Bacterial lignin degradation for enhancement of municipal waste breakdown

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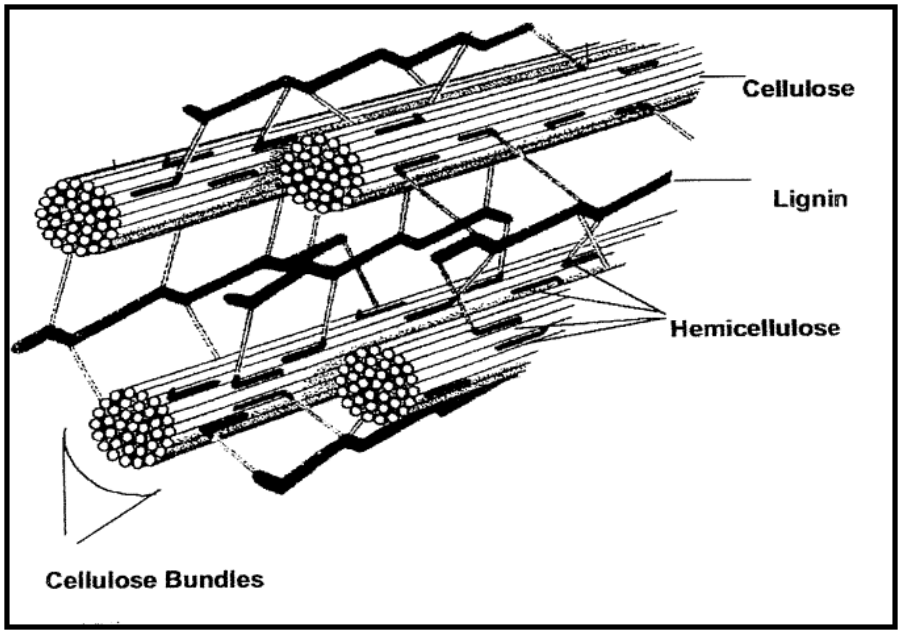
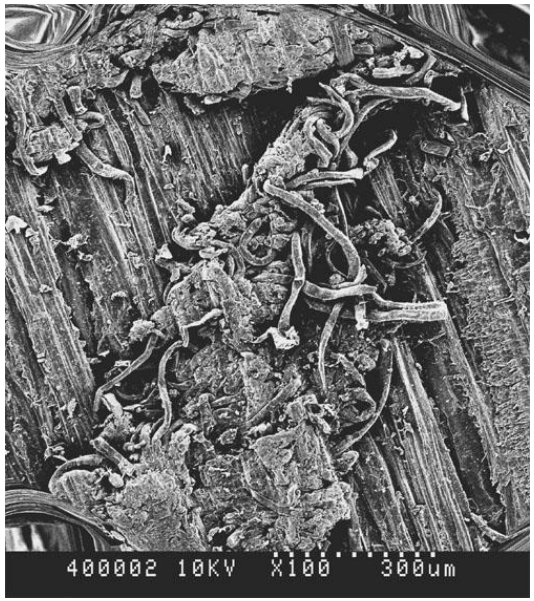
Lignocellulose is the main component of plant cell walls





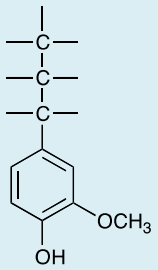
Lignocellulose	Cellulose	Hemicelluloses	**Lignin**
Spruce wood	42	22	**27**
Pine wood	38	12	**28**
Birch wood	38	20	**23**
Poplar wood	50	25	**18**
Corn stover	36	23	**17**
Wheat straw	38	25	**23**
Switchgrass	31	24	**18**

Table 1. % composition of various lignocelluloses.

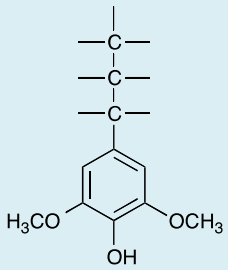
Molecular Structure of Lignin

- High molecular weight aromatic polymer
- Heterogenous structure, highly cross-linked
- Contains aryl-C3 units:

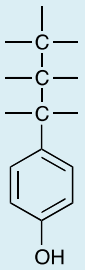
- Different substitution patterns in different plant types:



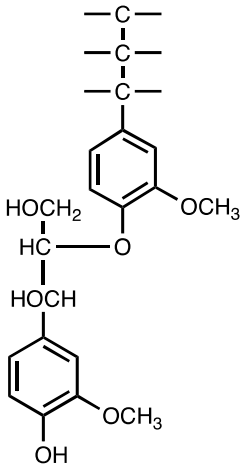
guaiacyl unit (G)



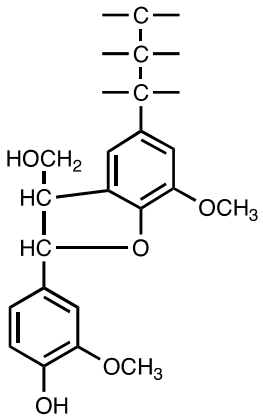
syringyl unit (S)



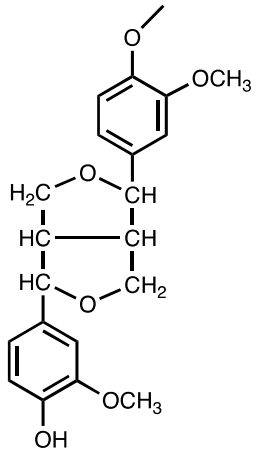
p-hydroxyphenyl unit (H)



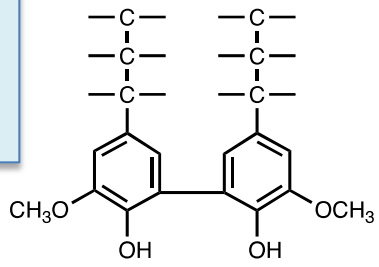
β-aryl ether



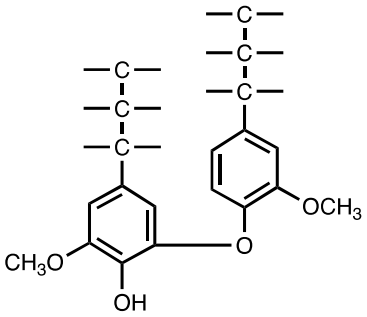
phenylcoumarane



pinoresinol



biphenyl

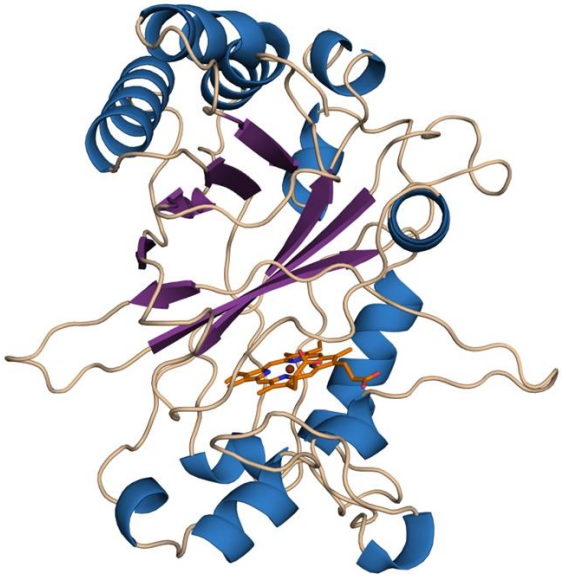


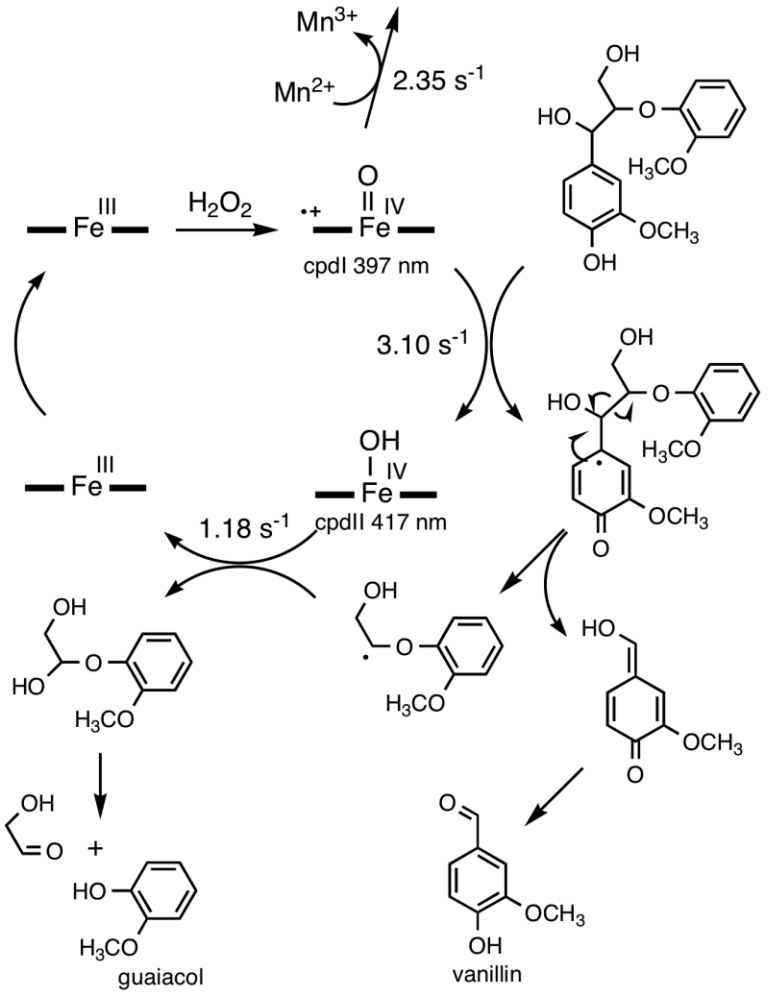
diaryl ether

Potential source of aromatic products

Identification of *Rhodococcus jostii* DypB as a Lignin Peroxidase

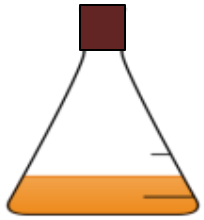
- first bacterial lignin peroxidase enzyme DypB identified in 2011
- oxidises β-aryl ether lignin model compound and Mn(II)
- Crystal structure of *R. jostii* DypB determined in collaboration with Prof. L. Eltis (UBC, Canada)



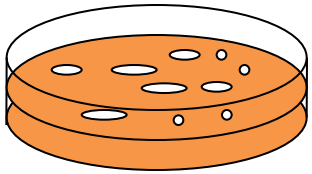


M. Ahmad, E.M. Hardiman et al., *Biochemistry*, 2011, **50**, 5096
J.N. Roberts et al., *Biochemistry*, 2011, **50**, 5108.

Screening for Novel Lignin Degrading Strains



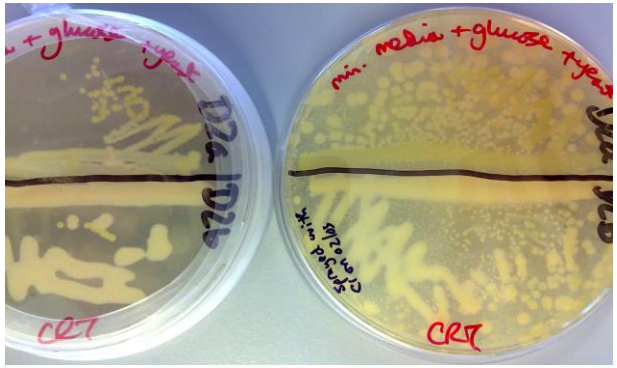
Culturing –
selective medium



Plating

Sampling

Nitrated lignin
spray assay



Pure cultures

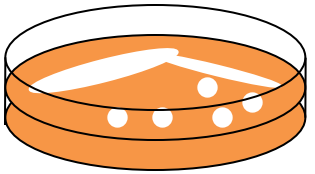


Plate assay

- Identification bacterial species (e.g.16S rRNA)
- Purify and identify extracellular enzymes
- Recombinant expression

Plan for Work Package 2 of NERC INSPIRE Project

2.1 Identification/isolation of bacterial species responsible for lignocellulose breakdown in landfill systems (months 1-9)

- Screen for lignin degraders from MSW, using colorimetric lignin degradation assay.
- Identify new strains by 16S rRNA sequencing

2.2 Testing of enzymatic/microbiological additives at batch scale (50-100 ml, month 9-18)

- Test lignin-degrading bacteria (or recombinant lignin-degrading enzymes) for:
  - delignification
  - Phenol release
  - Enhancement of methane production

2.3 Column studies of MSW +/- inoculum (with Cardiff, month 18-30)

Task 2.1 Lignin-oxidising bacteria isolated from MSW

Sample	Growth temp (ºC)	Highest identity sequence match from 16S rRNA sequence	GenBank accession	Sequence identity	Bacterial family
MSW-1	30	*Ochrobactrum* sp.	Growth in K.L.	99%	α-Proteobacteria
MSW-1	30	*Pseudomonas* sp.	-	98%	γ-Proteobacteria
MSW-1	30	*Microbacterium oxydans*	-	99%	Actinobacteria
MSW-1	30	*Agrobacterium* sp.	Growth in K.L.	99%	α-Proteobacteria
MSW-1	30	*Ochrobactrum pecoris*	Growth in K. L.	99%	α-Proteobacteria
MSW-2	30	*Ochrobactrum pituitosum*	NR 115043	99%	α-Proteobacteria
MSW-2	30	*Comamonas testosteroni*	KJ 806363	96%	β-Proteobacteria
MSW-2	30	*Enterobacter ludwigii*	GQ 284566	99%	γ-Proteobacteria
MSW-2	30	*Enterobacter cloacae*	KF 017288	98%	γ-Proteobacteria
MSW-2	45	*Lysinibacillus sphaericus*	HQ 259956	99%	Bacilli

Task 2.1 Testing of bacterial lignin degraders for delignification and phenol release

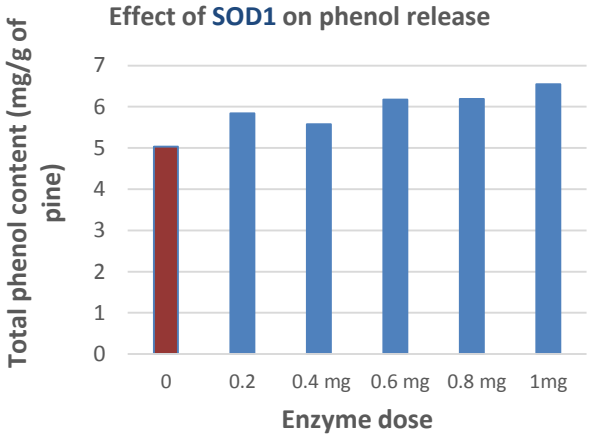
Bacterial strain	% Lignin decrease 7 days	% phenol increase 7 days	% phenol Increase 4 days
Pseudomonas putida	21.2	3.0	18.8
Pseudomonas fluorescens	20.6	0.6	NT
Microbacterium phyllosphaerae	7.8	31.3	16.3
Microbacterium sp.	17.6	31.3	NT
Rhodococcus jostii	9.5	38.7	NT
Rhodococcus erythropolis	7.3	63.7	39.1
Sphingobacterium sp.	8.5	43.0	13.2
Paenibacillus	1.0	1.8	21.0
Ochrobactrum sp.	12.9	6.7	26.8
Agrobacterium sp.	16.1	22.7	22.8
Ochrobactrum pecoris	17.4	18.0	NT
Ochrobactrum pituitosum	5.6	18.5	NT
Enterobacter ludwigii	17.3	20.0	NT
Enterobacter cloacae	22.7	14.1	9.7
Lysinibacillus sphaericus	24.0	10.0	19.1
Microbacterium oxydans	4.8	1.6	NT
Comamonas testosteroni	9.8	74.6	17.6

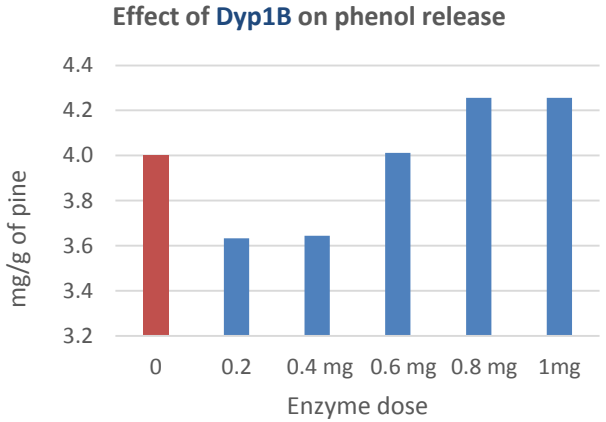
Testing of Lignin-Oxidising Enzymes for Delignification and Phenol Release

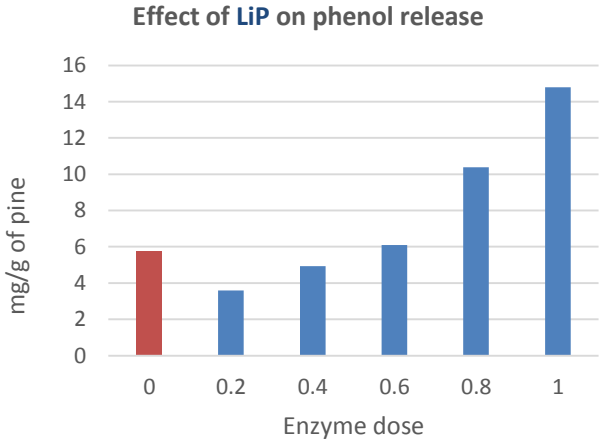
Sphingobacterium sp. T2 manganese superoxide dismutase

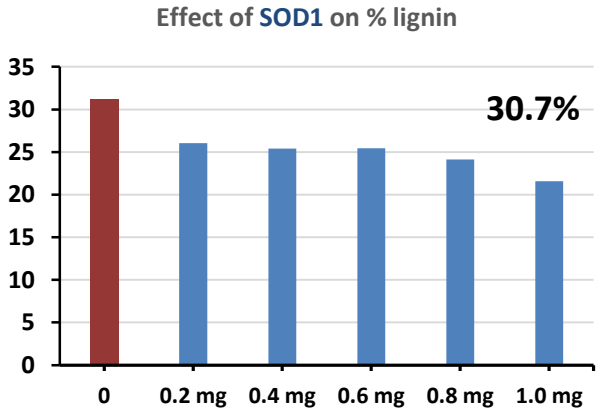
Pseudomonas fluorescens Dyp1B

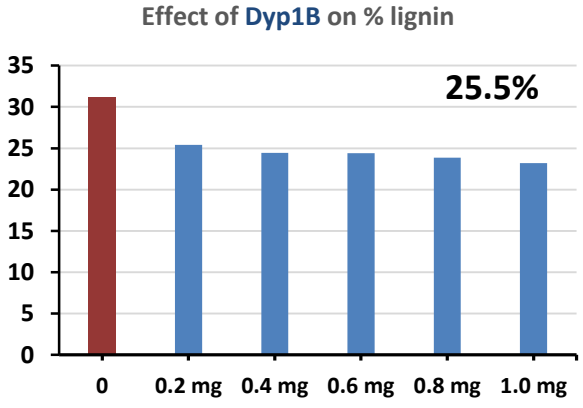
Compare with fungal lignin peroxidase

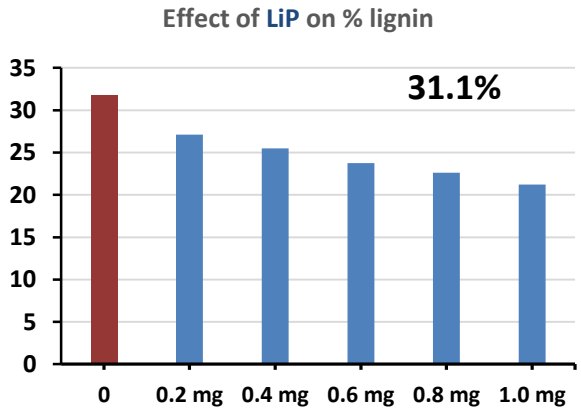




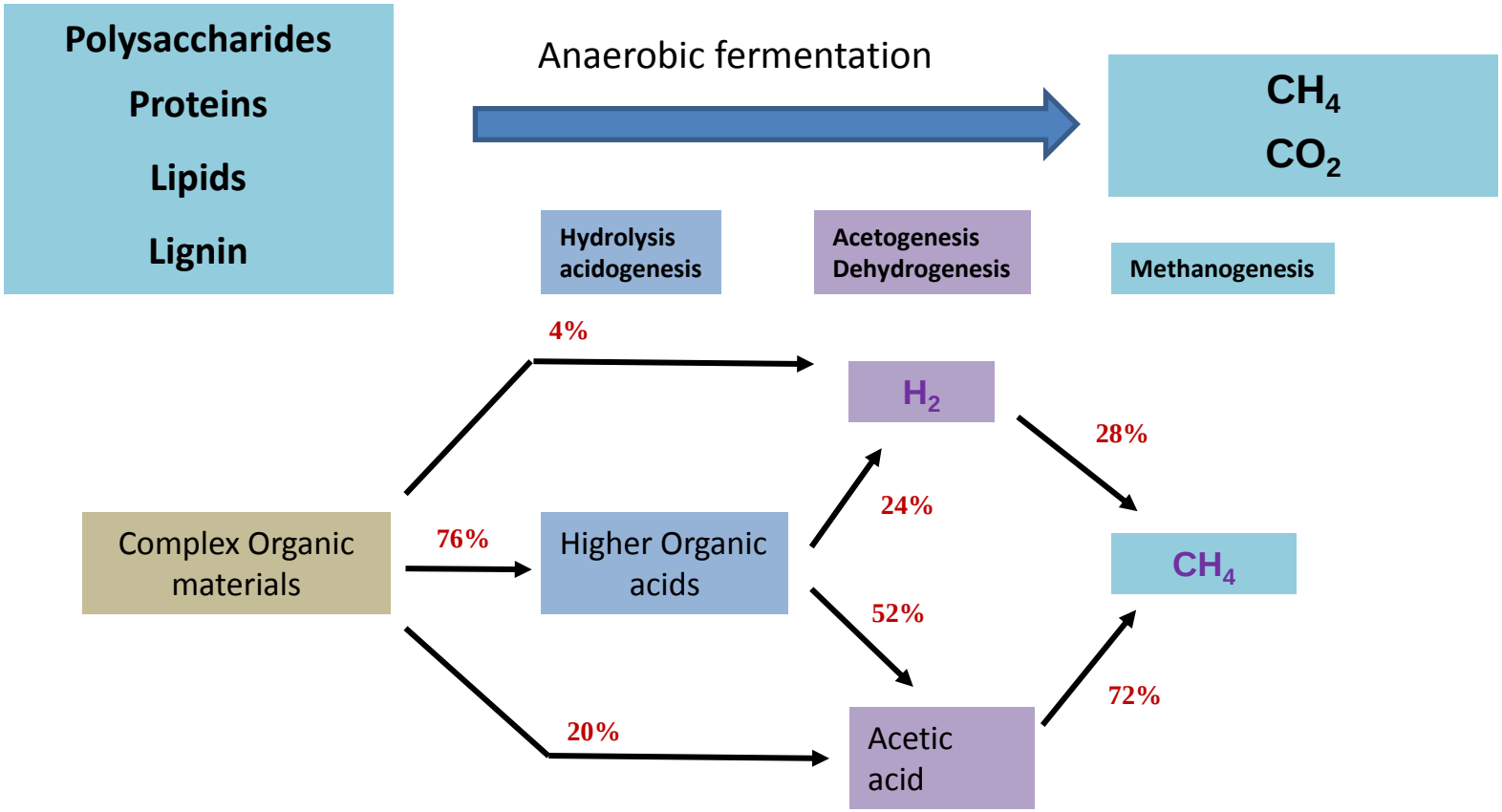








Enhancement of biogas production

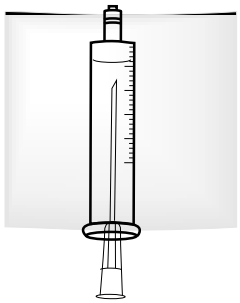


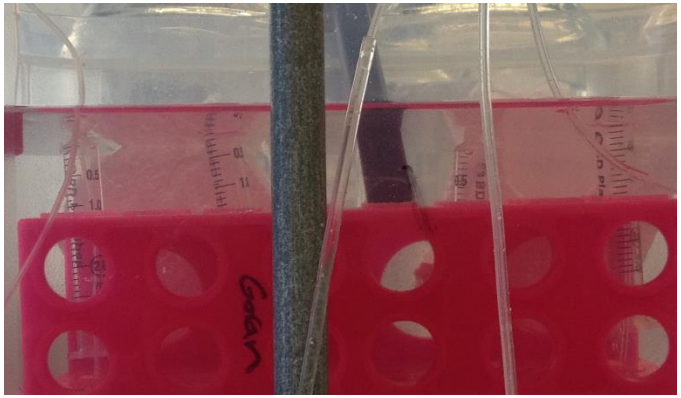
Examine the effect of lignin degrading bacteria on methane production

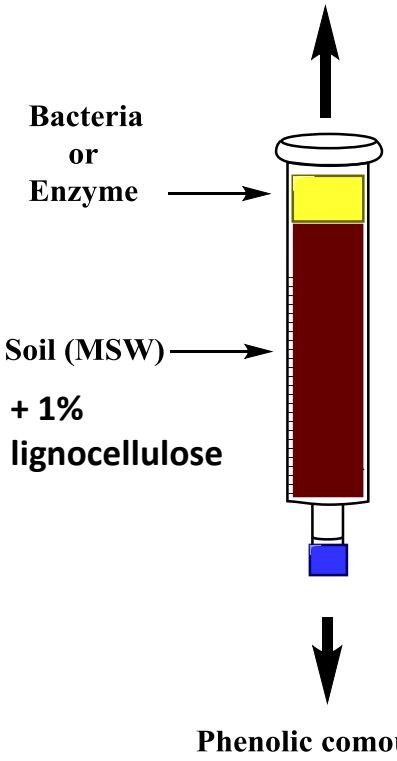
Agrobacterium sp., Paenibacillus, Pseudomonas putida, Ochrobactrum sp., Comamonas testosteroni and *Lysinibacillus sphaericus*

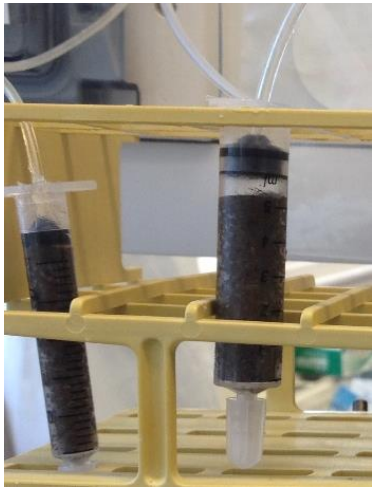
BUT lignin degradation is an aerobic process!

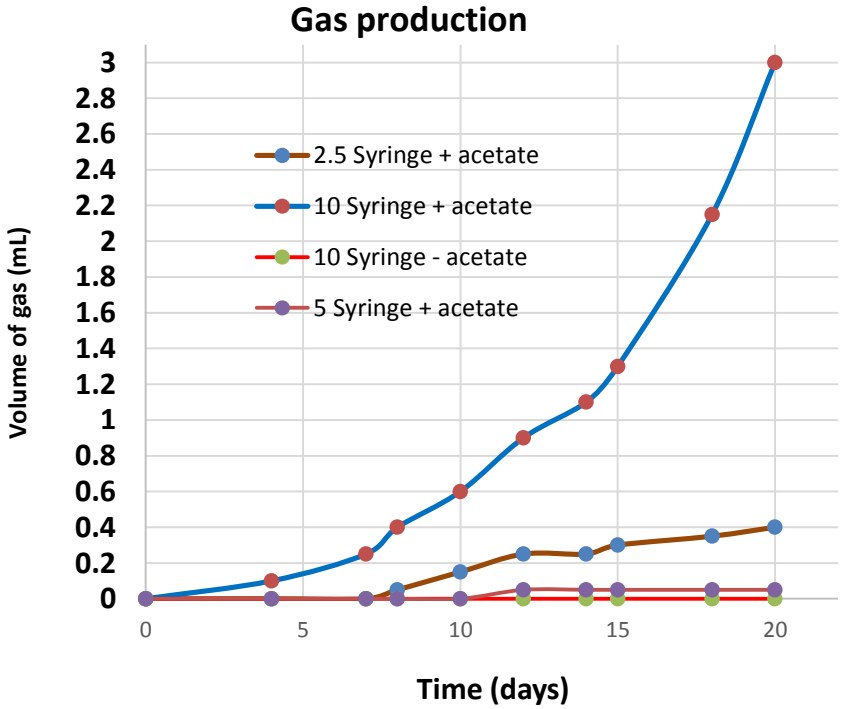
Experimental set-up for small scale gas production from MSW (no additive)



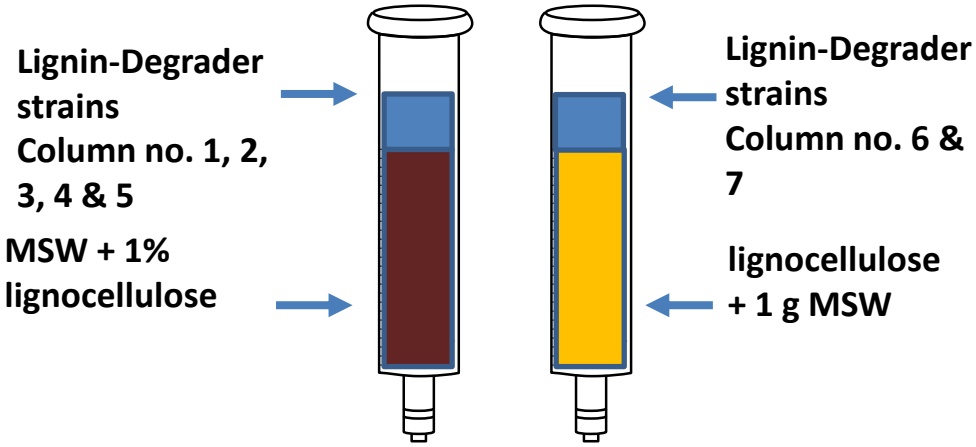


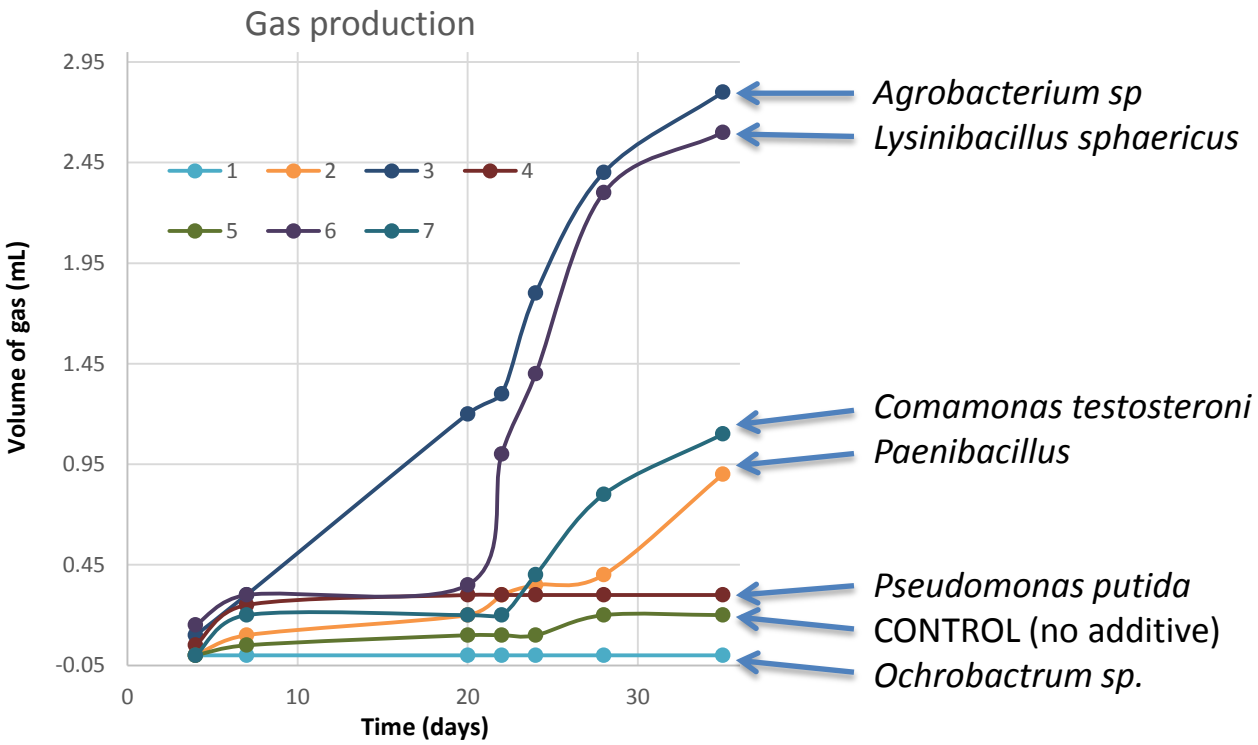




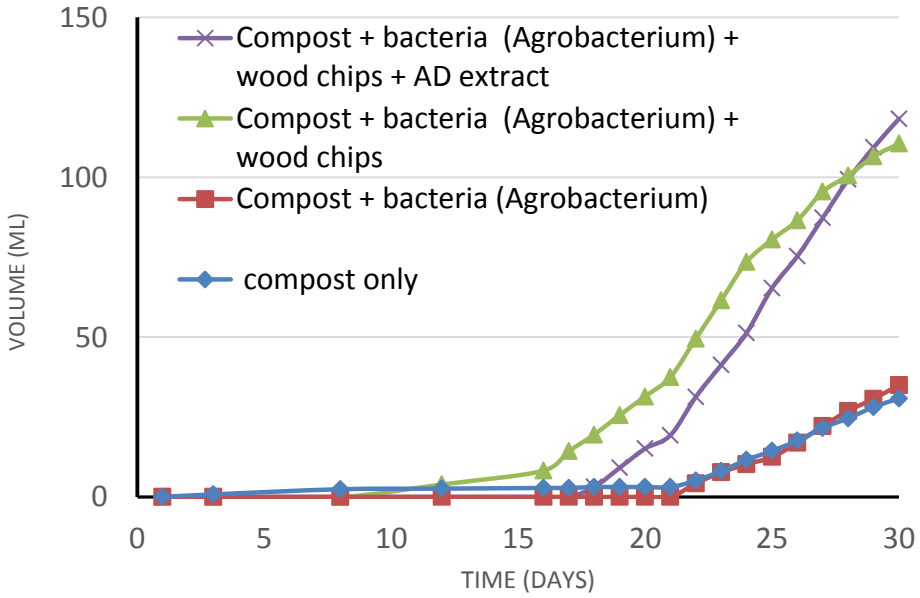


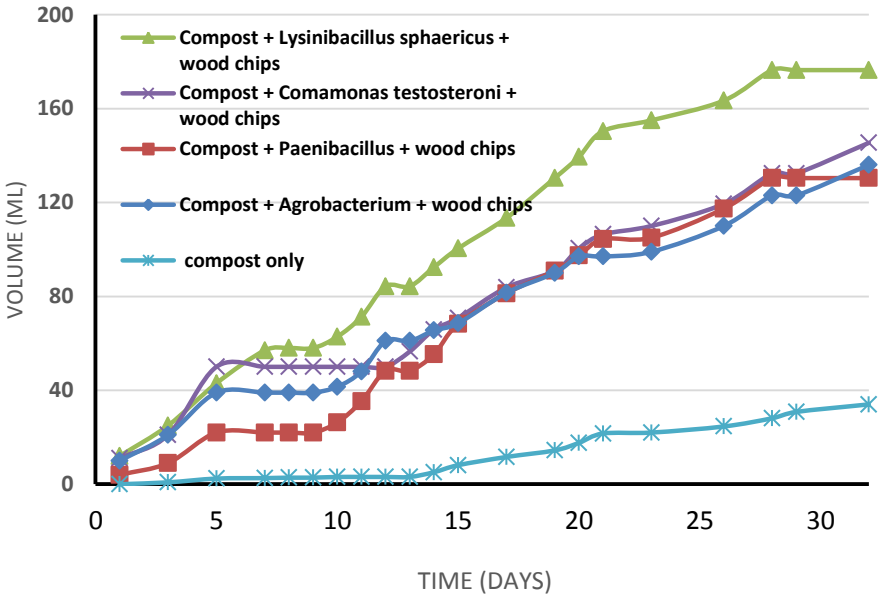
Enhancement of gas release using bacterial lignin-degrading strains



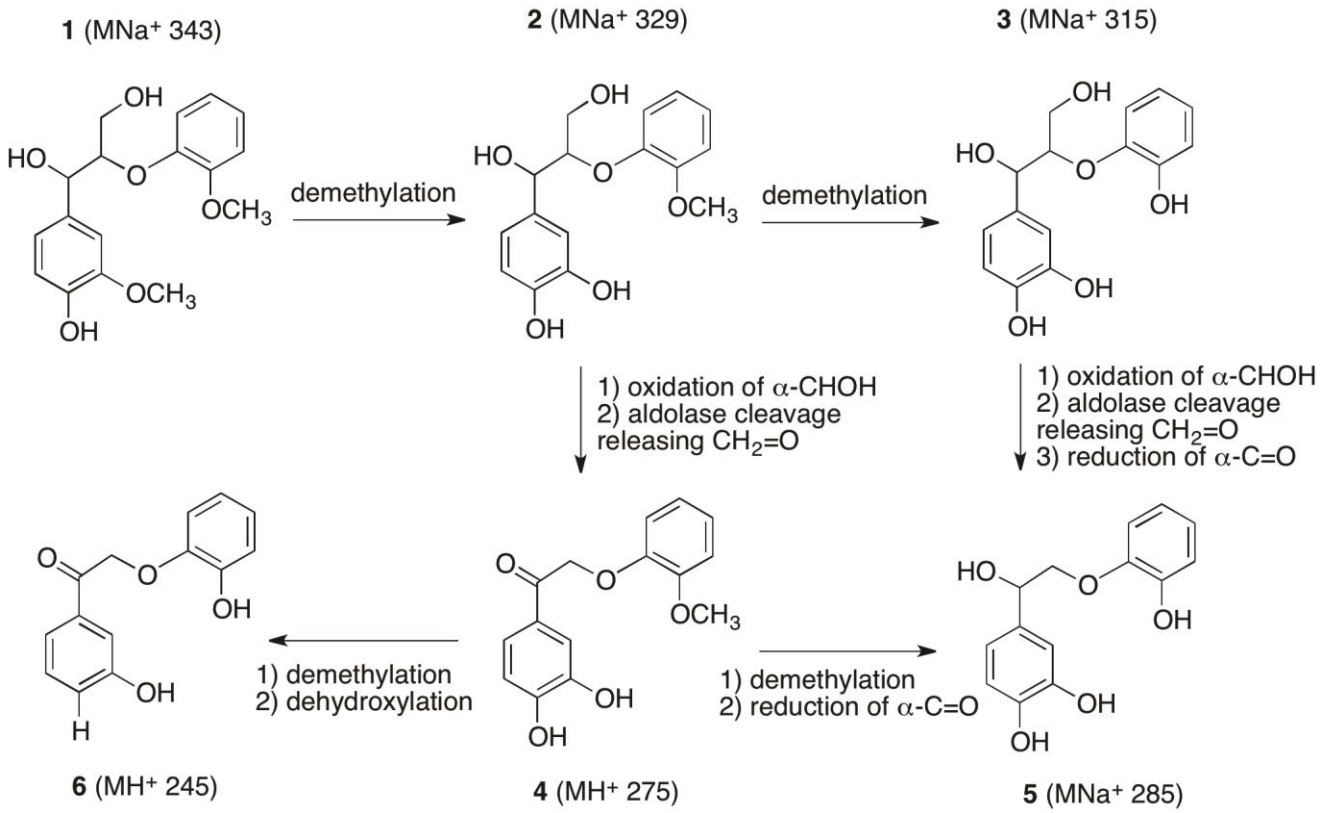


0.5 litre scale methanogenesis





Transformation of Lignin Model Compound



Compounds identified by LC-MS analysis over 4 week experiment

Conclusions

- Bacterial lignin-degrading strains isolated from MSW soil, some similar to strains isolated from woodland soil, all in related bacterial classes

- A number of isolates show activity for delignification of lignocellulose as a bacterial treatment, also recombinant enzymes can be used for delignification

- Four isolates show enhancement of gas release on 0.5 litre scale.

Two possible scenarios for treatment:

A) Treat under aerobic conditions, then anaerobic fermentation

B) *In situ* bacterial treatment, facultative anaerobes appear to work better

Publication: "Delignification and enhanced gas release from soil containing lignocellulose by treatment with bacterial lignin degraders" G.M.M. Rashid, M.J. Duran-Pena, R. Rahmanpour, D. Sapsford, and T.D.H. Bugg, *J. Appl. Microbiol.*, **123**, 159-171 (2017)

Agrobacterium genome determined in 2017, contains genes for aerobic AND anaerobic aromatic degradation, currently being studied at Warwick.

Acknowledgements
Goran Rashid, Maria Jesus Duran-Pena (Warwick Univ)
Dr Devin Sapsford (Cardiff Univ.)

NERC SCIENCE OF THE ENVIRONMENT