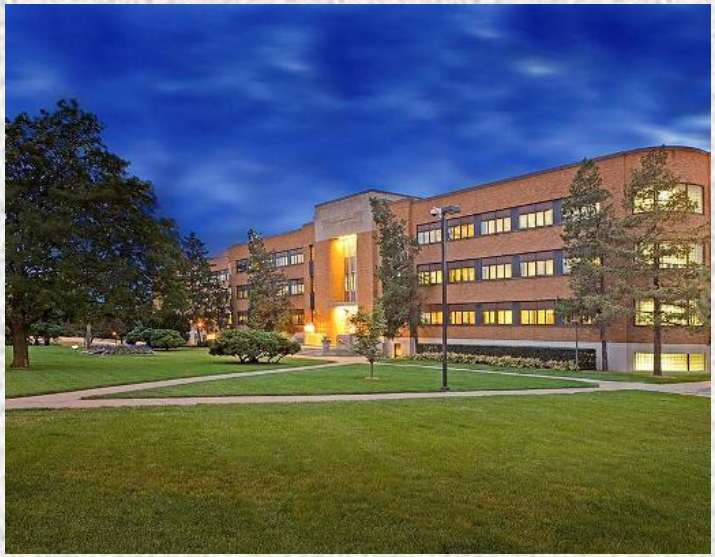
Biological Abatement of Fermentation Inhibitors

Nancy N. Nichols, Badal C. Saha, Bruce S. Dien, Michael A. Cotta

National Center for Agricultural Utilization Research
ARS-USDA, Peoria, IL



Biomass to ethanol

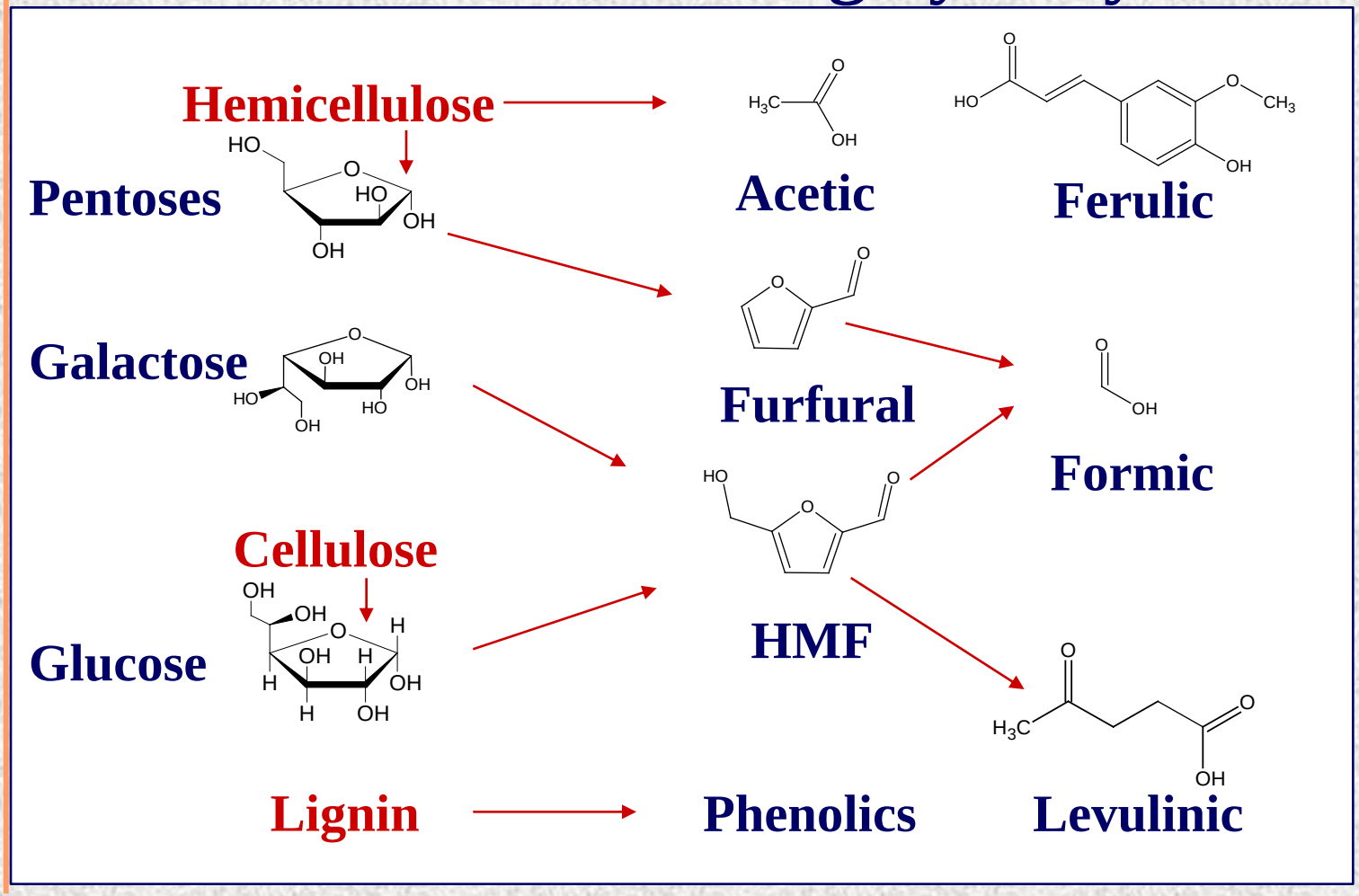
Cellulose
Hemicellulose

→ Enzymes
Acid

Hexoses
Pentoses
Inhibitors

→ **Ethanol**
Recombinant
Yeast or Bacteria

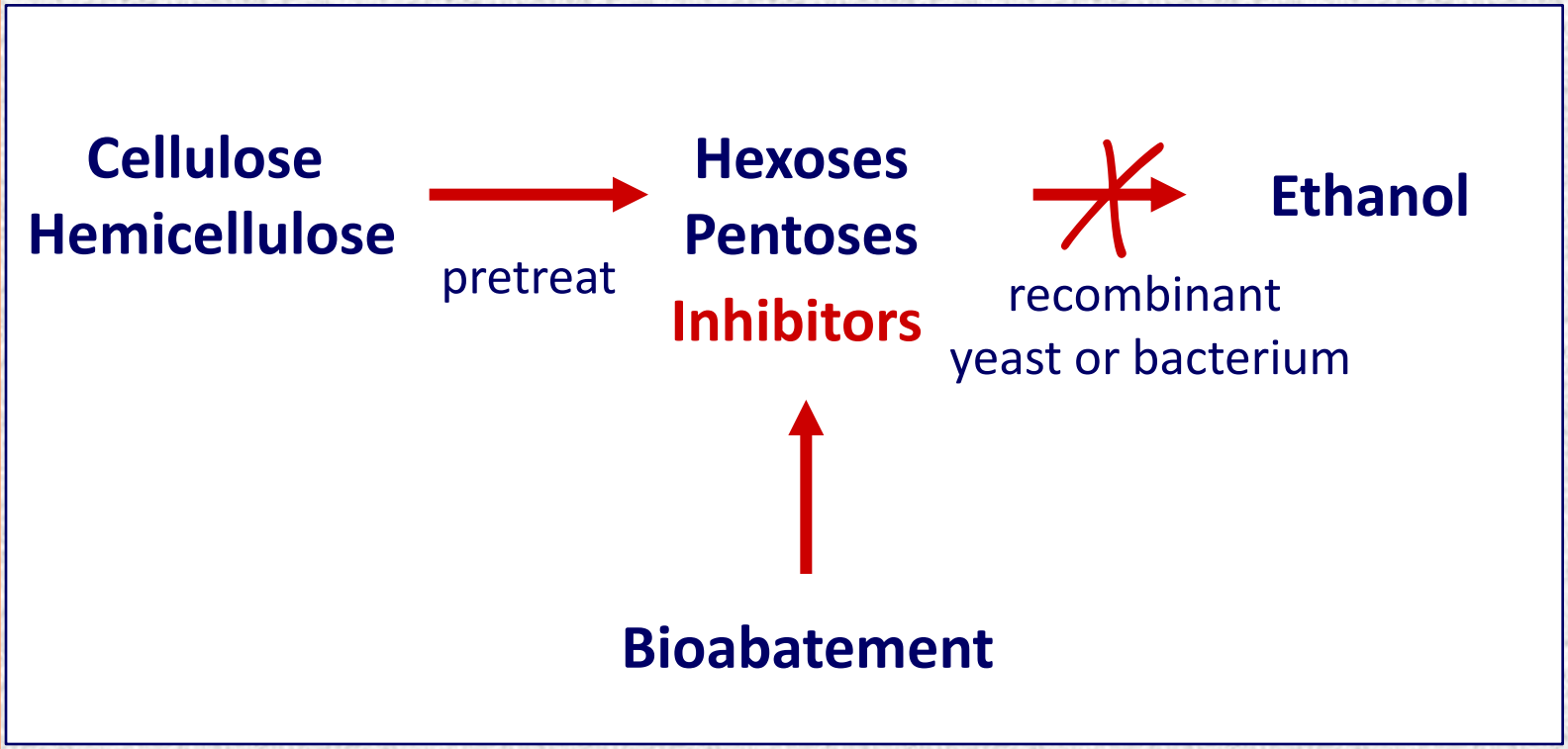
Inhibitors formed during hydrolysis



Inhibitor abatement

- Inexpensive
- Easy to integrate
- Selective removal of inhibitors
- Dilution
  Vacuum evaporation
  pH adjustment
  Polymeric adsorption

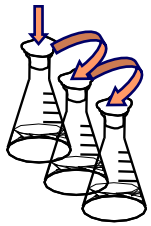
A microbiological approach to inhibitor abatement



Enrichment of detoxifying microbes

Environmental sample: contaminated soil

Enrichment in defined media



- Furfural
- 5-HMF
- Ferulic acid

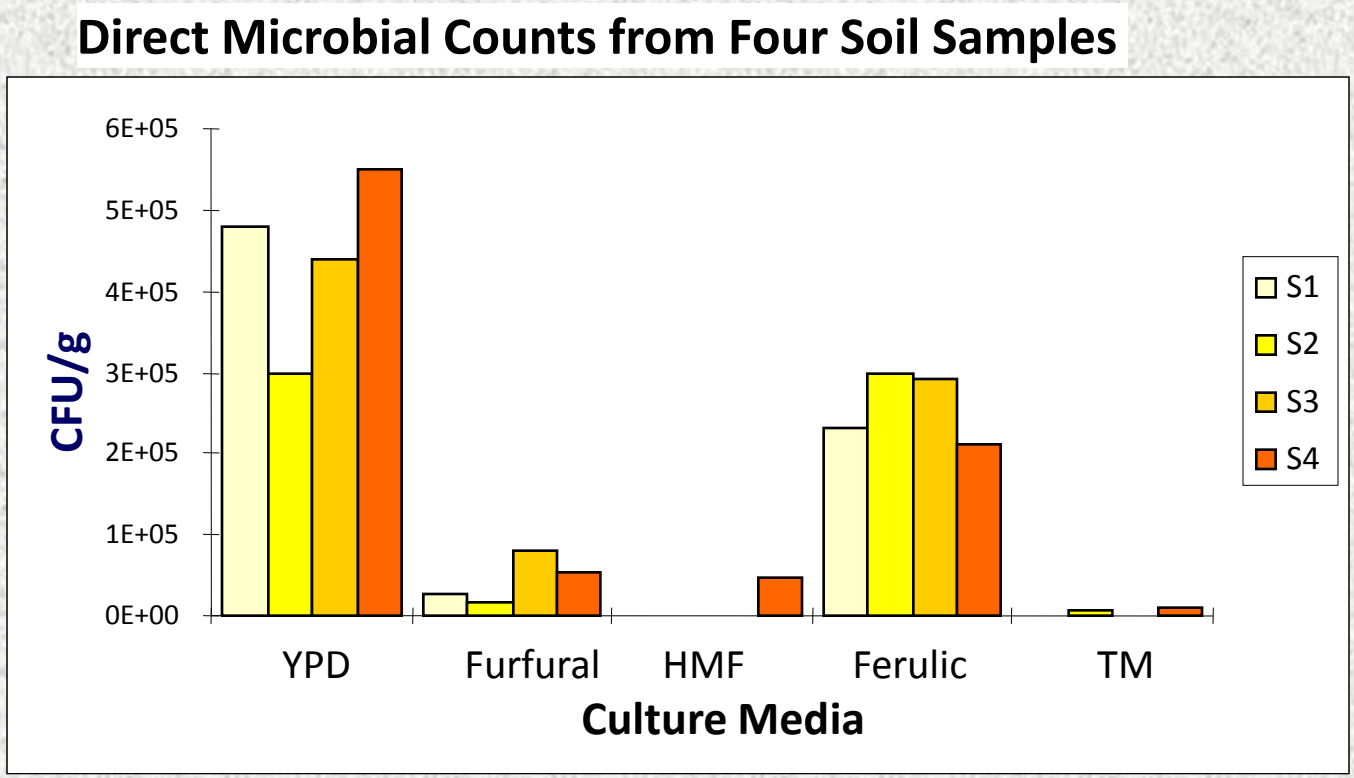
Microorganisms metabolize toxic compounds

Enrichment in Hydrolysate

Microorganisms survive/grow in dilute acid hydrolysates

Isolation and identification

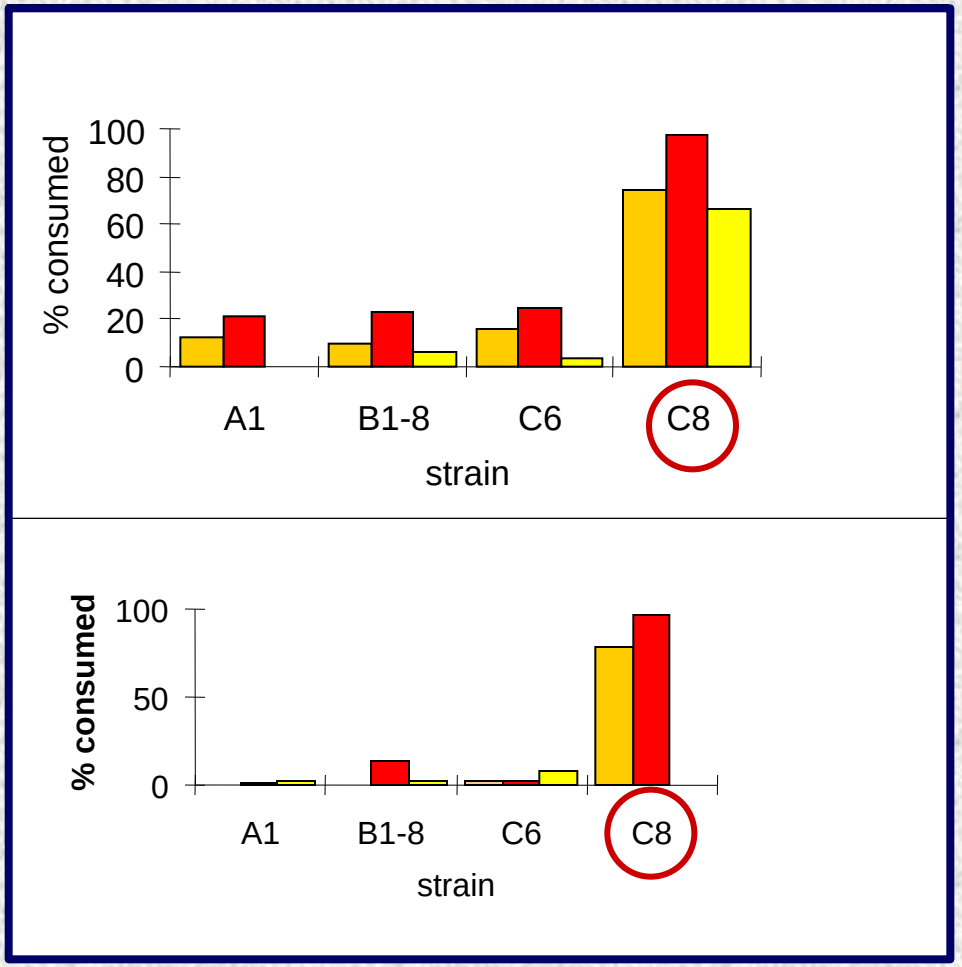
Microbes enriched from contaminated soil



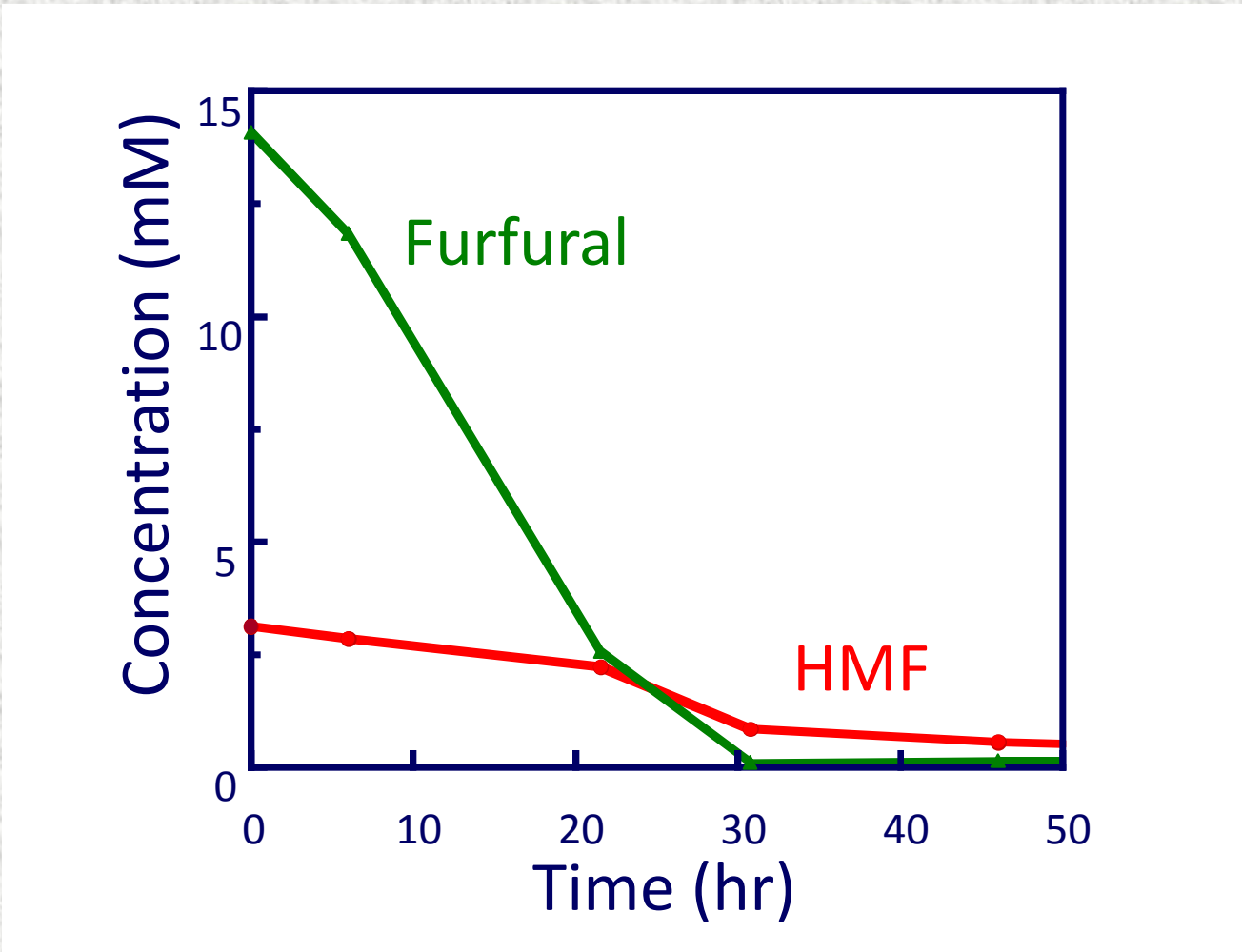
Identification of isolates

- A3, A6, B1-1: *Pseudomonas sp.*
- A1: *Methylobacterium extorquens*
- A5: *Arthrobacter aurescens*
- B1-1: *Acinetobacter sp.*
- C6: *Flavobacterium indologenes*
- C7: *Stenotrophomonas maltophylia*
- C8: Ascomycete fungus

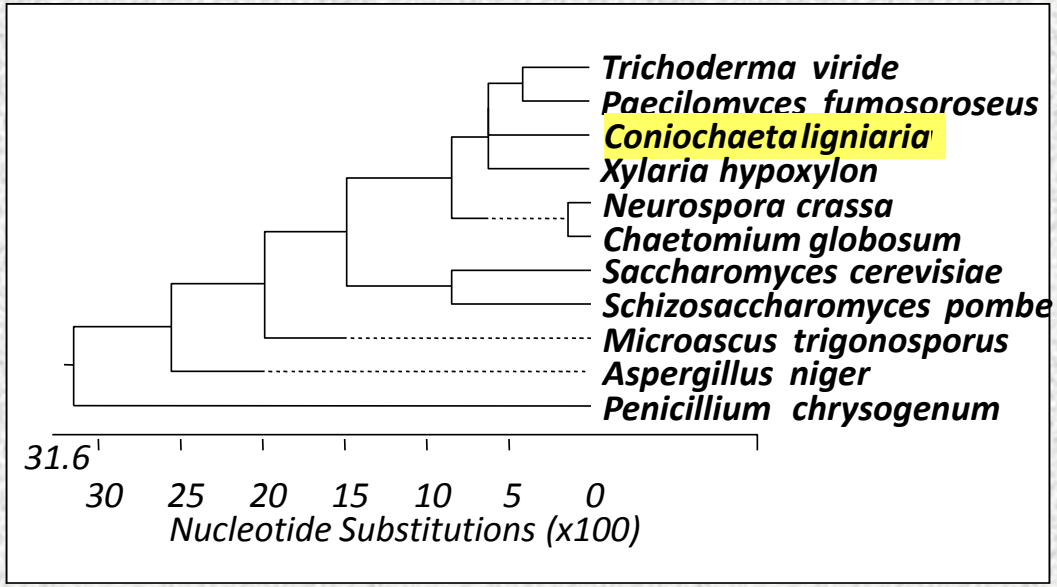
Treatment of hydrolysate



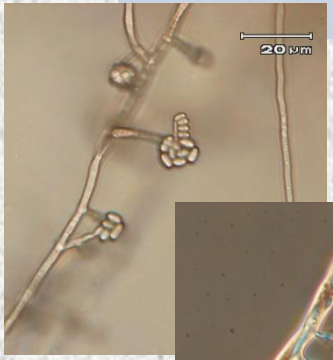
Removal of furans from corn stover hydrolysate

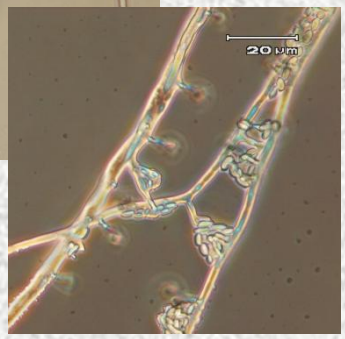


Coniochaeta ligniaria C8 (NRRL30616)



Alignment of the large ribosomal subunit (D1D2 domain) of representativeAscomycetes by the ClustalW method. (Adapted from Weber et al (2002)).

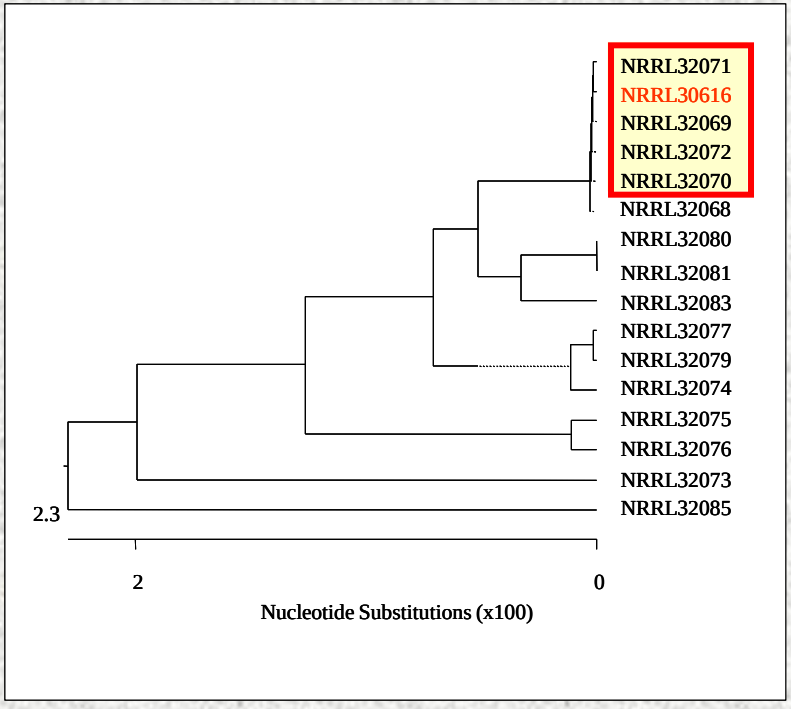




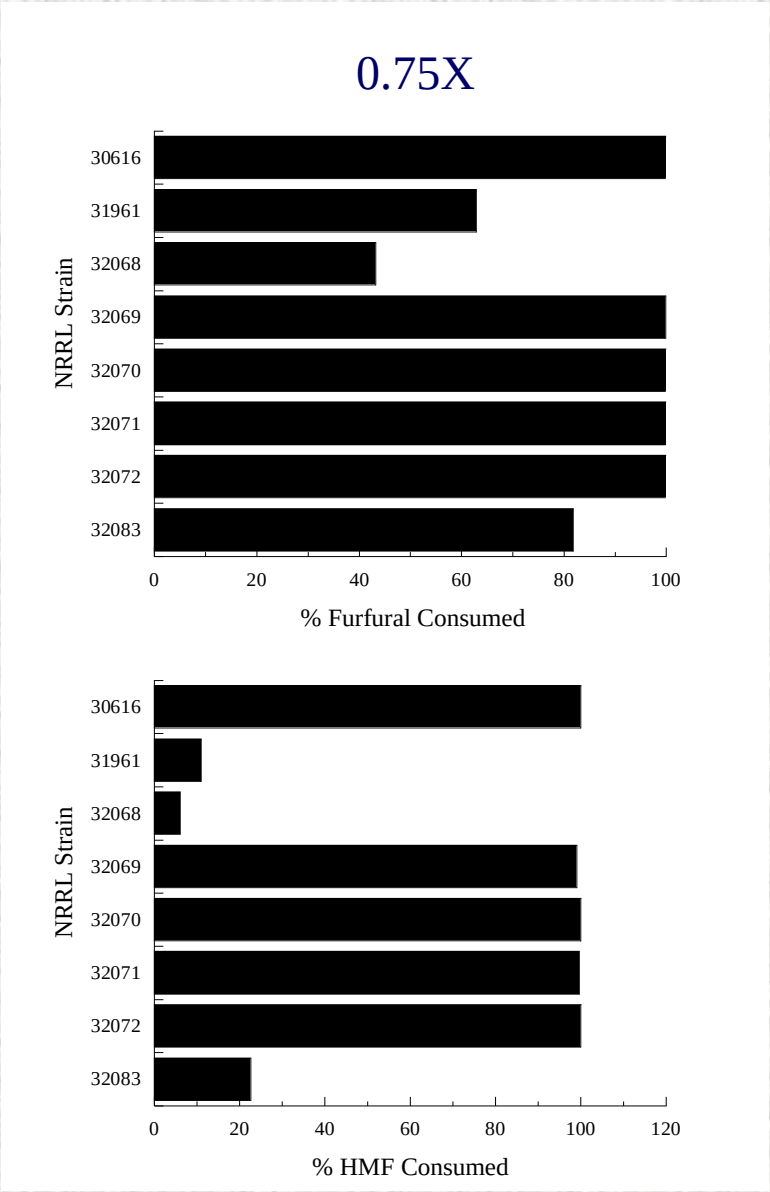
Comparison of *Coniochaeta* strains

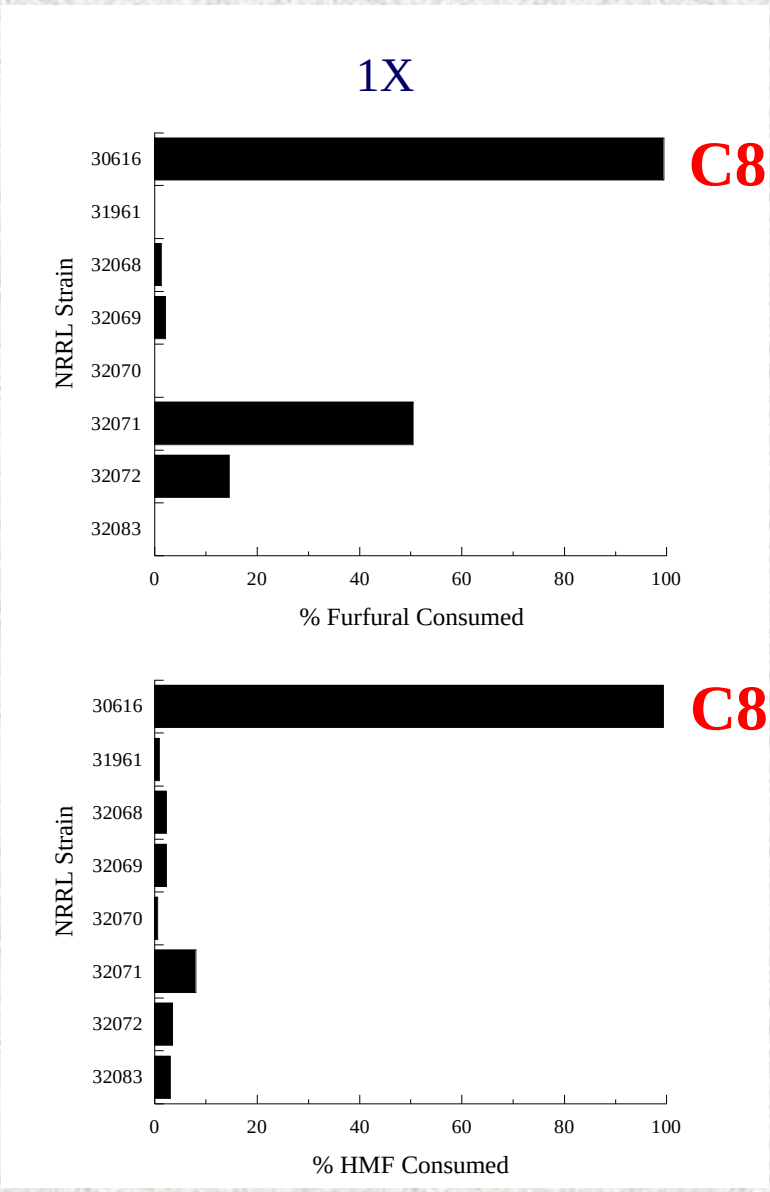
Organism	NRRL	Other ID	Furfural	5-HMF	Levulinic Acid	*p*-Hydroxy-benzaldehyde
Coniochaeta ligniaria	30616	C8	0.605	0.090	0.006	0.081
Lecythophora hoffmannii	31961	DSM2693	0.560	0.148	0	0.086
Lecythophora mutabilis	31962	DSM10716	---	---	n.d.	---
Lecythophora lignicola	31963	DSM63551	---	---	n.d.	---
Coniochaeta ligniaria	32068	95.605	0.278	0.023	0.308	---
Coniochaeta ligniaria	32069	98.1105	0.577	0.166	0.145	0.063
Coniochaeta ligniaria	32070	98.1126	0.640	0.101	0.073	0.196
Coniochaeta ligniaria	32071	F3331	0.005	0.104	n.d.	0.136
Coniochaeta ligniaria	32072	F3343	0.723	0.214	0.148	0.073
Phialophora decumbens	32073	CBS153.42	---	---	n.d.	---
Phialophora fasciculatus	32074	CBS205.38	---	---	n.d.	---
Lecythophora hoffmannii	32075	CBS245.38	---	---	n.d.	---
Lecythophora hoffmannii	32076	CBS140.41	---	---	n.d.	0.053
Lecythophora lignicola	32077	CBS267.33	---	---	n.d.	---
Phialophora luteoviridis	32079	CBS206.38	---	---	n.d.	---
Lecythophora mutabilis	32080	CBS157.44	---	---	n.d.	---
Lecythophora mutabilis	32081	CBS303.62	---	---	n.d.	0.09
Coniochaeta ligniaria	32082	CBS620.69	---	---	n.d.	---
Coniochaeta ligniaria	32083	CBS178.75	0.186	0.071	0.088	---
Coniochaeta malacotricha	32084	CBS323.72	---	---	n.d.	---

Comparison of *Coniochaeta* strains



Alignment of the large ribosomal subunit (D1D2 domain) of the strains in Table 1 by the ClustalW method (Adapted from Weber et al (2002)). Strains that were the best at removing furfural from corn stover hydrolysate are highlighted.


0.75X
NRRL Strain
30616
31961
32068
32069
32070
32071
32072
32083
0
20
40
60
80
100
% Furfural Consumed
0
20
40
60
80
100
120
% HMF Consumed


1X
C8
NRRL Strain
30616
31961
32068
32069
32070
32071
32072
32083
0
20
40
60
80
100
% Furfural Consumed
C8
% HMF Consumed

General bioabatement scheme

10 % corn stover and 0.66% H_2S0_4

Steel pipe reactors – fluidized heating bath –180°C, 10 min.

↓

Adjust pH with $Ca(OH)_2$

↙

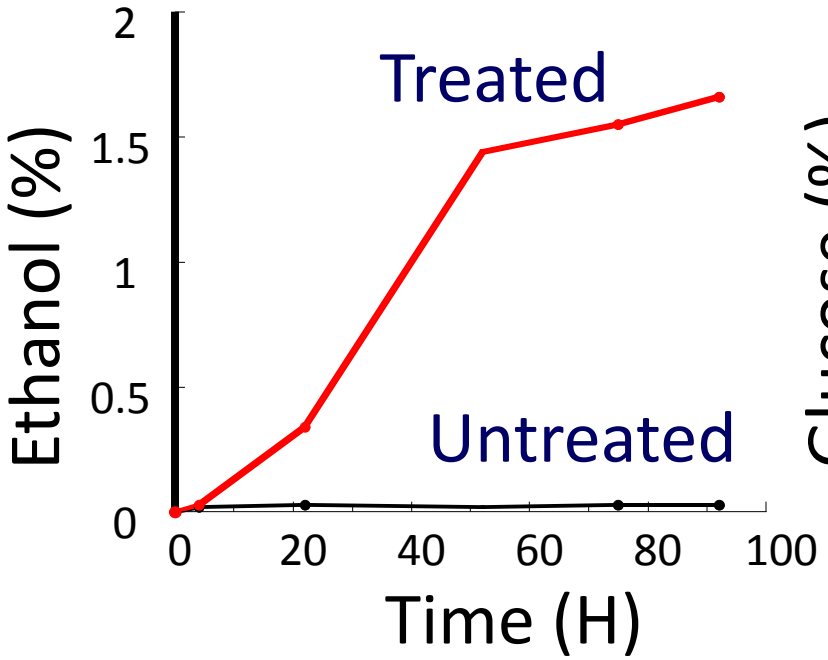
Wash solids → liquid → ↓

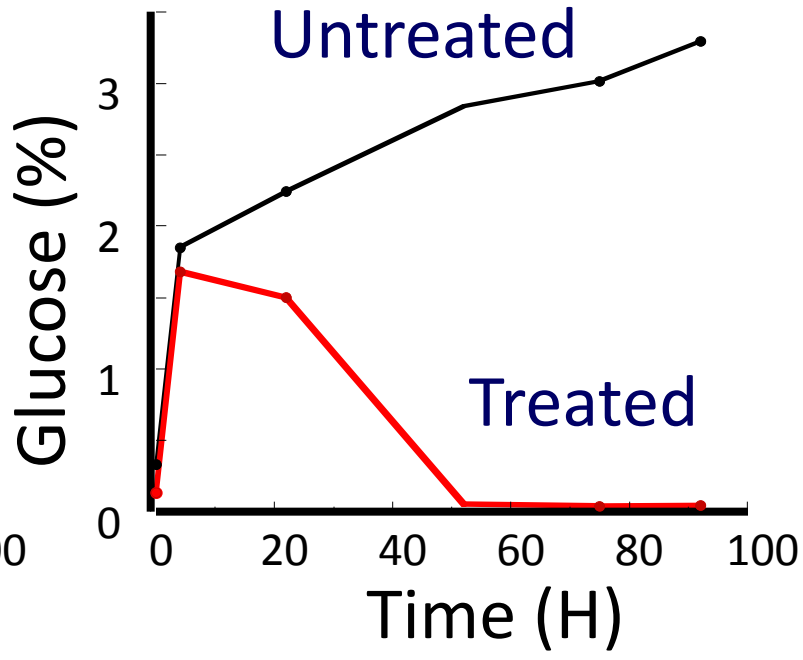
Bioabatement: Inoculate with *C. ligniaria* cell pellet, incubate 30°C, shaking, 18-24 h.

solids ↘ ↓

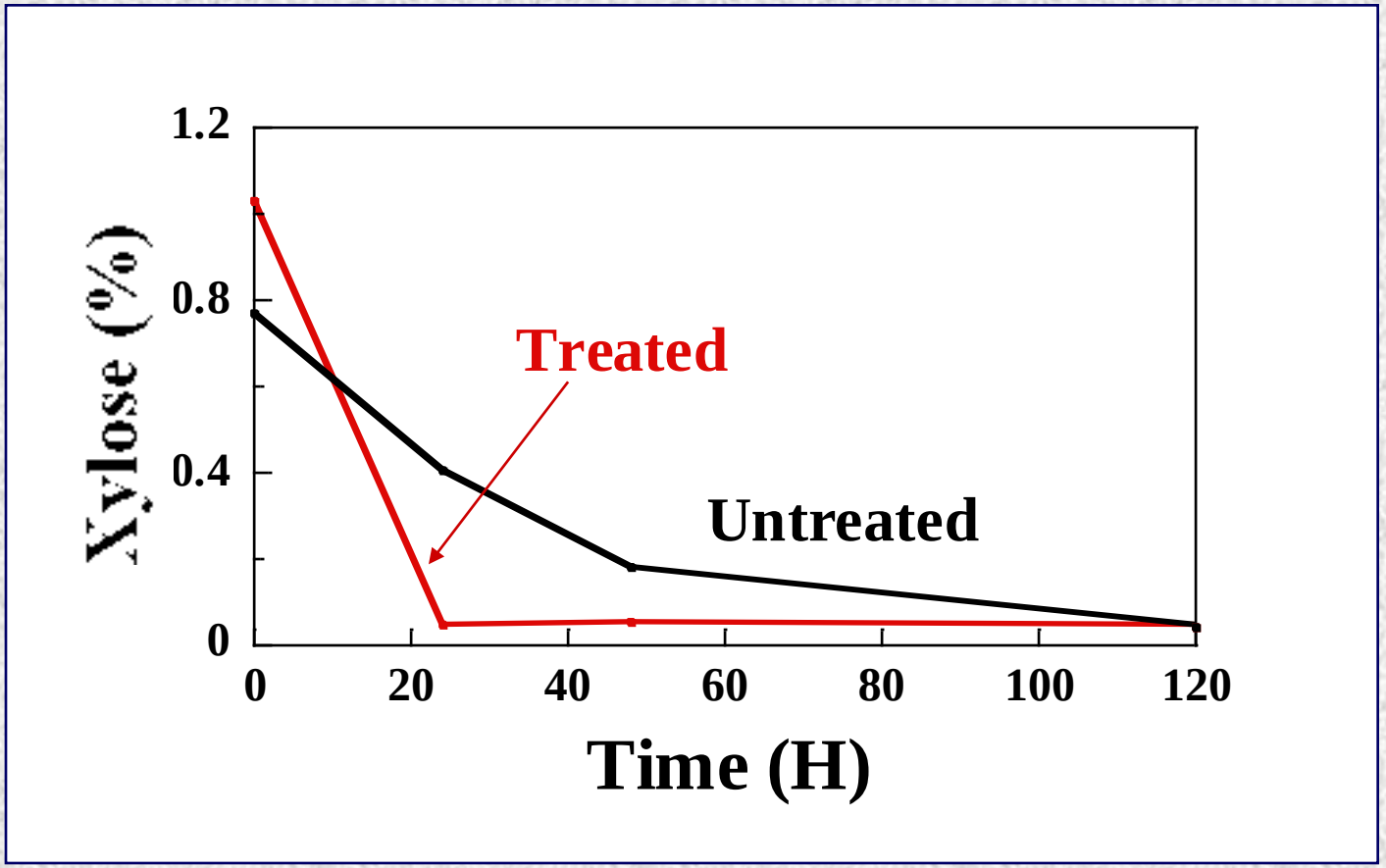
Saccharification & Fermentation

Fermentation of glucose in corn stover



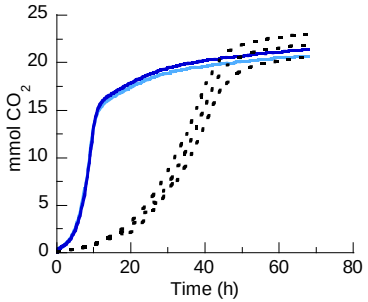


Fermentation of xylose in corn stover

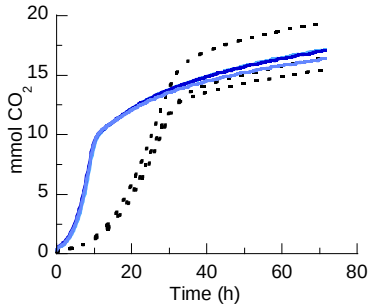


Reed Canarygrass

Vegetative

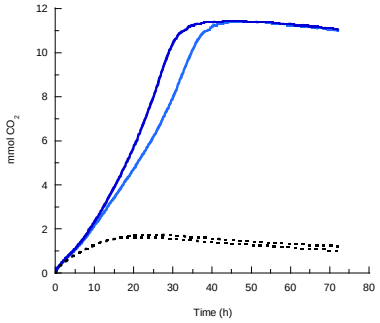


Ripe Seed

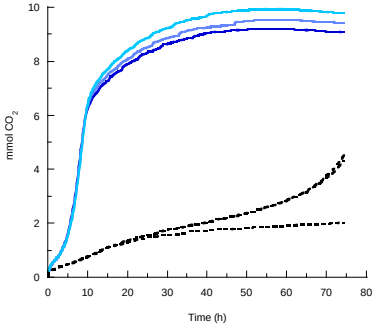


Alfalfa Stems

Bud

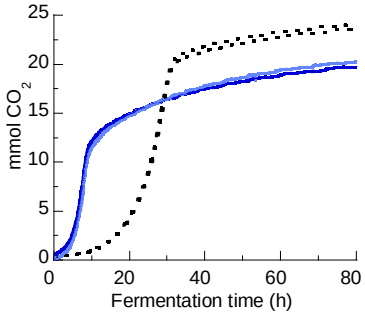


Flower

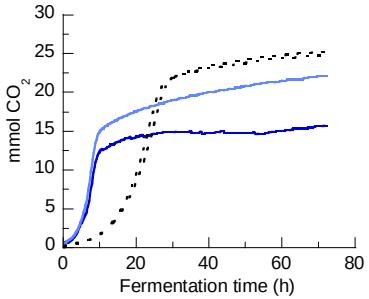


Switchgrass

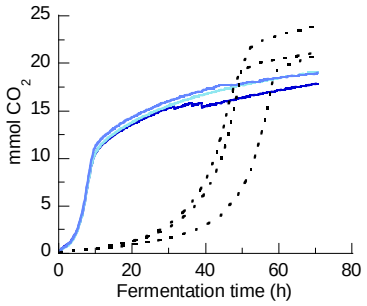
Preboot



Anthesis

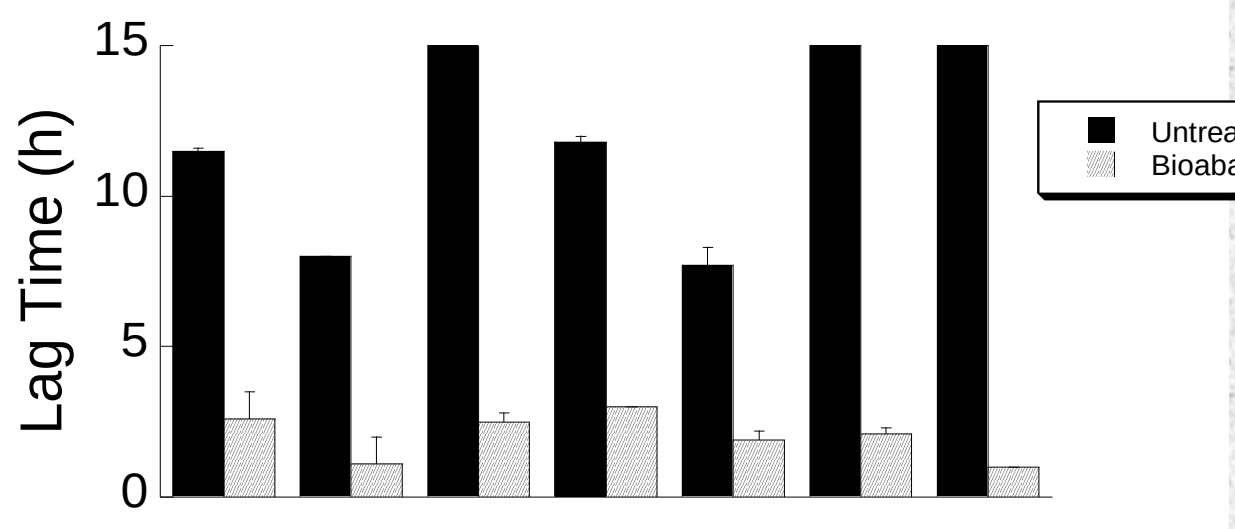


Post frost

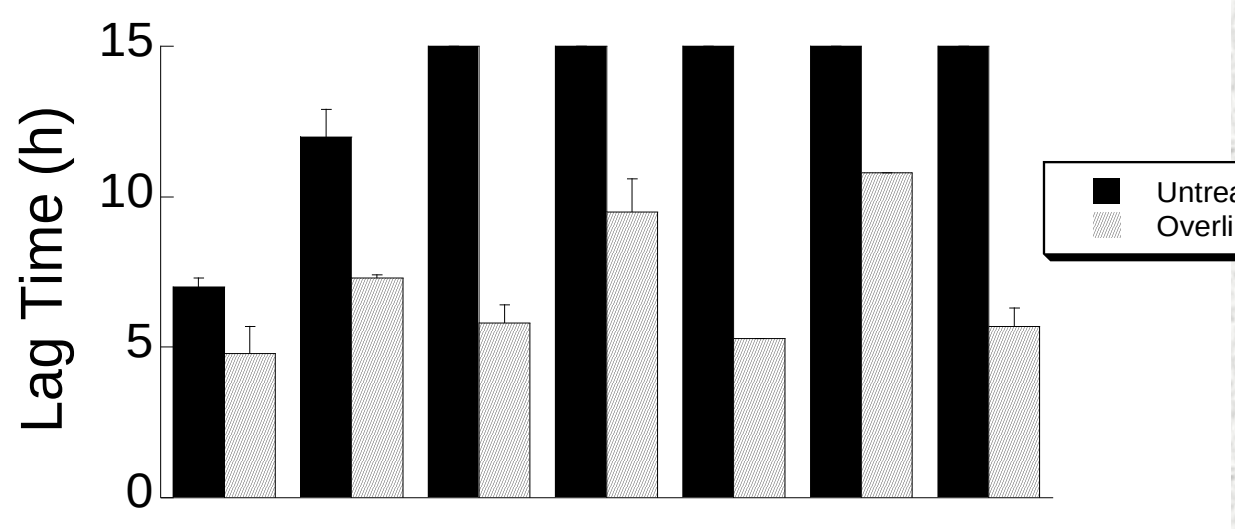


Fermentation lag time

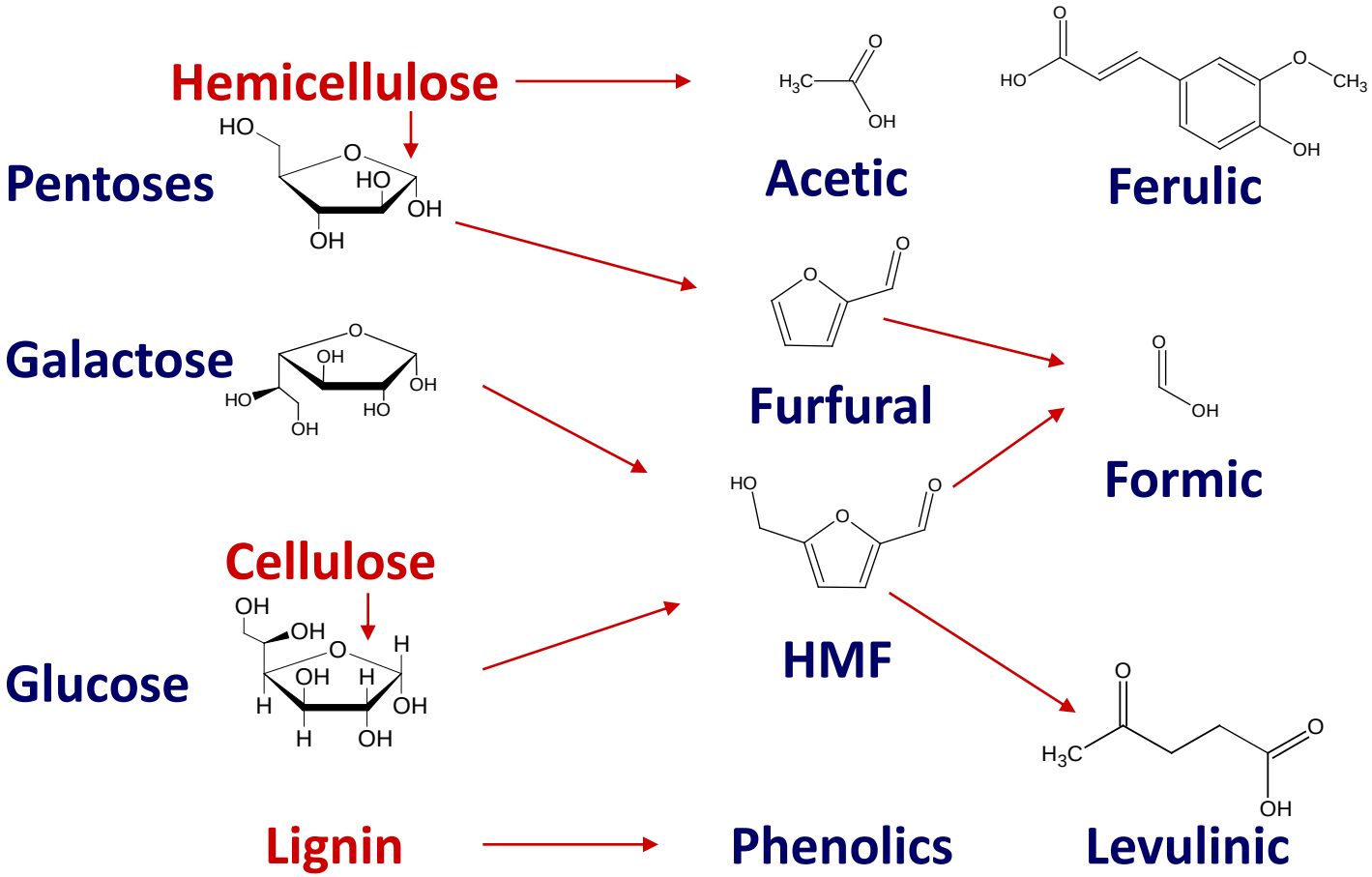
Bioabatement



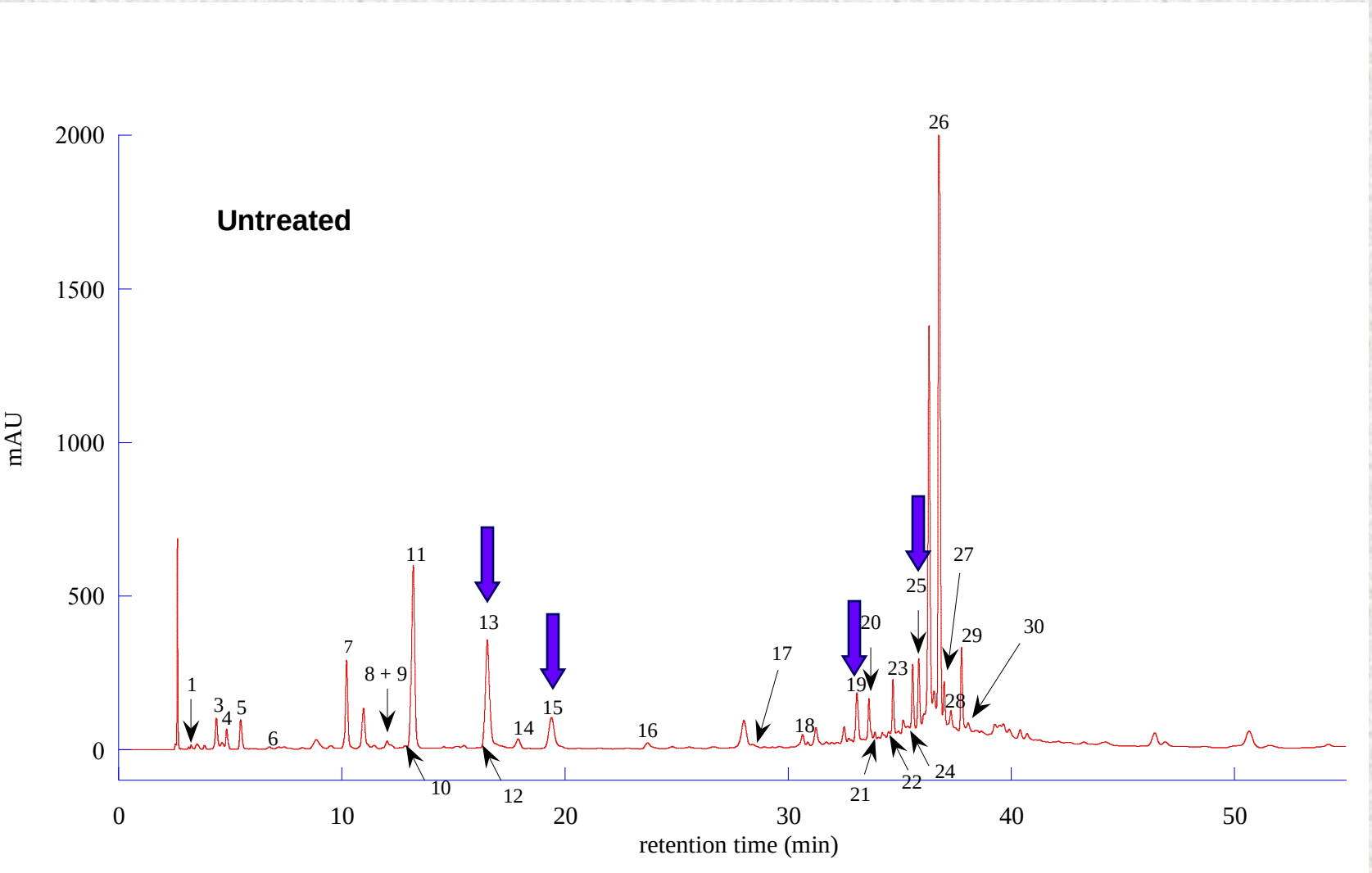
Overliming



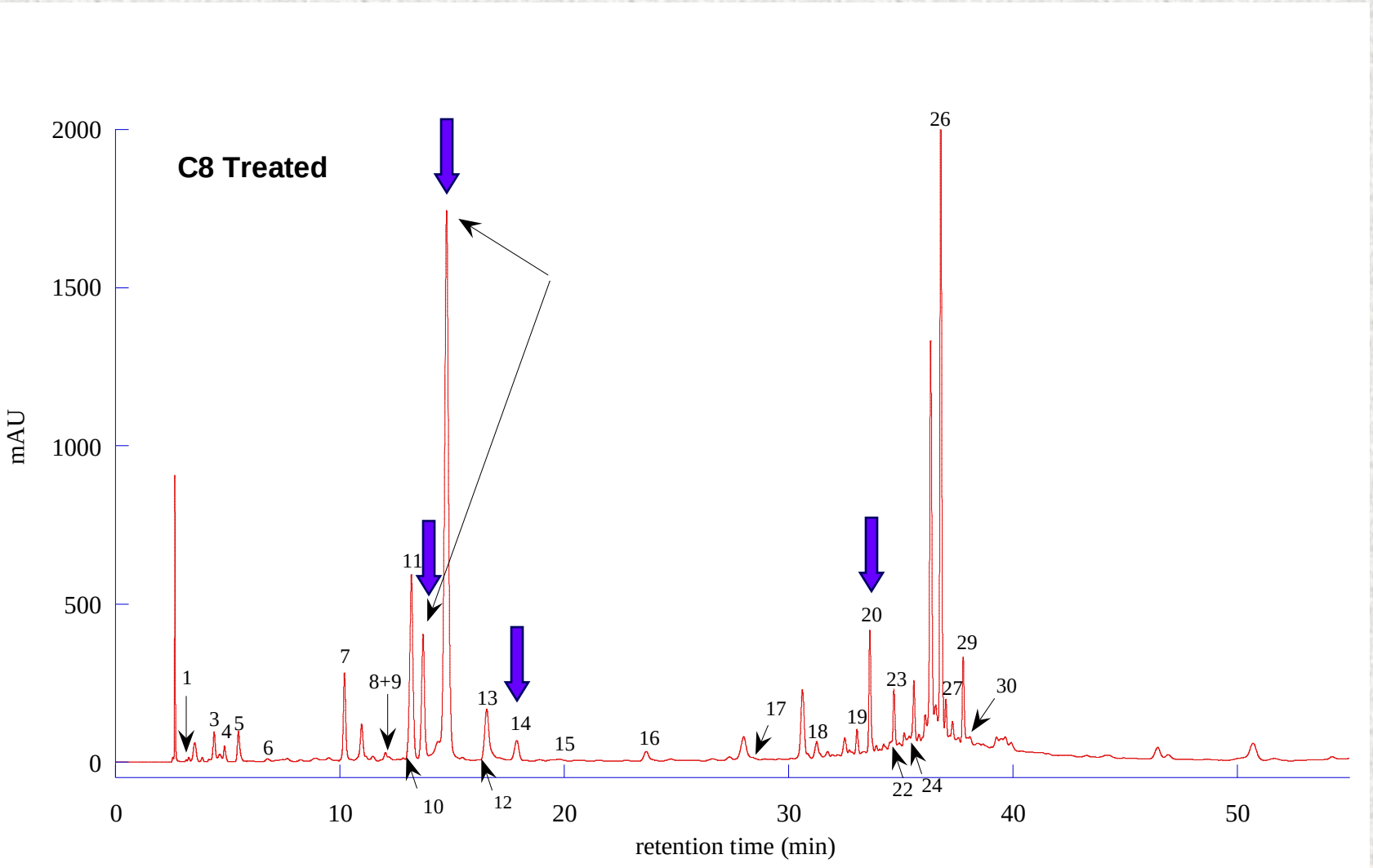
Inhibitors formed during hydrolysis



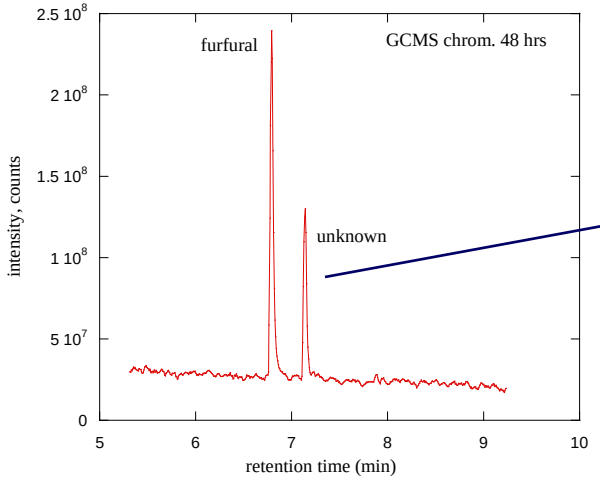
Untreated corn stover hydrolysate

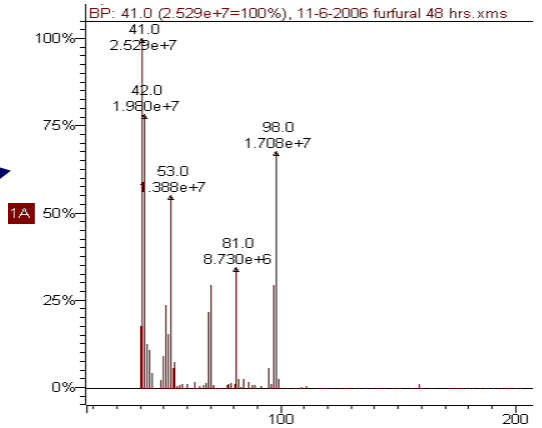


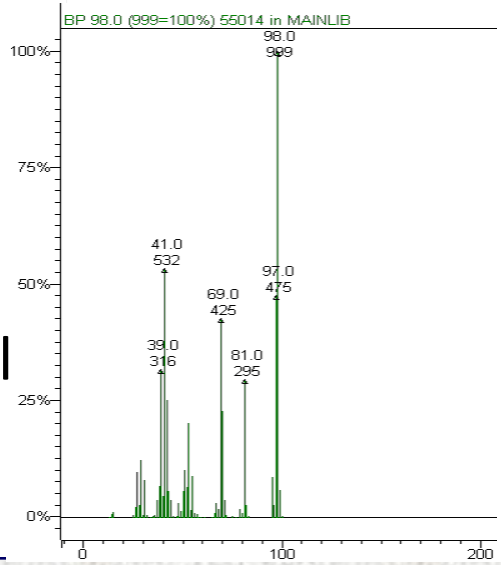
C8-treated corn stover hydrolysate



New peak is furfuryl alcohol





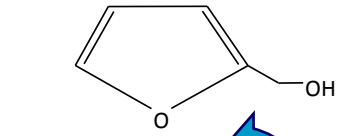


NIST
2-furanmethanol

Furan metabolic pathway

Furfuryl Alcohol

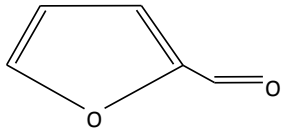
Furfuryl Alcohol Dehydrogenase



NAD

$NADH_2$

Furfural

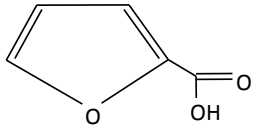


Furfural Dehydrogenase

ACC_{ox}

ACC_{red}

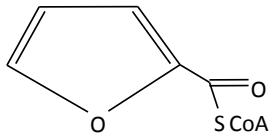
2-Furoic Acid



2-Furoate-CoA Synthetase

ATP, CoASH

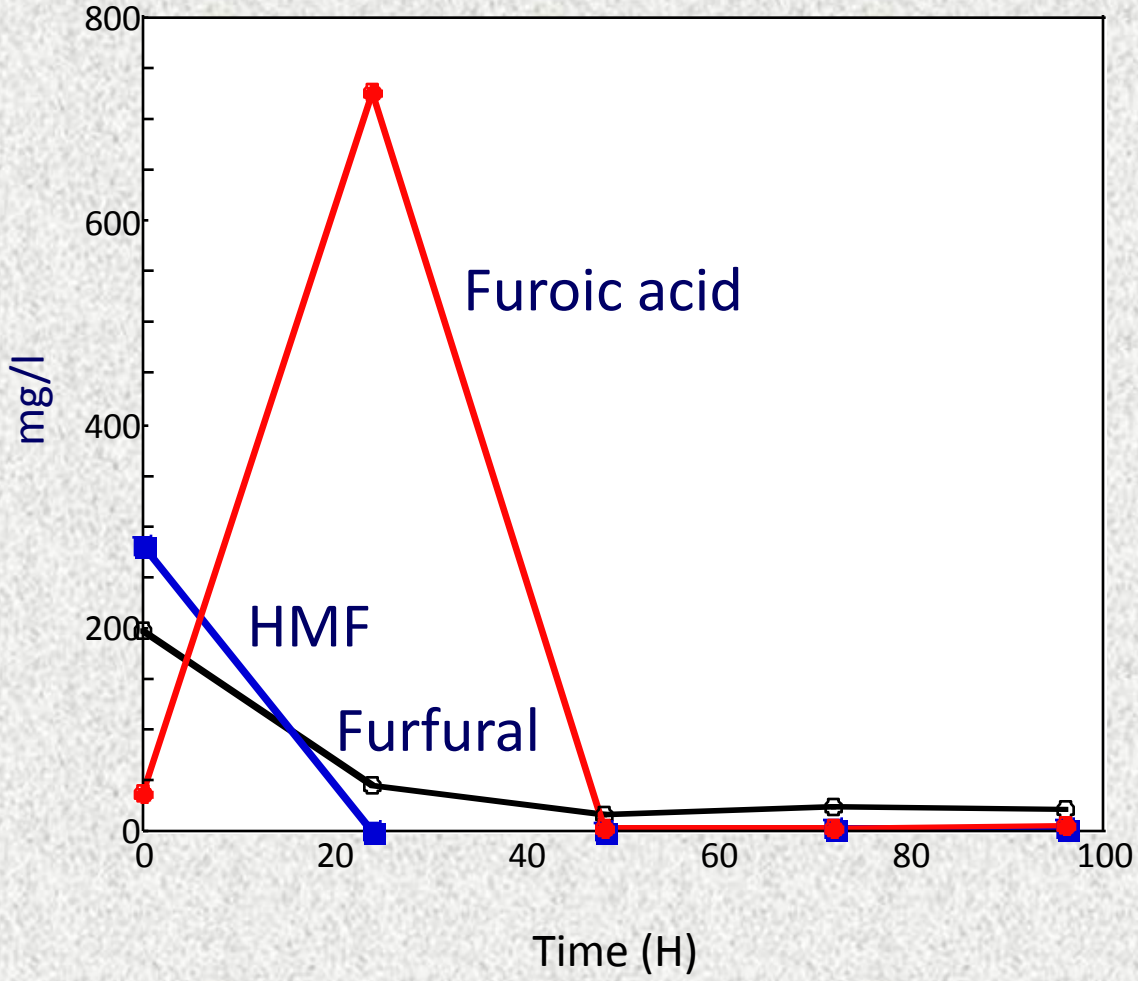
2-Furoyl-CoA



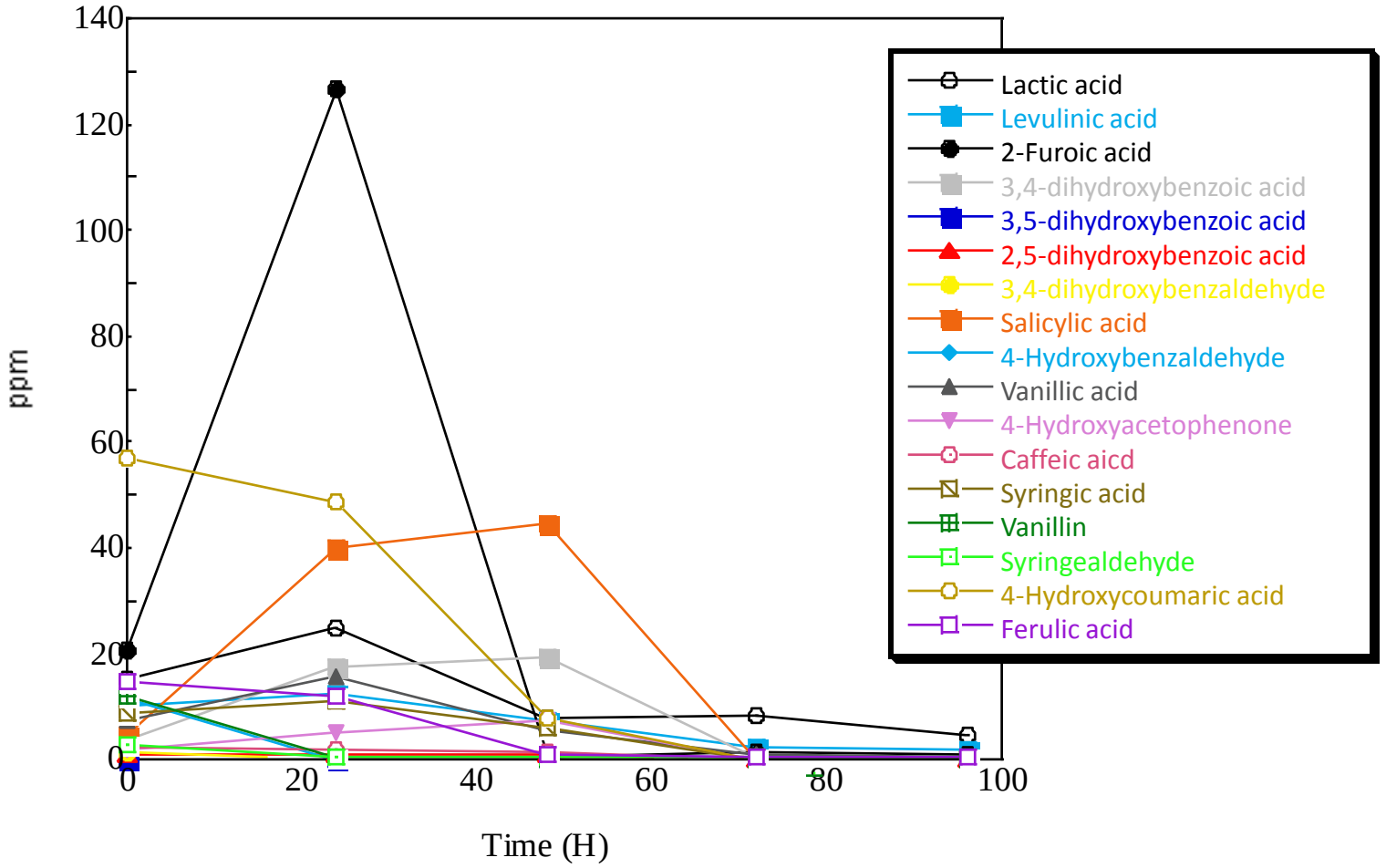
→ → →

Citric Acid Cycle

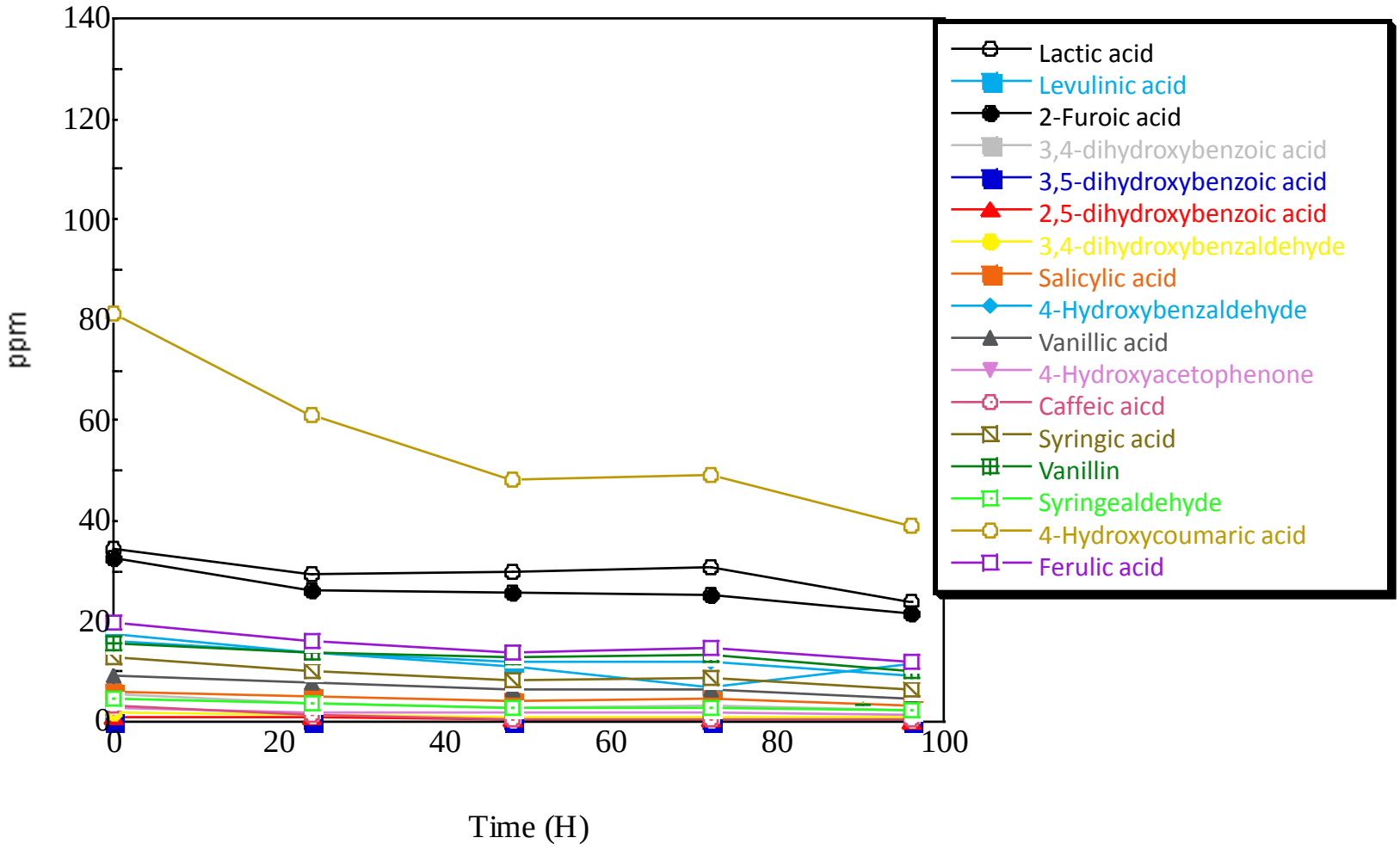
Time course of furfural & HMF metabolism



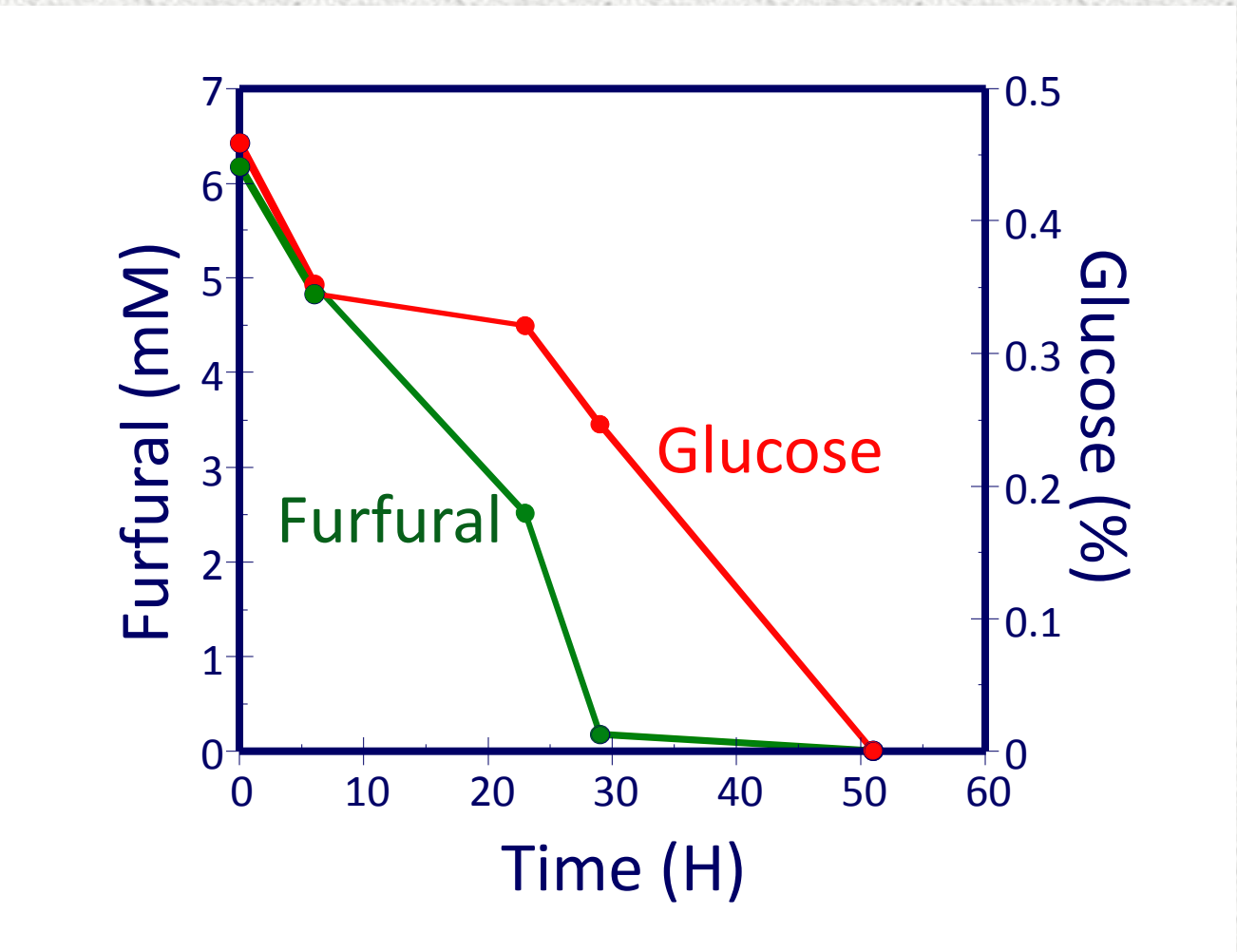
C8-treated hydrolysate



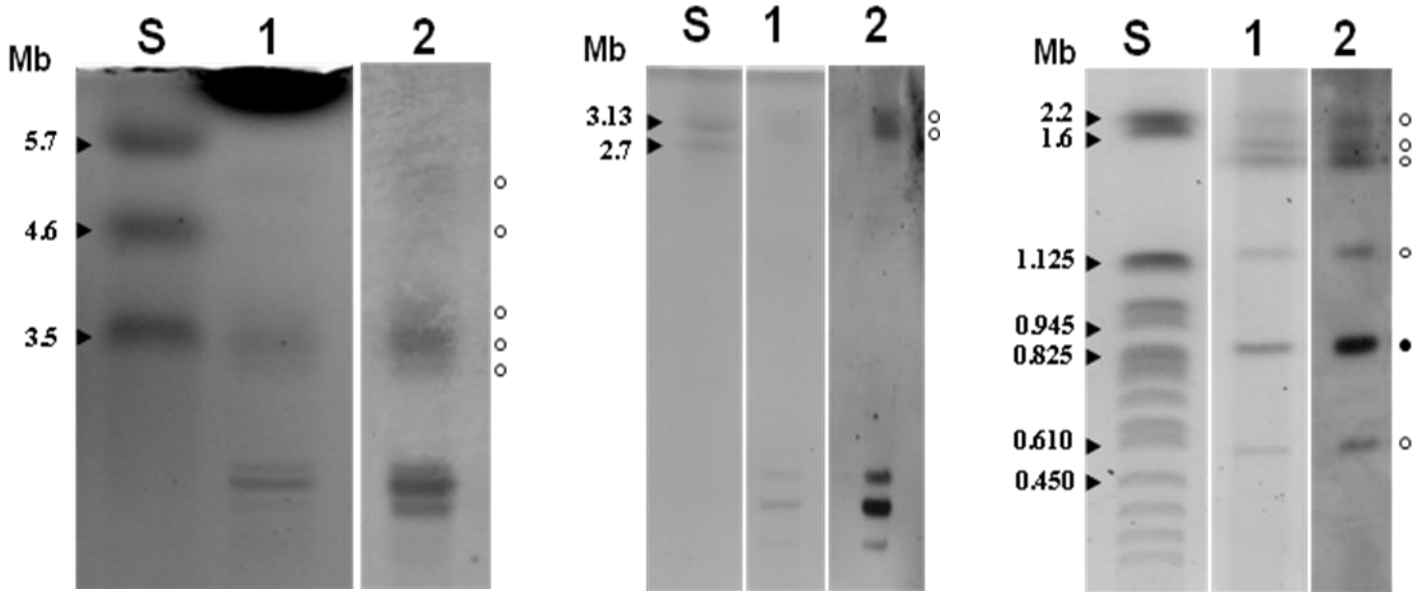
Untreated corn stover hydrolysate



Metabolism of furfural before glucose



Coniochaeta ligniaria genome



13chromosomes (0.5-5.0 Mb)

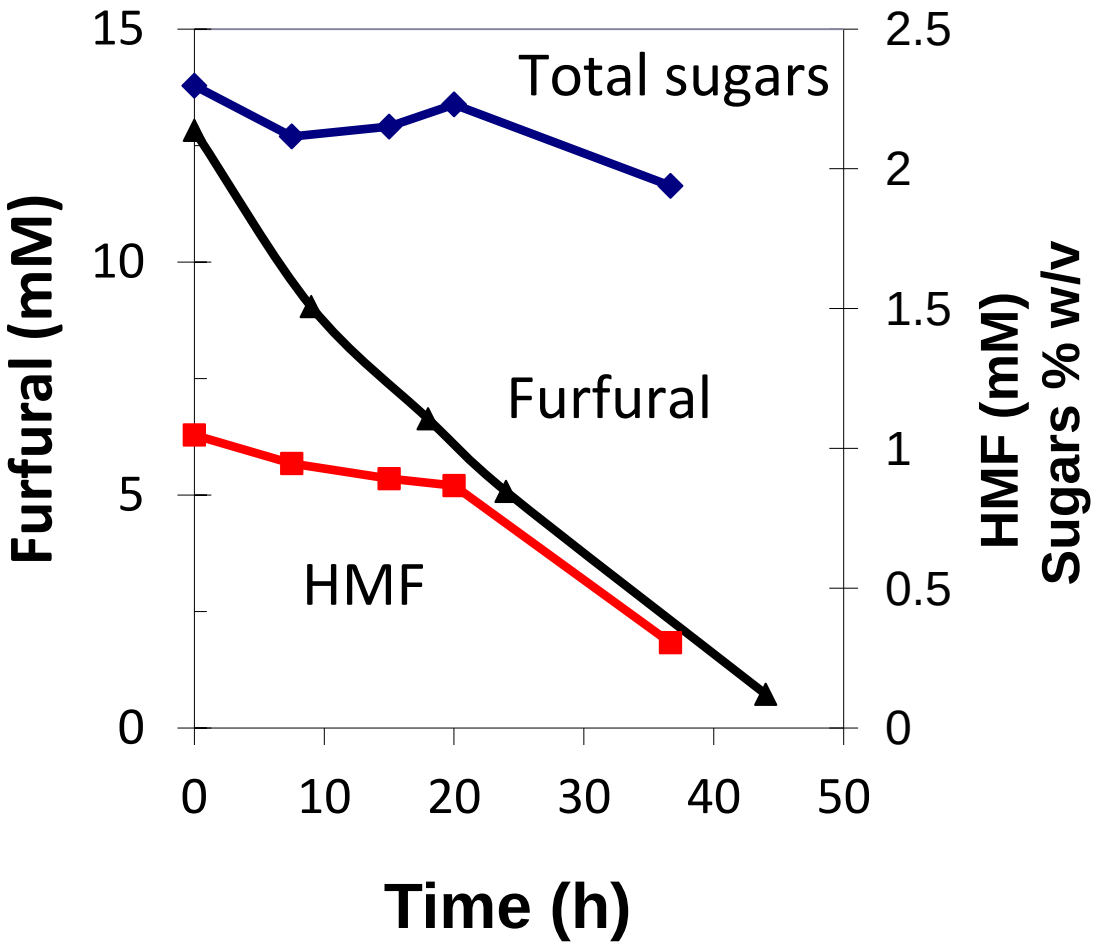
Estimated total size 30.1 Mb

2: $(TTAGGG)_6$

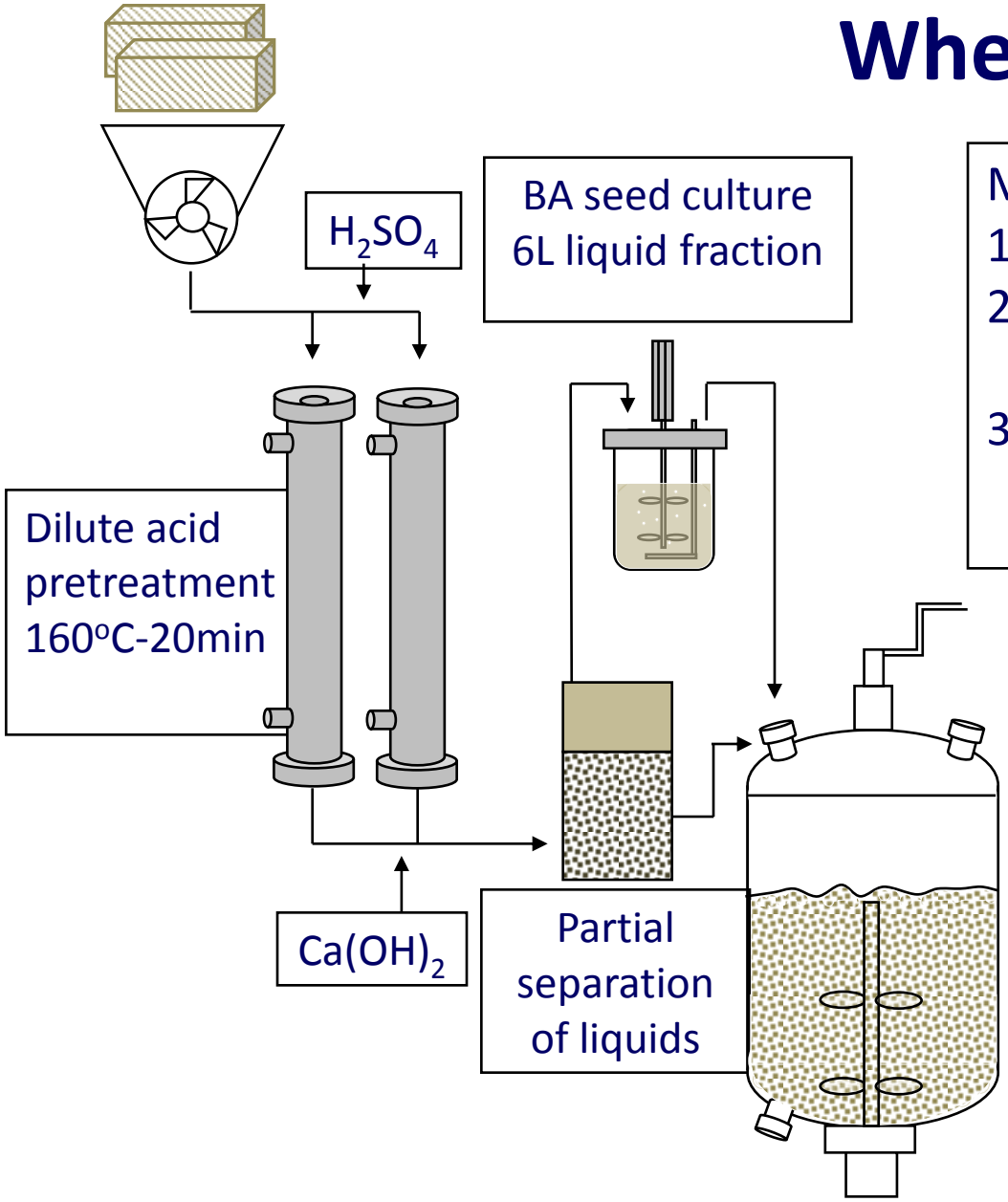
Characterizing *C. ligniaria*

- G + C content 49.2% (coding regions 51.9%)
- No unusual codons
- Haploid
- UV mutagenesis
- Transformation by protoplasting
- Antibiotic resistance: hygromycin, G418, benomyl

Wheat straw--bioabatement



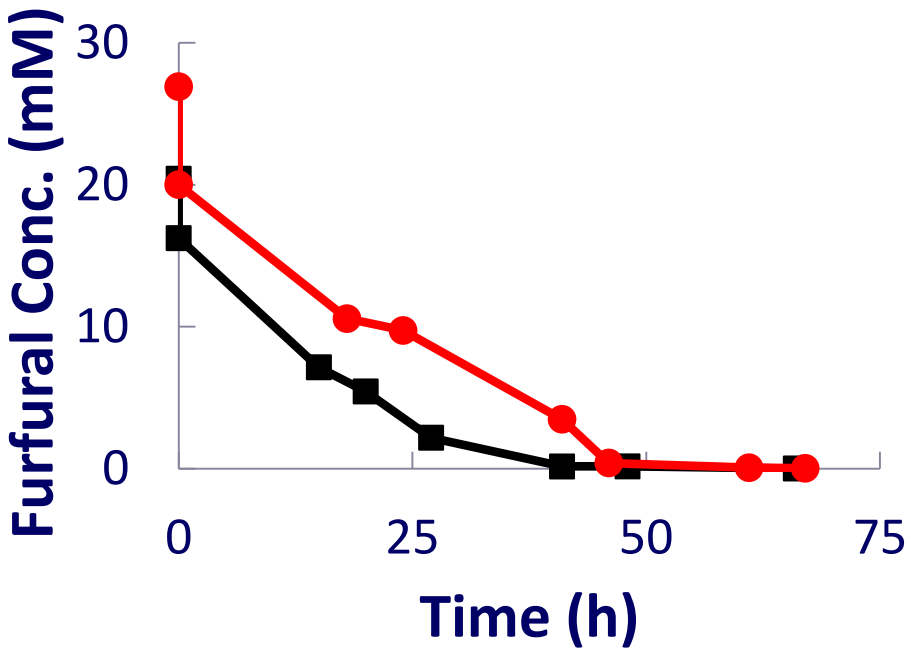
Wheat Straw SSF



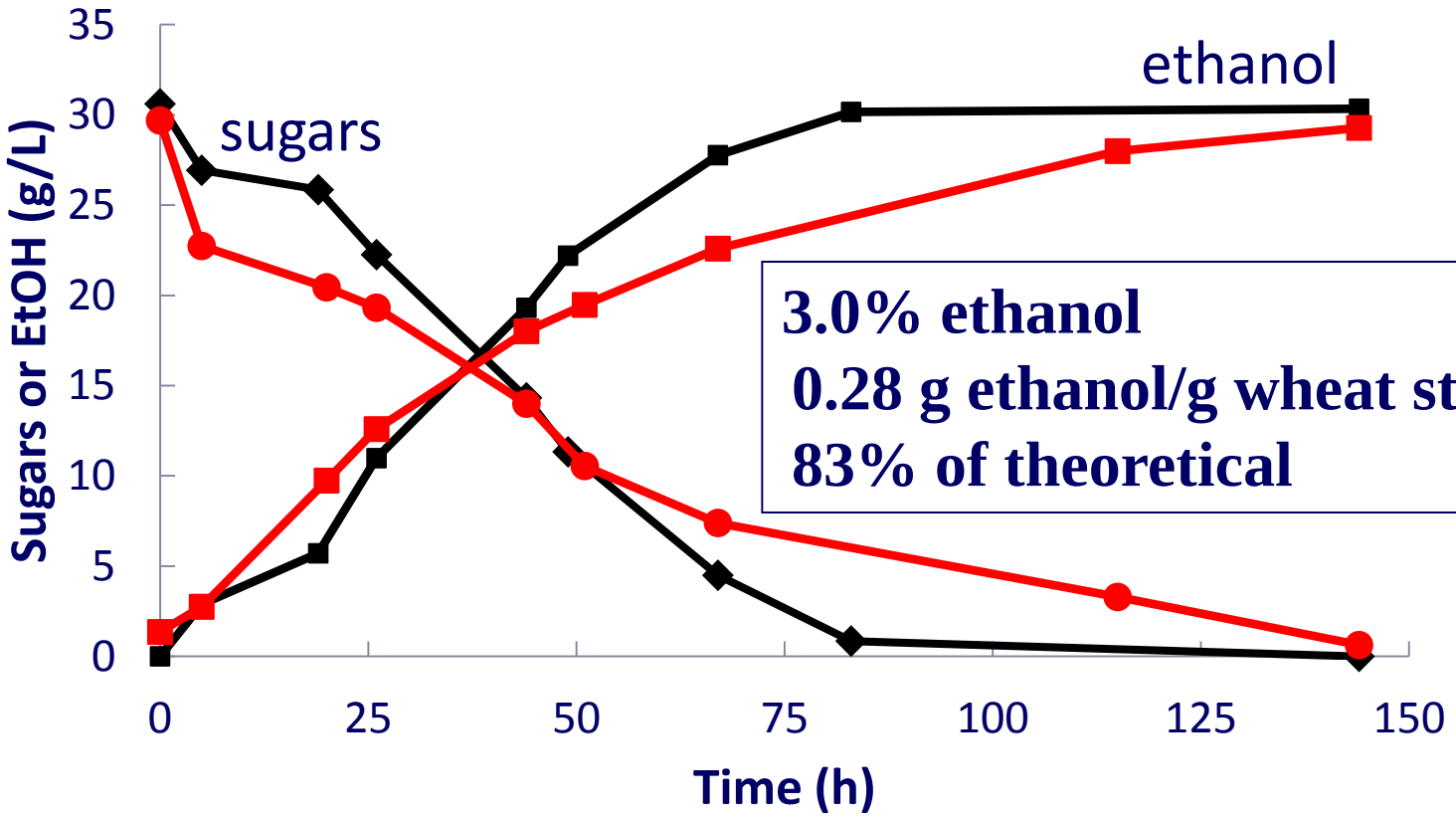
Multistep 60L SSF

1. NRRL30616 mitigation
2. *E. coli* pentose fermentation
3. Enzyme hydrolysis/ hexose fermentation

Wheat straw bioabatement: furfural degradation



Wheat straw bioabatement: SSF



3.0% ethanol
0.28 g ethanol/g wheat straw
83% of theoretical

Summary

- A biological approach to inhibitor abatement
- Best isolate: Ascomycete *Coniochaeta ligniaria* C8
- Consumes furfural and HMF from hydrolyzed corn stover; also phenolics and organic acids
- Enhanced fermentation of biomass feedstocks: crop residues & energy crops

Acknowledgements

USDA/ARS/NCAUR

- Sarah Frazer, Greg Kennedy, Loren Iten

Baylor University

- Kevin Chambliss, Lekh Nath Sharma

University of Almeria (Spain)

- Maria Lopez

Illinois State University

- Matt Szynkarek, Wade Nichols

Purdue University

- Nancy Ho