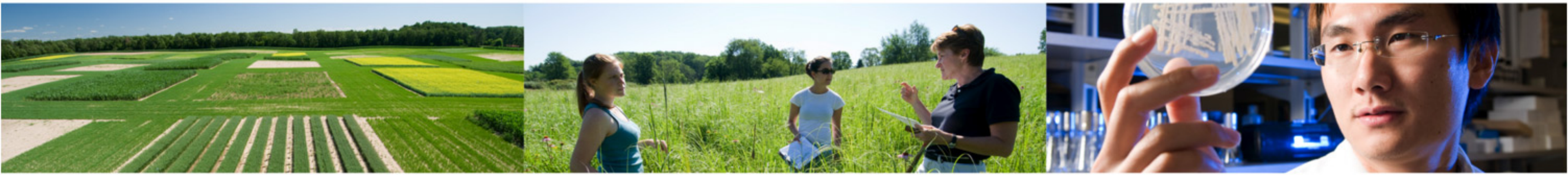
GREAT LAKES BIOENERGY RESEARCH CENTER



BERAC Update

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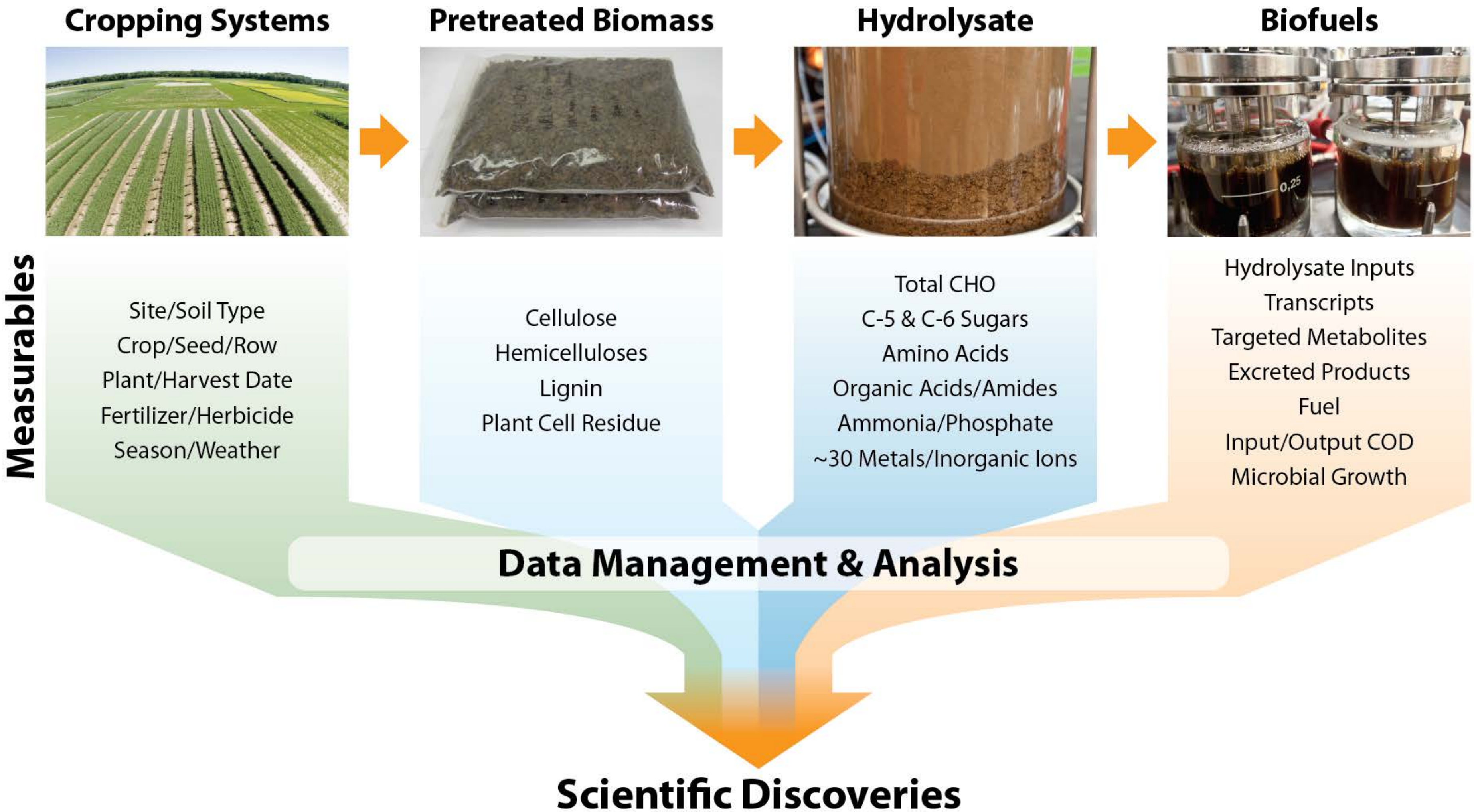
February 27, 2015

Systems Analysis of Biomass & Biofuel Production



Collaboration Enables Discoveries

GLBRC

Sustainability | Plants | Deconstruction | Conversion | Enabling Technologies

DOE BER

BESC BioEnergy Science Center

jbei Joint BioEnergy Institute

KBASE predictive biology DOE Systems Biology Knowledgebase

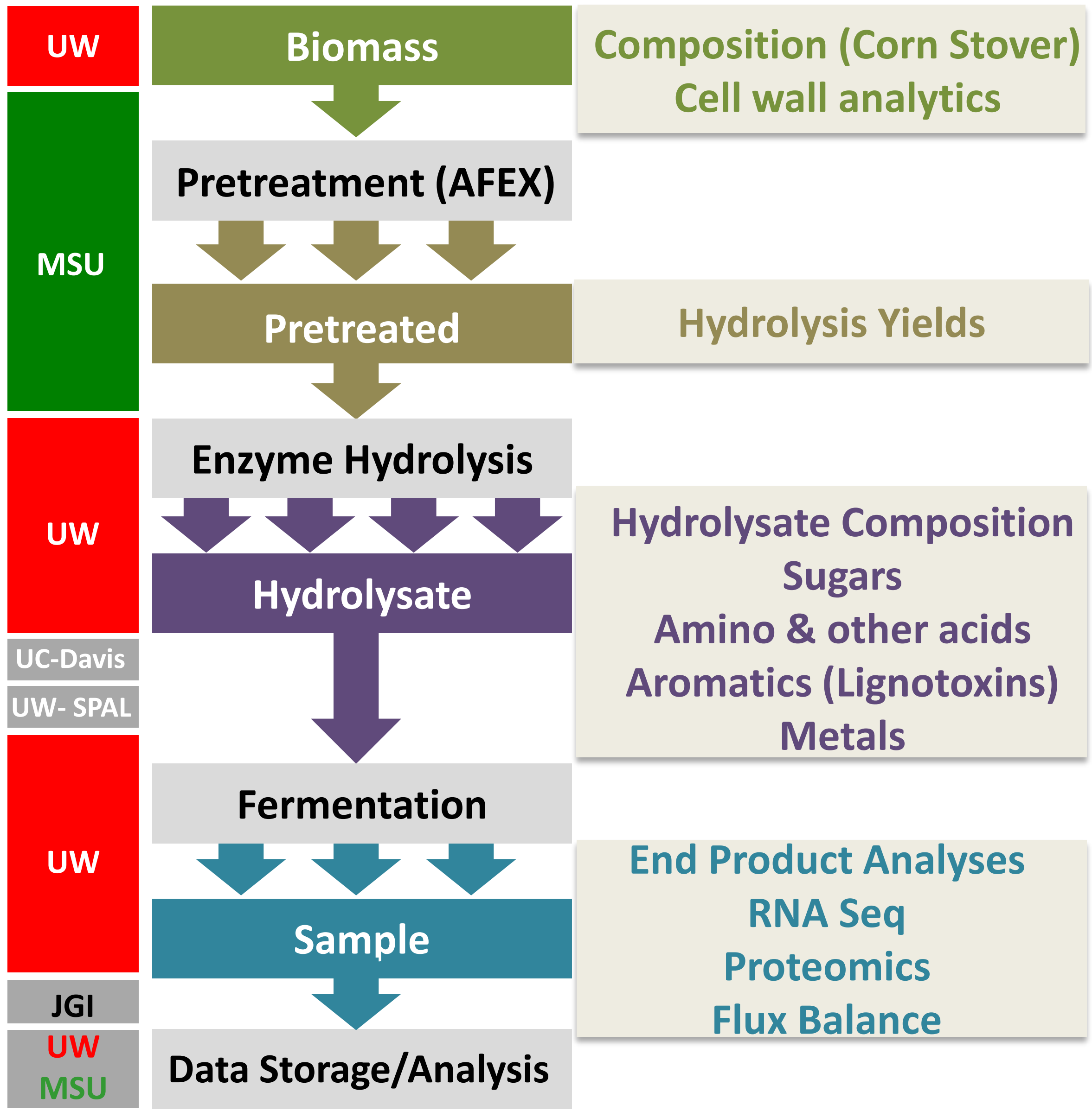
JGI DOE JOINT GENOME INSTITUTE US DEPARTMENT OF ENERGY OFFICE OF SCIENCE

"External"

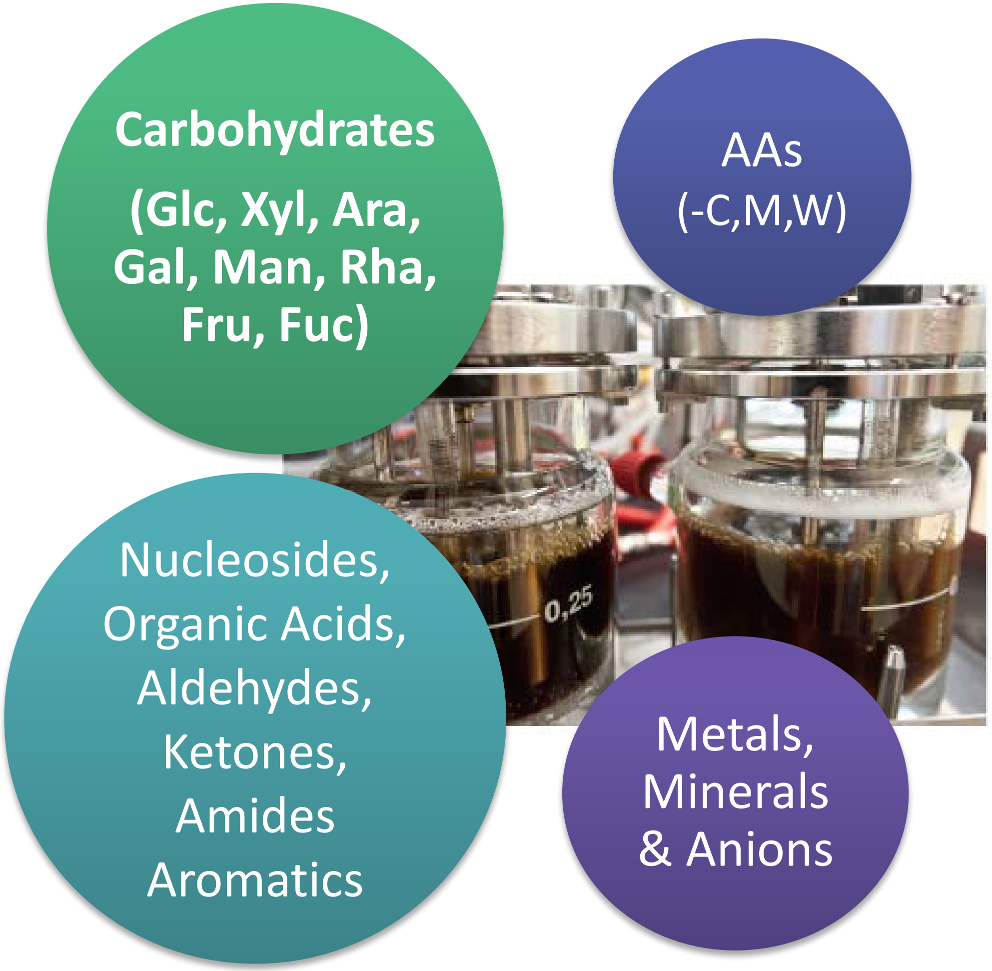
USDA, LTER, Forest Service U.S. Department of Agriculture, NRCS, U.S. Fish & Wildlife Service Department of the Interior, Engine Research Center University of Wisconsin - Madison est 1946, PRL

Farmers
Landowners
Universities

Biomass Field to Fuel Analysis



Chemical composition of hydrolysates



Keating, *et al*. 2014 *Frontiers Micro.* 5:402

Schwalbach, *et al.* 2012 *App. Env. Micro.* 78:3442

Media component	ACSH	SynH1	SynH2-	SynH2
CARBOHYDRATES (mM)[a]				
D-Glucose	343	333	333	333
D-Xylose	205	200	200	200
L-Arabinose[b]	31	–	–	–
D-Arabinose[b]	–[c]	–	34	34
D-Galactose	7.0	–	6.4	6.4
D-Mannose	3.5	–	2.6	2.6
L-Rhamnose	0.9	–	–	–
L-Fucose	0.2	–	–	–
D-Fructose	8.2	–	8.2	8.2
MISC. COMPOUNDS (mM)[a]				
Lactate	0.5	–	0.5	0.5
Pyruvate	–	–	30	30
Citrate	–	–	10	10
Nitrate	–	–	0.1	0.1
Formate	11.2	–	10	10
Malate	9.3	–	10	10
Succinate	0.8	–	0.5	0.5
Acetate	30	–	33	33
Acetamide	78	–	95	95
Glycerol	5.5	–	3	3
Glycine betaine	0.7	–	0.7	0.7
Choline	0.7	–	0.7	0.7
Carnitine	0.2	–	0.2	0.2
SALTS (mM)				
KH_2PO_4	–	22	3.4	3.4
K_2HPO_4	–	42	0.5	0.6
KCl	–	45	45	45
NaCl	–	25	25	25
$(NH_4)_2SO_4$	–	30	30	30
$MgCl_2$	–	1	1	1
$CaCl_2$	–	0.09	0.09	0.09
AMINO ACIDS (μM)[a]				
Alanine	760	700	700	700
Arginine	370	400	400	400
Asparagine	165	200	200	200
Aspartate	379	350	350	350
Cysteine	n.d.[d]	60	60	60
Glutamine	102	100	100	100
Glutamate	459	450	450	450
Glycine	942	400	400	400
Histidine	66	80	80	80
Isoleucine	0	250	250	250
Leucine	460	380	380	380
Lysine	187	200	200	200
Methionine[d]	n.d.	100	100	100
Phenylalanine	189	200	200	200
Proline	214	225	225	225
Serine	272	275	275	275
Threonine[d]	216	225	225	225
Tryptophan	n.d.	50	50	50
Tyrosine	171	175	175	175
Valine	270	225	225	225
NUCLEOBASES (μM)				
Adenine	–	100	100	50
Cytosine	–	100	100	50
Uracil	–	100	100	50
Guanine	–	100	100	50
TRACE COMPONENTS (μM)				
Thiamine-HCl	–	10	10	10
Pantothenate	–	10	10	10
p-Aminobenzoic acid	–	10	10	10
p-Hydroxybenzoic acid	–	10	10	10
2,3-di-Hydroxybenzoic acid	–	10	10	10
$CuCl_2$	–	0.010	0.010	0.010
$CoCl_2 \cdot 6H_2O$	–	0.025	0.030	0.030
H_3BO_3	–	0.400	10	10
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	–	0.003	0.003	0.003
$FeCl_3$	–	18.6	17	17
$ZnCl_2$	–	12	12	12
$MnCl_2 \cdot 4H_2O$	–	100	100	100

LC-derived inhibitors (mM)[e,f]	w/o autoclaving[g]	w/autoclaving			
Feruloyl amide	5.5	3.5 ± 0.6	–	–	2.75
Coumaroyl amide	5.5	2.1 ± 1.3	–	–	2.75
Hydroxymethylfurfural	1.1	<0.03	–	–	0.55
p-Coumaric acid	2.1	1.4 ± 0.3	–	–	1.05
Ferulic acid	0.71	0.091 ± 0.003	–	–	0.355
Benzoic acid	0.48	0.32 ± 0.01	–	–	0.48
Syringic acid	0.03	0.035 ± 0.004	–	–	0.03
Cinnamic acid	0.09	–	–	–	0.09
Vanillic acid	0.09	0.15 ± 0.02	–	–	0.09
Caffeic acid	0.01	0.000 ± 0.001	–	–	0.01
Vanillin	0.122	0.24 ± 0.04	–	–	0.122
Syringaldehyde	0.162	0.017 ± 0.002	–	–	0.162
4-Hydroxybenzaldehyde	0.157	0.15 ± 0.02	–	–	0.157
4-Hydroxyacetophenone	0.025	0.017 ± 0.002	–	–	0.025
Osmolality (mol/kg)	1.16 ± 0.03		0.97	1.17 ± 0.01	1.19 ± 0.01

[a]ACSH data are from Schwalbach et al. (2012). Sugar concentrations are averages of HPLC-MS and NMR determinations.

[b]In the SynH2 recipe, D-Arabinose was substituted for the L-Arabinose present in ACSH to avoid AraC-mediated repression of xylose-utilization genes (Desai and Rao, 2010). In other contexts, use of L-Arabinose in SynH2 would be appropriate.

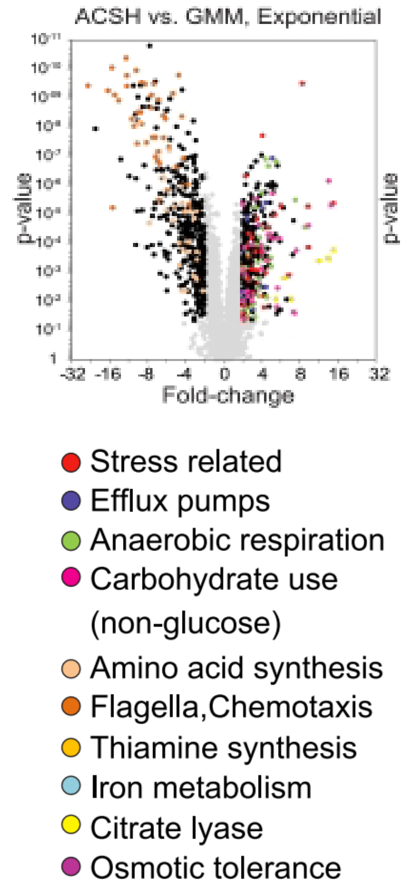
[c]–, not determined in ACSH or not added in SynH.

[d]n.d., not detectable by methods used.

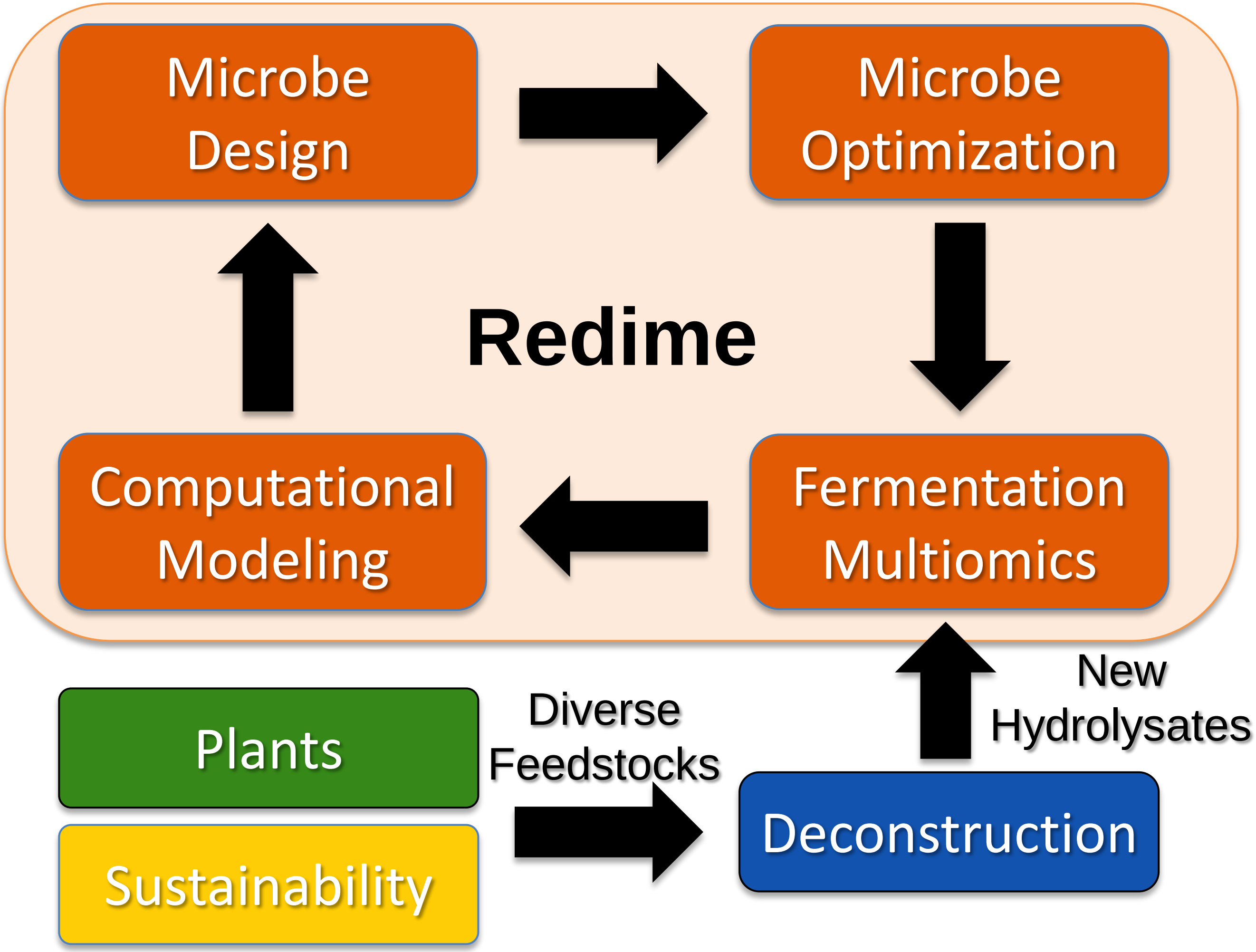
[e]Aromatic compounds detected at less than 20 μM in ACSH are not reported here.

[f]The sets of acids, amides, and aldehydes used for supplemental studies in formulating SynH2 consisted of p-Coumaric acid, Ferulic acid, Benzoic acid, Syringic acid, Cinnamic acid, Vanillic acid, and Caffeic acid (acids), Feruloyl amide and Coumaroyl amide (amides), and HMF, Vanillin, Syringaldehyde, 4-Hydroxybenzaldehyde, and 4-Hydroxyacetophenone (aldehydes) at the concentrations listed for non-autoclaved ACSH or fractions thereof as described in the Supplemental Results.

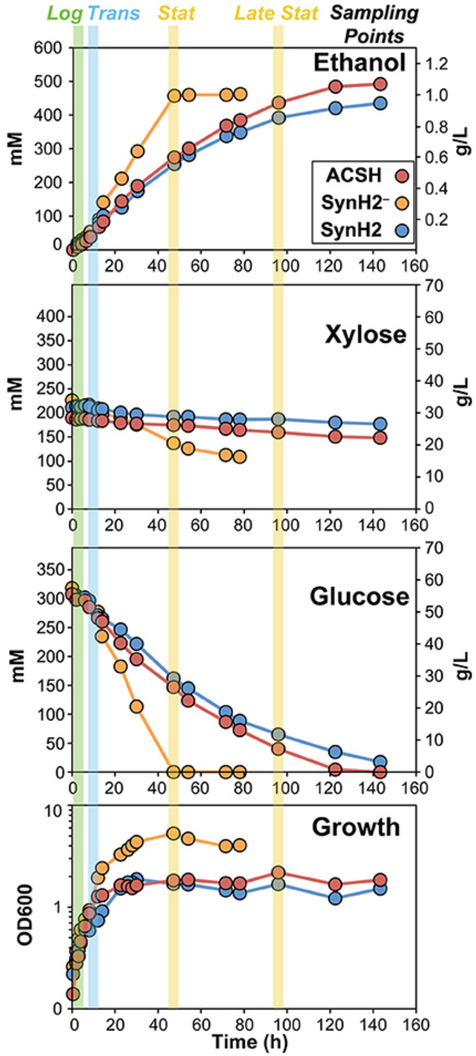
[g]ACSH inhibitor concentrations for non-autoclaved CS hydrolysate are from (Tang et al., submitted). Hydrolysate preparations are described in Materials and Methods.



Reiterative microbial design targets sites for strain improvement



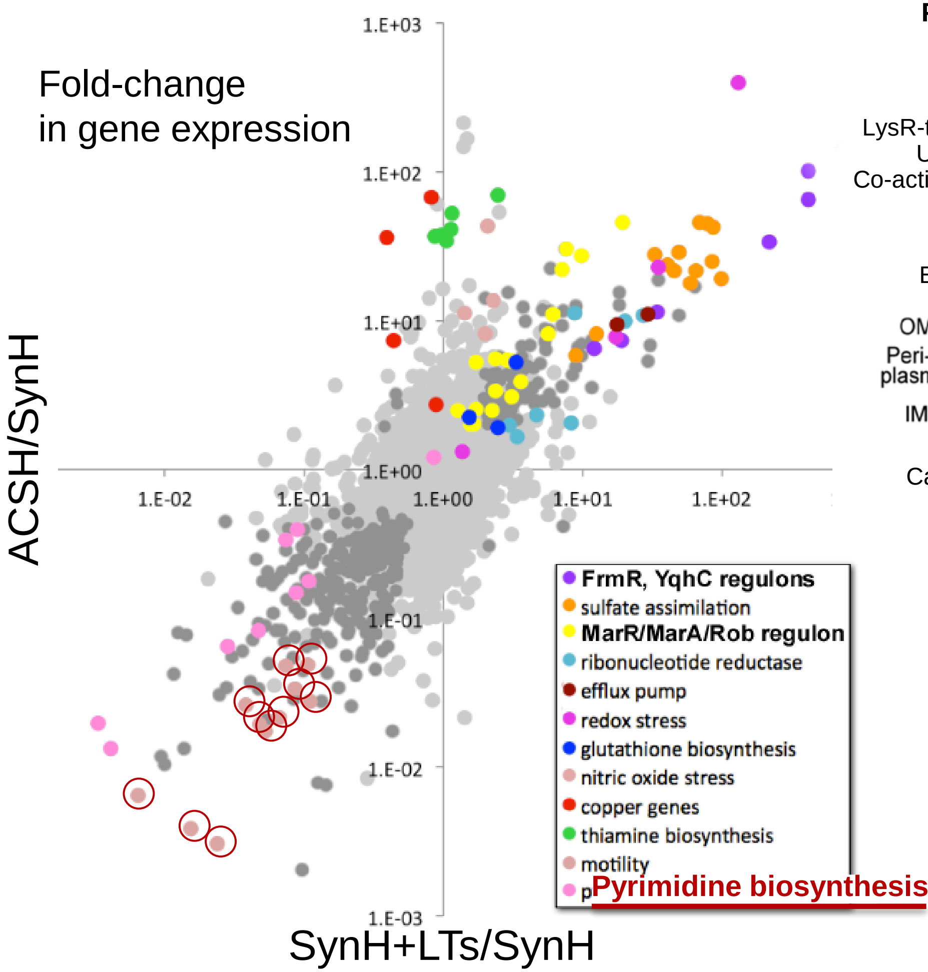
Hydrolysate composition negatively impacts biofuel fermentations

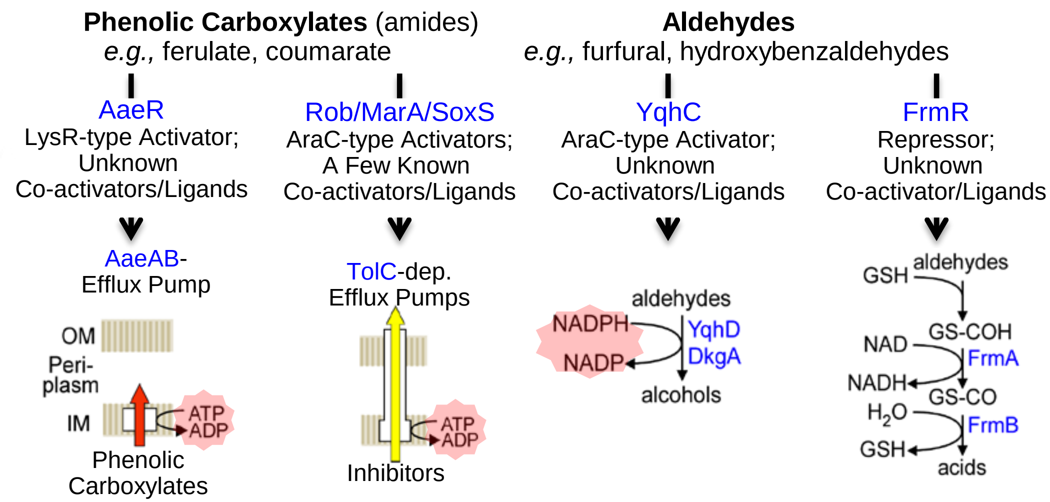


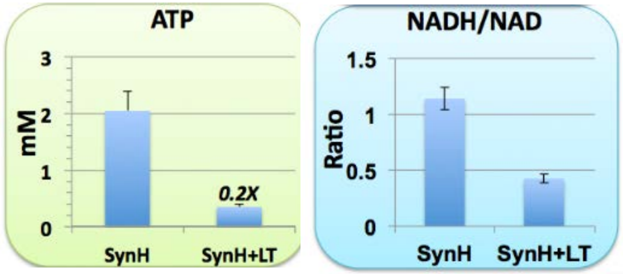
- Negative impact of ACSH on growth/biomass production, xylose and glucose utilization & ethanol production
- Recapitulate behavior with synthetic hydrolysate (SynH2)
- Negative impact overcome in SynH2^{-} (SynH2 minus aromatics from ACSH)

<u>Lignotoxins (LTs)</u>
5.5 mM Feruloyl amide
5.5 mM Coumaroyl amide
2.1 mM Coumaric acid
0.5 mM Benzoic acid
1.1 mM Hydroxymethylfurfural
0.7 mM Ferulic acid
8 minor LTs (<0.5 mM)

LTs induce energy-consuming pathways & deplete ATP/NADH

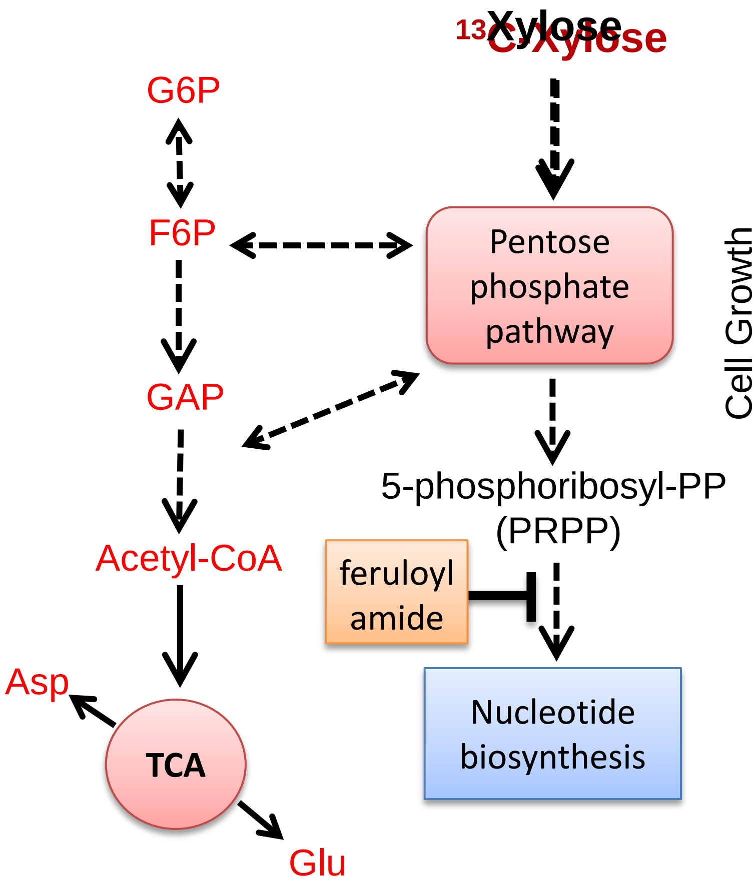


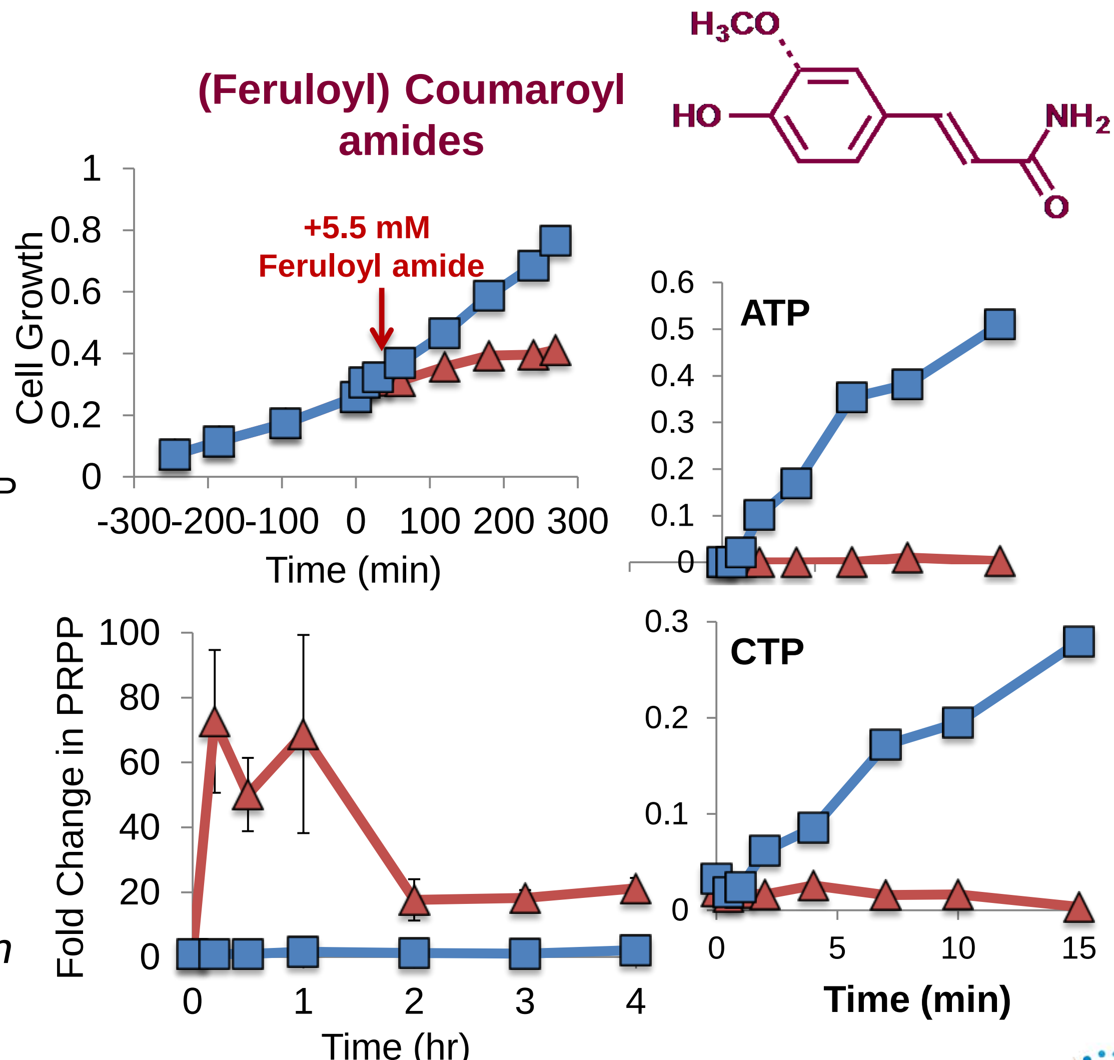




Keating, *et al*. 2014 *Frontiers Micro.* 5:402

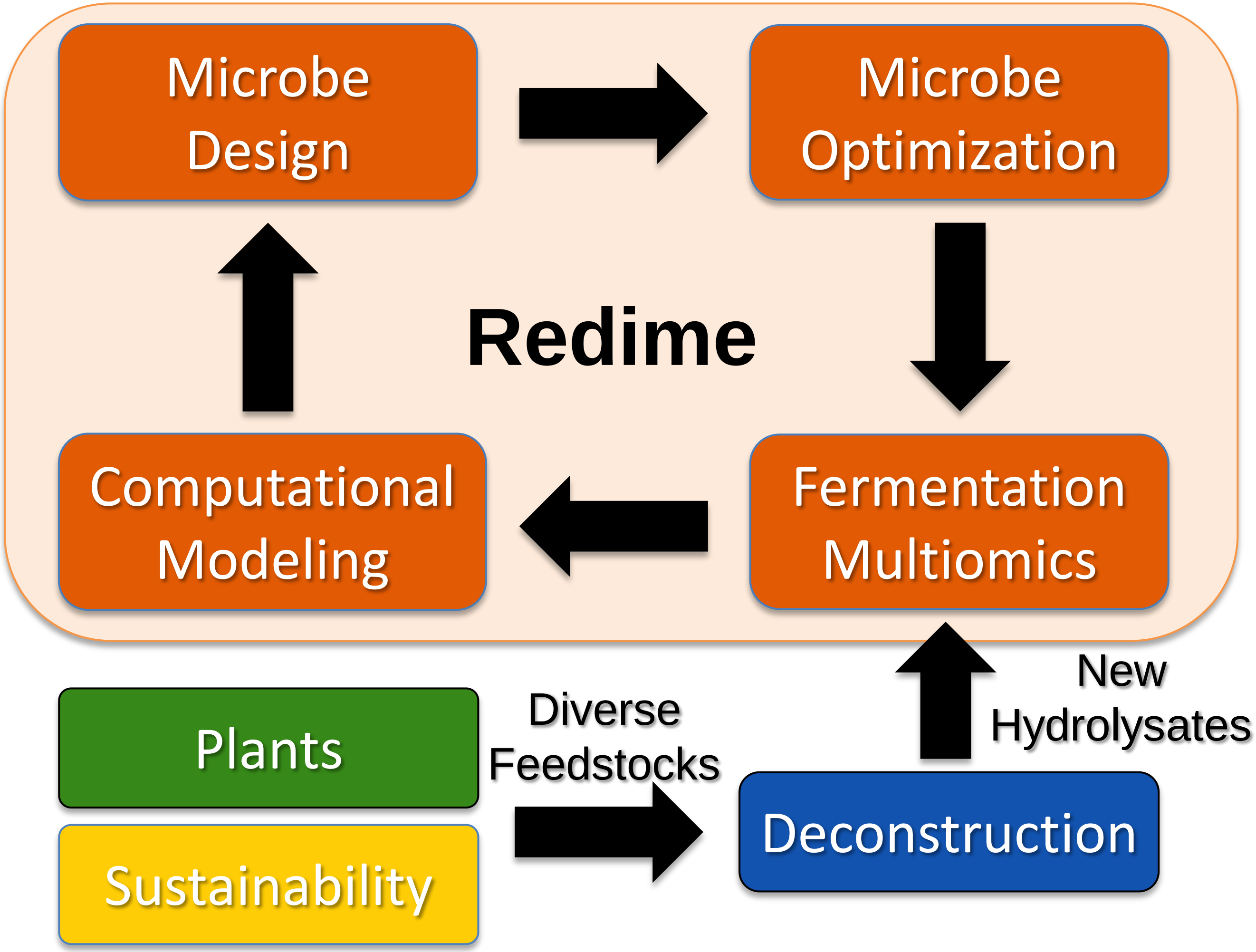
Feruloyl amide inhibits growth, increases PRPP pools & blocks nucleotide synthesis



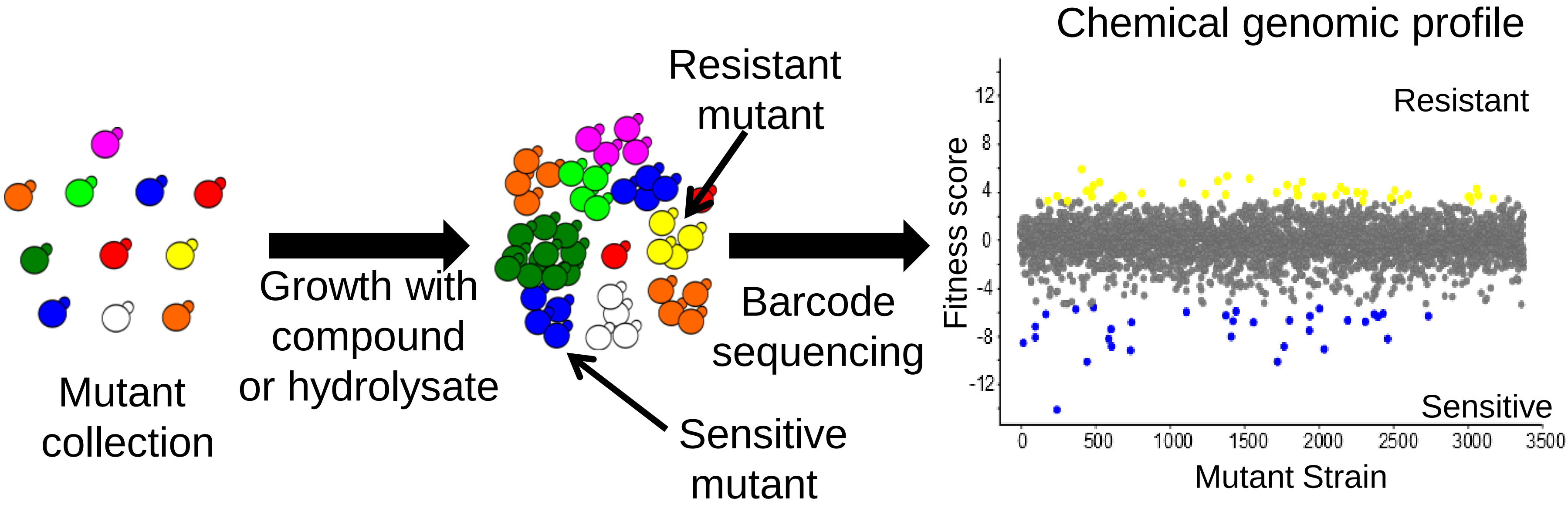


Amador-Noguez, *et al. in preparation*

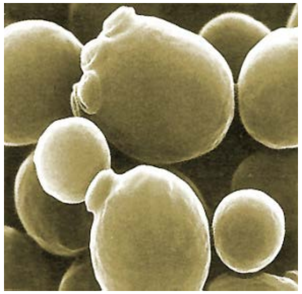
Reiterative microbial design targets sites for strain improvement



Genomic fingerprinting of inhibitors

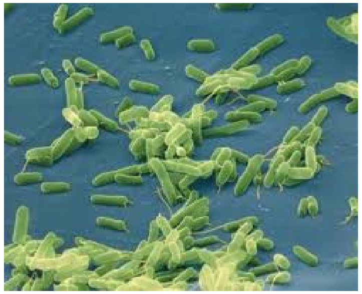


S. cerevisiae



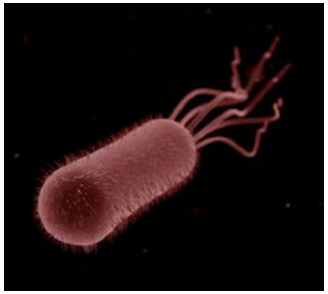
Parsons, *et al*. 2006
Piotrowski, *et al*. *in press PNAS*

Z. mobilis



Skerker, *et al*. 2013

E. coli



Otsuka, *et al*. 2015

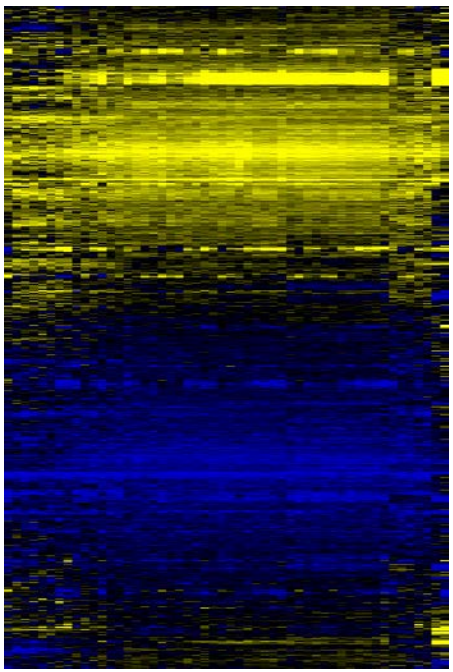
Conserved mode of action of LT's

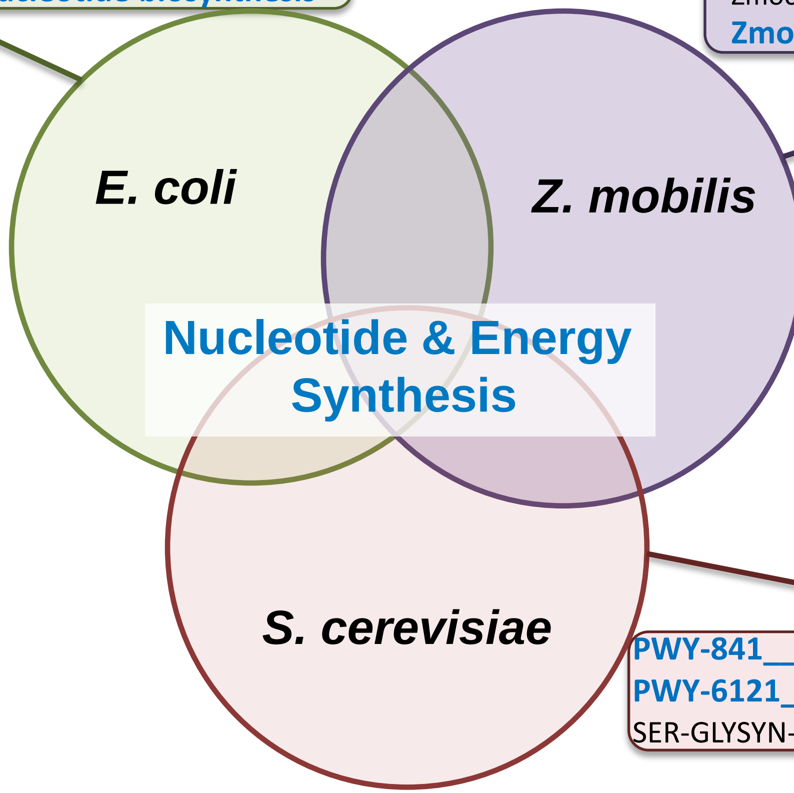
Chemical genomic analysis of 29 hydrolysate LT's against 3 "disparate" microbes (*E. coli*, *Z. mobilis*, *S. cerevisiae)*

- Nucleotide & energy metabolism responsive across inhibitors & microbes

PWY0-1334__electron transfer from NADH to cytochrome *bd* oxidase
PURINE2-PWY__*de novo* purine nucleotide synthesis
PWY-7219__*de novo* adenosine nucleotide biosynthesis

Zmo00290 Valine, leucine and isoleucine biosynthesis
Zmo00340 Histidine metabolism
Zmo00230 Purine metabolism

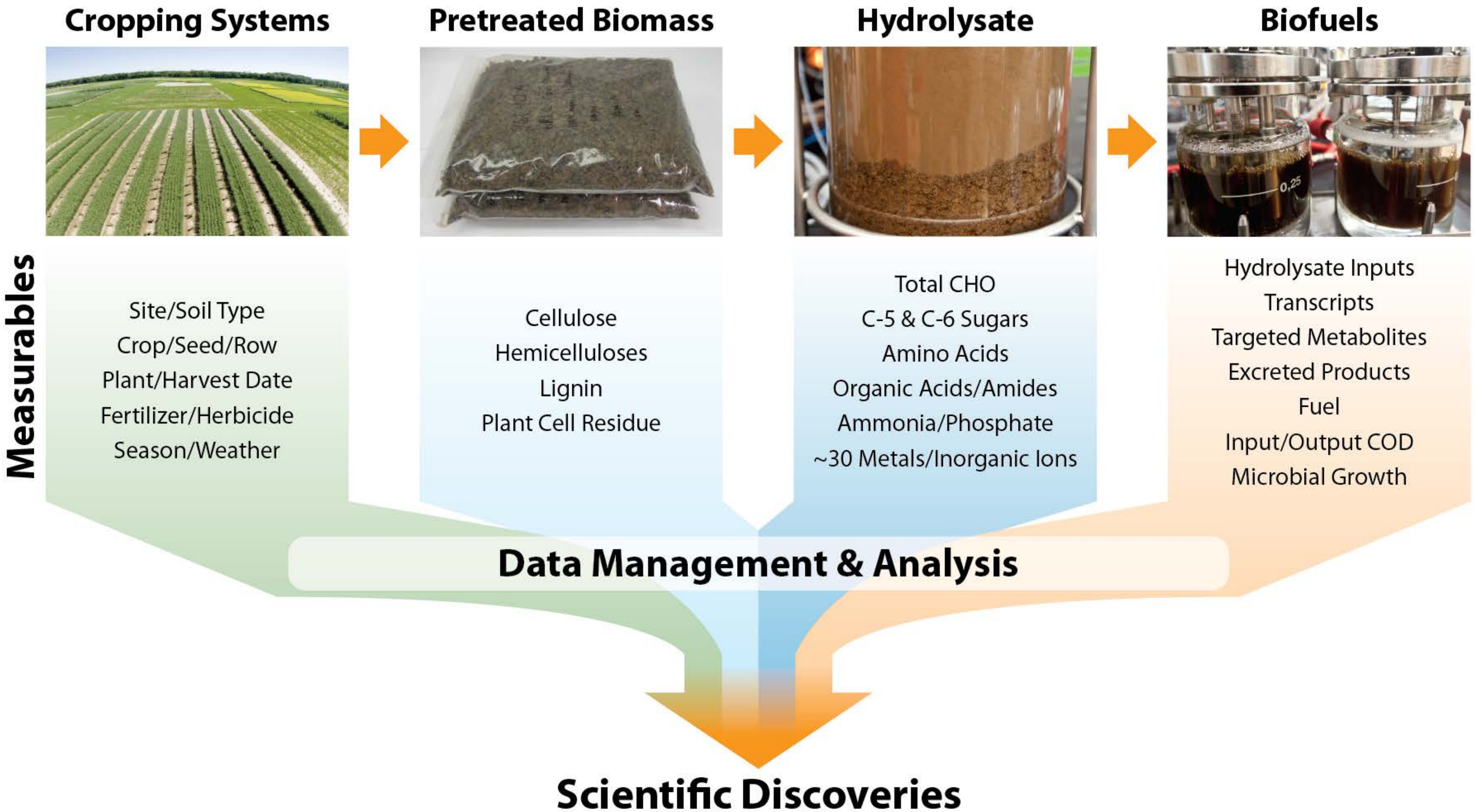




E. coli: Otsuka, *et al*. 2015
Z. Mobilis: Skerker, *et al*. 2013

PWY-841__*de novo* purine biosynthesis
PWY-6121_5-aminoimidazole ribonucleotide synthesis
SER-GLYSYN-PWY__Serine & glycine synthesis

Systems Analysis of Biomass & Biofuel Production



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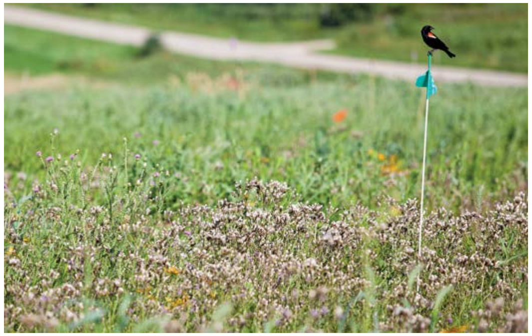
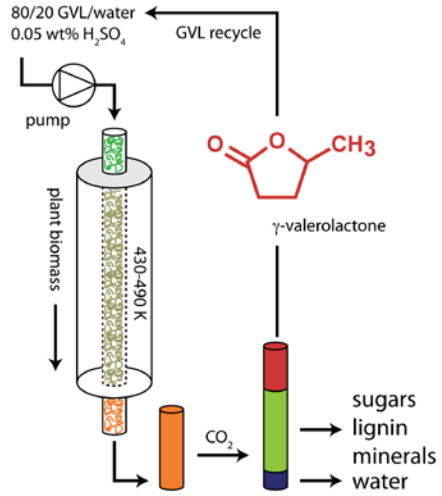


Analysis of additional biomass crops

- Poplar
- Miscanthus
- Switchgrass
- Native Prairie
- Mixed Feedstocks

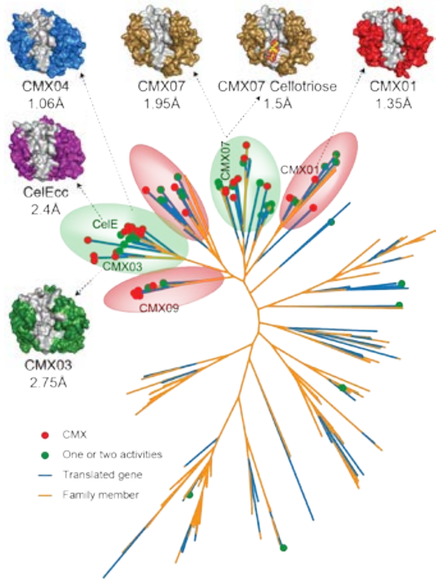
- Assess yearly/regional feedstock variations
- Impact of biomass trait modifications (Zip-Lignin & others)

Systems Analysis of Biomass & Biofuel Production



Analysis of other "polysaccharide hydrolysates" & *lignin streams* from other pretreatments

- γ-Valerolactone (GVL)
- Alkaline (alkaline hydrogen peroxide, extractive ammonia)
- Ionic liquids
- Monitor variations due to changes in enzyme cocktails



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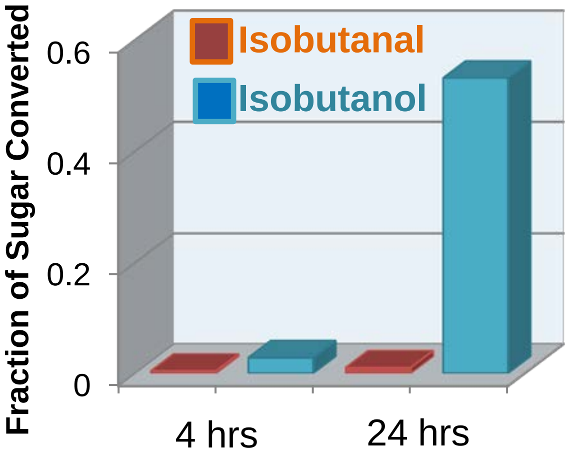
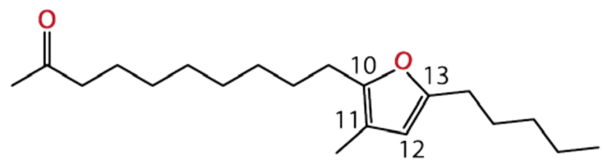
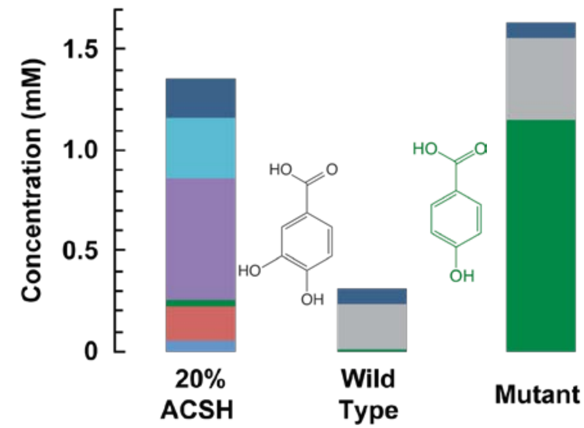




10,13-epoxy-11-methyl-octadecadienoate (FFA)



Analysis of other microbial catalysts

- Additional fuels & chemicals (long chain alcohols, hydrocarbons, aromatics, etc.)
- Impact of changes on producing microbes
- Different single species/consortia

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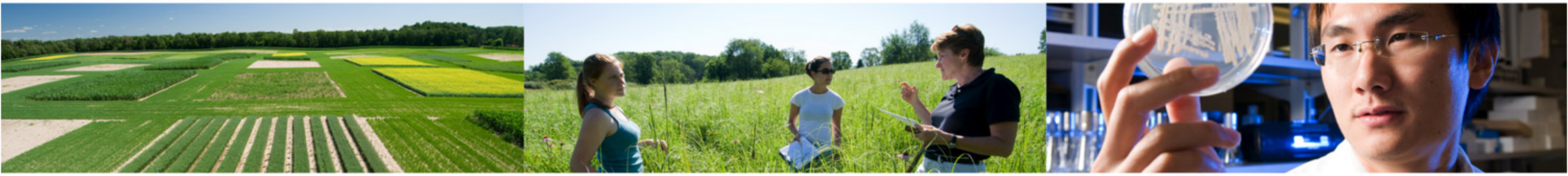

Questions?