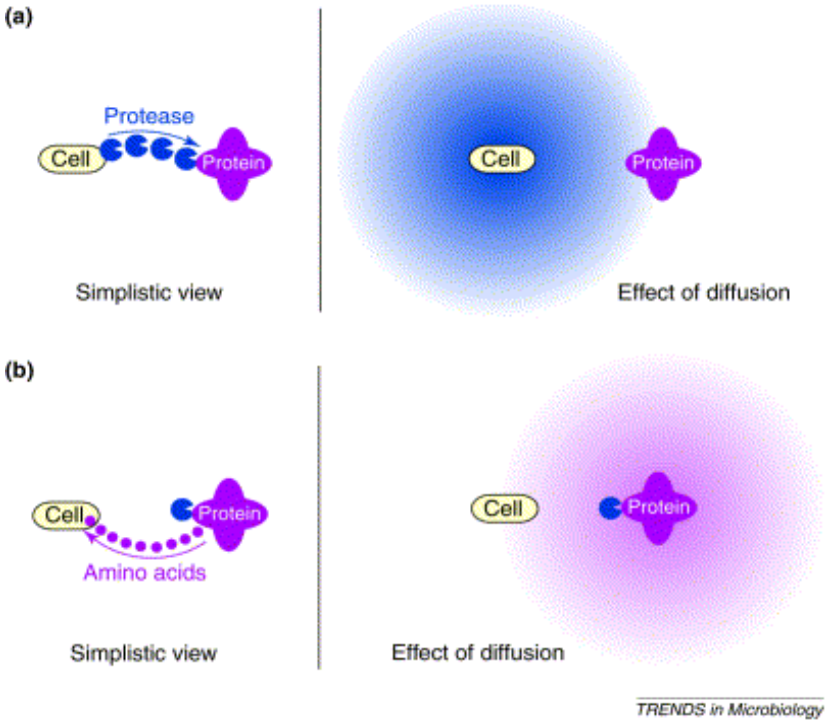
- The one hour assay = existing pre-generated enzyme (accumulated, mural, solution, etc)
- The six hour assay = the above plus new enzymes from *r*-strategists and zymogenous bugs
- The 24-hour assay = the above plus new enzymes from *K*-strategists and autochthonous bugs
- The 72-hour assay = the above plus activity due to selection pressures and resulting *new* interactive microbial communities

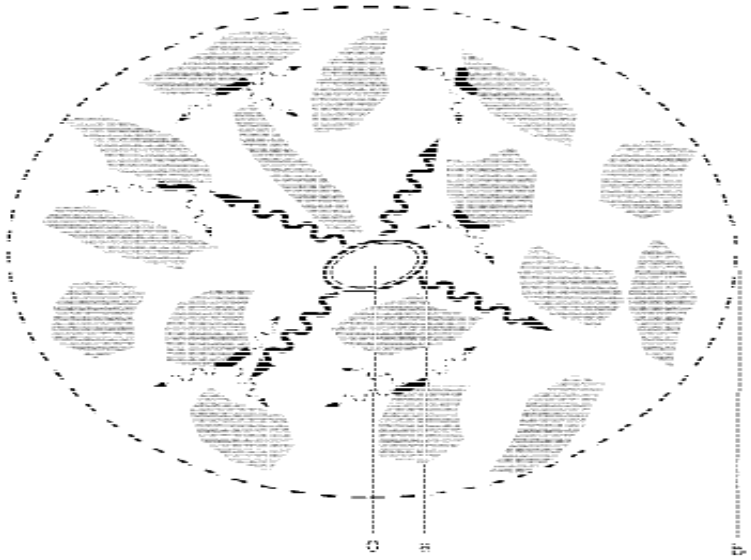
How do microbes and their enzymes locate and degrade natural and synthetic polymers in soil?

And should we call this soil enzyme (nano)ecology?

What are the various strategies for the effective functioning of these extracellular enzymes?

Strategy 1: microbes constitutively secrete enzymes to forage for substrates - *but* dilution and sorption reduce efficiency





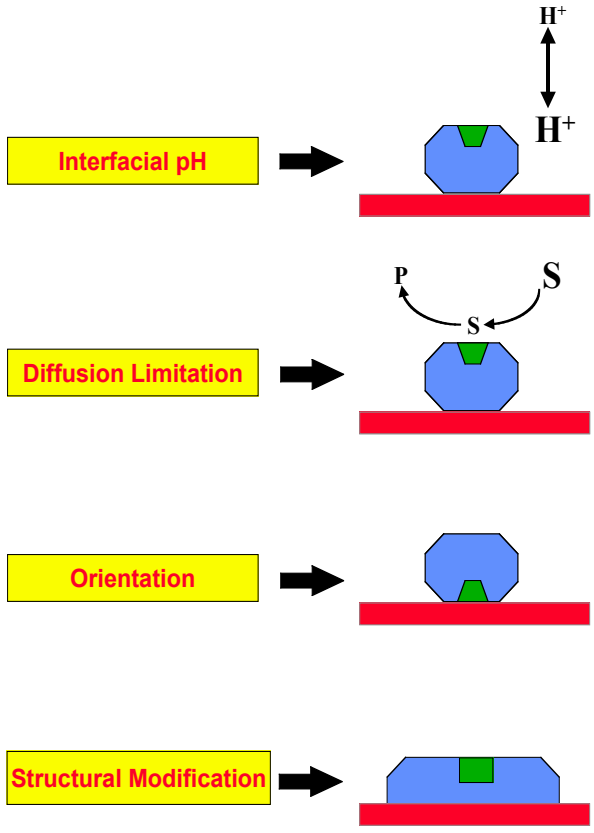
And many other factors that make enzyme secretion in soil a high risk strategy

1. Attacked by proteolytic microbes
2. Substrate doesn't exist
3. Substrate never found (remote or hidden within microaggregates)
4. Wrong mix or sequence of enzymes
5. Others ('cheaters') cash in = no rewards

Ways around these problems: (i) pre- or post-secretion modifications and behaviour will help stabilise enzymes in solution

- ***Stabilization*: conformational changes and polymerisation; disulphide bonds; complexing with glycoproteins and polysaccharides; proteolysis yielding active subunits; co-secretion with protective proteases and antibiotics**
- ***Optimization*: combining with other secreted enzymes (sequence) ; orientation at cellulose surface; binding modules; catalytic domains; and 2-D migration**

Ways around these problems: (ii) enzymes protected and retain activity when complexed with clays and humates



Quiquampoix & Burns (2007)

Are clay- and humic-immobilized enzymes a good strategy for substrate degradation?

Stable humic-*B*-glucosidase generates glucose from cellobiose

Synthesis and secretion of new microbial cellobiase induced or up-regulated after *signal recognition* and chemotaxis

Stable humic-clay-urease rapidly hydrolyses urea

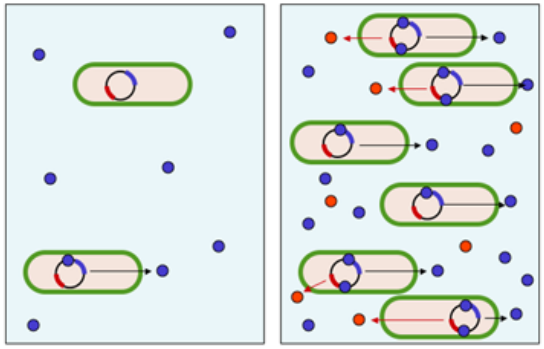
Ammonia provides energy for chemolithotrophs and then nitrate (good rhizosphere sequence)

Difficult to see a key role in ligno-cellulose decay

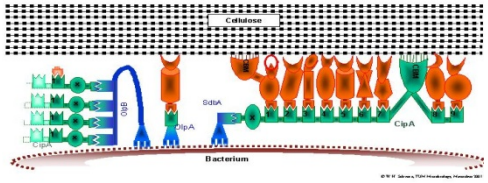
(although colloids are mobile/diffusive)

Strategy 2: take your time - don't secrete (many) enzymes until the substrate is detected

- Many plant phytopathogens (the *Erwinia* story) only secrete cell wall depolymerases when substrate quality, quantity, and proximity is appropriate and when microbial density is high
- Homoserine lactones and oligopeptides provide one answer to this problem – *quorum sensing*



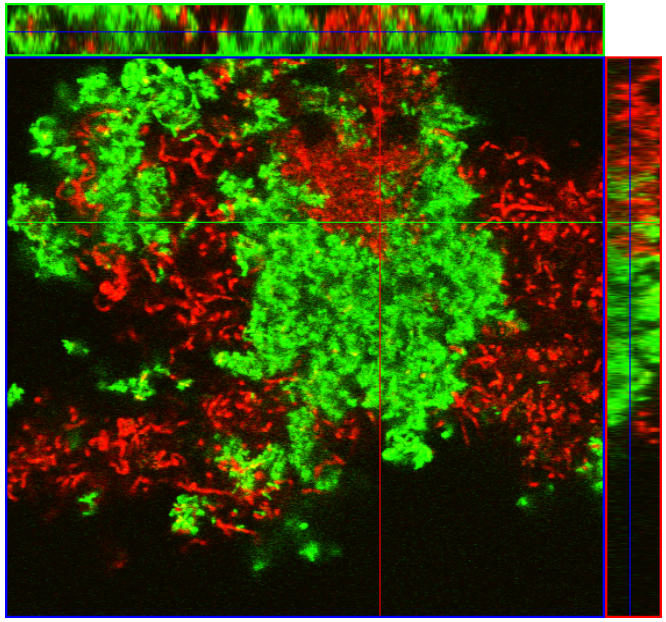
Strategy 3: keep the enzymes close (i) the 'somes'



- Some soil bacteria and fungi retain their extracellular enzymes at the cell wall whilst others package cellulases within a 'cellulosome' (18-200nm, 100MDa)
- Cellulosomes are attached to the outside wall of the cell and contain numerous hydrolases (c, hc, pc) arranged on a protein scaffold to optimise attachment to substrate
- Other poysaccharide-degrading enzymes (e.g. xylanases) are contained in the cellulosome ('celluloxylanosome') or exist as separate 'somes (xylanosome, pectinosome)
- Are there 'peroxidosomes' and 'laccasosomes' and 'dehalagenosomes' and, if not, can we construct them?

Strategy 3: keep the enzymes close (ii) the community biofilm

Motility - attachment - biofilms - community development - process competence - and then enzyme secretion



Summary

- Microbes have a number of strategies to efficiently degrade insoluble/non-bioavailable organics in soil
- Different substrates require different combinations of strategies at different times
- Understanding these strategies may enable their manipulation to 'improve' the soil environment
- Combinations of microbes, enzymes (and plants) generate high levels of appropriate catalytic activity

Immobilized and persistent cell and enzyme inoculants can be designed for soils, crop and climate?

As can transgenic plants containing and secreting useful enzymes?

Enzymes and substrates in soil from Pollock to Miro to Rothko - getting easier to understand all the time