

Supplemental Information for

Complex physiology and compound stress responses during fermentation of alkaline-pretreated corn stover hydrolysate by an *Escherichia coli* ethanologen

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Hours	ACSH																			
	amino acid concentration (μM)																			
	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
0	717	0	379	459	189	484	56	0	187	519	0	163	214	102	370	272	216	239	0	171
3	752	0	279	397	197	486	62	0	166	299	0	0	264	101	331	63	159	249	0	137
5	786	0	0	342	191	502	53	0	140	273	0	0	278	96	295	0	119	254	0	131
6	801	0	59	340	194	503	0	0	142	277	0	0	216	106	287	0	120	238	0	138
8	802	0	61	197	185	503	0	0	104	364	0	0	325	97	239	0	62	210	0	203
10	822	0	71	101	167	506	0	0	73	330	0	0	314	116	229	0	0	171	0	155
12	840	0	0	0	164	505	0	0	0	264	0	0	294	62	203	0	0	157	0	166
14	829	0	62	0	158	479	0	0	0	204	0	0	263	0	177	0	0	141	0	161
16	853	0	0	0	163	468	0	0	0	166	0	0	269	0	167	0	0	124	0	145
22	878	0	0	0	170	428	0	0	0	127	0	0	275	0	141	0	0	54	0	125
28	899	0	0	0	169	398	0	0	0	105	0	0	252	0	134	0	0	0	0	111
52	809	0	0	0	229	403	0	0	0	158	0	0	330	0	105	0	0	0	0	0
72	991	0	50	0	252	351	0	0	0	80	0	0	259	0	130	0	0	39	0	0
82	996	0	0	0	267	358	0	0	0	104	0	0	261	0	111	0	0	0	0	0
98	1010	0	0	0	285	383	0	0	0	131	0	0	364	0	100	0	0	52	0	0
112	1041	0	0	0	275	395	0	0	0	157	0	0	331	0	104	0	0	58	0	184
124	1051	0	104	106	270	405	0	0	0	180	0	0	372	0	99	0	0	75	0	207

Hours	SynH																			
	amino acid concentration (μM)																			
	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
0	659	0	315	382	190	376	71	262	172	349	77	163	203	134	366	250	295	246	0	165
3	631	0	164	287	165	350	56	228	128	285	67	0	88	77	312	0	142	347	0	143
4	617	0	161	211	154	338	49	209	103	250	52	0	53	60	291	0	84	315	0	133
10	495	0	162	0	94	297	13	108	11	84	0	0	0	0	195	0	6	196	0	79
14	392	0	168	0	62	282	0	59	7	0	0	0	0	0	139	0	6	155	0	52
24	525	0	184	5	82	225	0	71	84	0	0	0	0	0	130	0	5	116	0	52
31	582	0	186	20	100	179	0	76	142	0	0	0	0	0	118	0	3	83	0	59
48	640	0	196	64	150	127	0	82	277	0	0	0	0	0	95	0	3	36	0	99
53	647	0	197	163	153	129	0	81	274	0	0	0	26	0	94	6	8	10	0	110
56	659	0	192	188	157	133	0	83	276	0	0	0	25	0	94	3	8	12	0	117
75	676	0	170	262	168	146	0	82	267	17	0	0	24	0	94	0	0	25	0	119

Hours	SynH 3x AA																			
	amino acid concentration (μM)																			
	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
0	1866	0	909	1065	550	1028	196	717	487	994	239	462	661	336	1002	695	616	0	0	488
3	1798	0	502	989	499	967	179	655	424	913	211	0	403	186	927	0	448	0	0	447
6	1663	0	465	708	421	806	150	495	269	697	124	0	78	0	774	0	109	0	0	387
8	1390	0	450	322	313	631	70	317	98	413	0	0	0	0	568	0	0	0	0	297
10	1161	0	415	57	225	487	0	163	0	217	0	0	0	0	420	0	0	203	0	231
12	988	0	456	0	166	464	0	0	0	0	0	0	0	0	328	0	0	0	0	206
14	1100	0	513	0	193	452	0	0	834	0	0	0	0	0	332	0	0	0	0	209
23	1315	0	449	230	181	387	0	0	221	0	0	0	0	0	303	0	0	0	0	193
28	1336	0	423	267	194	398	0	0	204	0	0	0	0	0	291	0	0	0	0	187
49	1394	0	449	299	208	402	0	0	216	0	0	0	0	0	299	0	0	362	0	214
96	1466	0	419	350	293	350	0	0	188	0	0	0	0	0	281	0	0	422	0	410

FIG. S1. Extracellular amino acid concentration in SynH and SynH supplemented with amino acids. The concentration in micromolar of amino acids present in the media was determined as described in Materials and Methods. **Upper panel.** Extracellular amino acid concentrations in ACSH during growth (Figure 3C) are repeated for comparative purposes. **Middle Panel.** Extracellular amino acid concentrations during growth of GLBRCE1 in SynH supplemented with amino acids to a concentration equivalent to what was measured in ACSH. **Lower Panel.** Extracellular amino acid concentrations during growth of GLBRCE1 in SynH supplemented with amino acids to a concentration three times what was measured in ACSH. “ACSH” refers to amino acid concentration during growth in ACSH, “SynH” refers to amino acid concentrations in media during growth in SynH, and “SynH 3x AA” refers to synthetic media supplemented with amino acids to concentration three times what was measured in ACSH. Shading represents amino acid concentration, and is shown as a gradient between dark blue, corresponding to the highest concentrations and red, representing the lowest concentration.

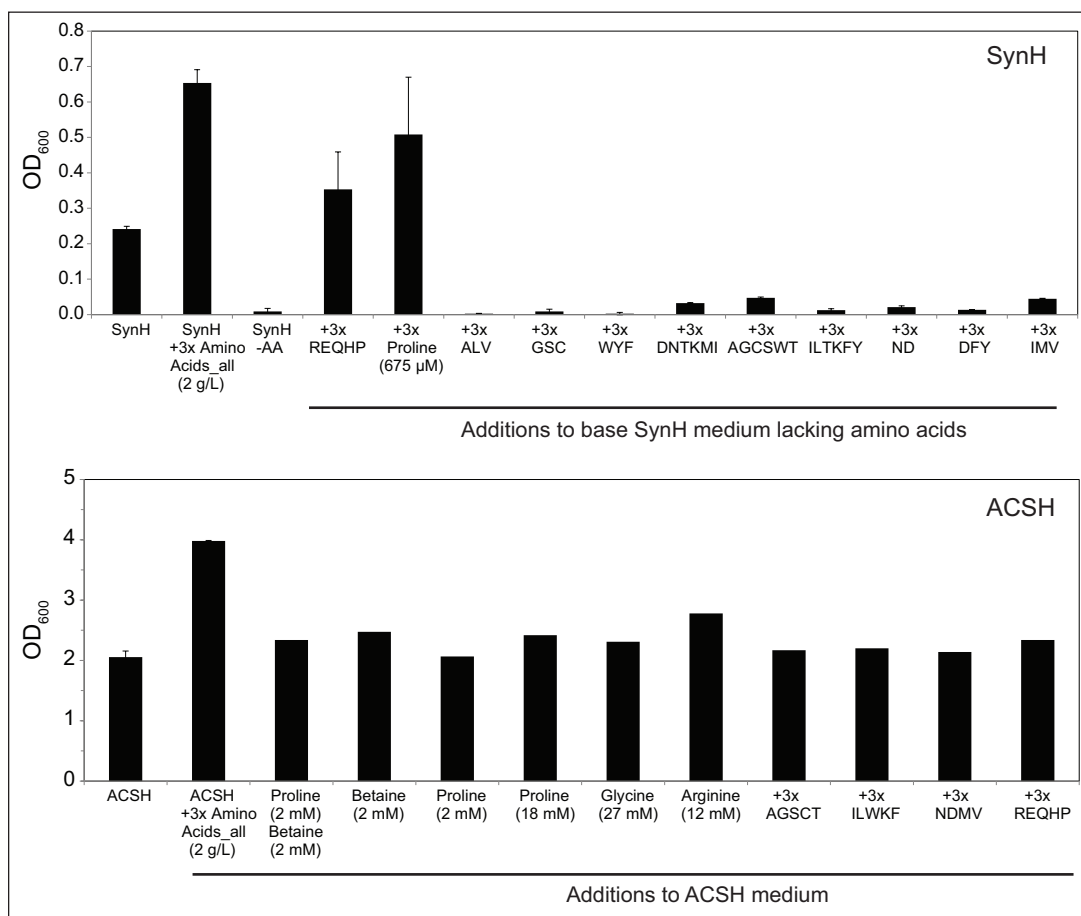
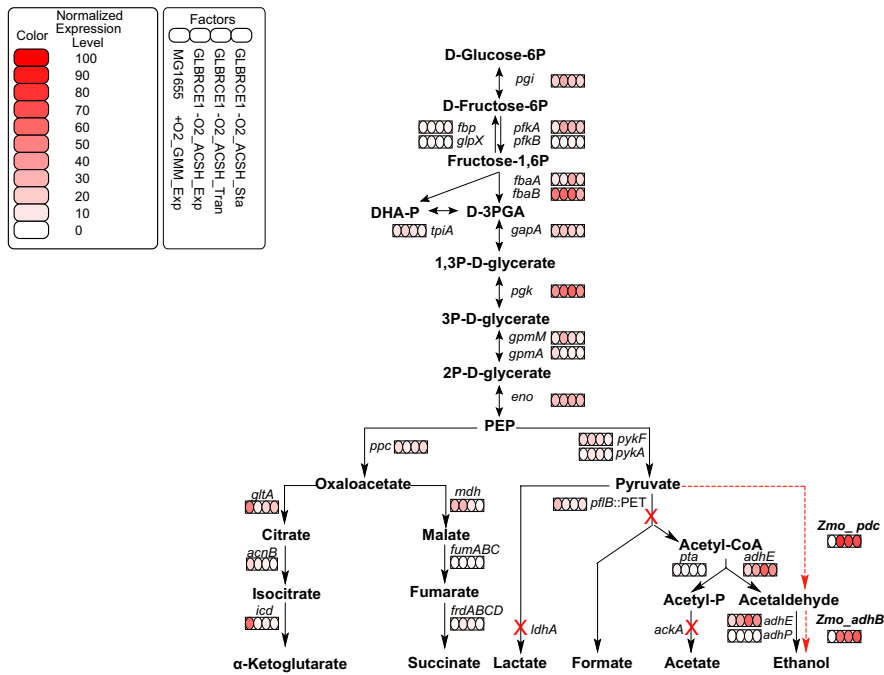


FIG. S2. Growth of the ethanologen in SynH and ACSH supplemented with protective osmolytes and amino acids. The ethanologen GLBRCE1 was cultured in SynH or ACSH in the presence of individual amino acids, groups of amino acids, or protective osmolytes as described in Materials and Methods. **Upper panel.** SynH media was prepared either without amino acids, supplemented with amino acids to a concentration equivalent to what was measured in ACSH, supplemented with all amino acids at a concentration equivalent to 3X the concentration that was detected in ACSH, individual amino acids or groups of amino acids at a concentration equivalent to 3X the concentration that was detected in ACSH. “SynH-AA”, refers to synthetic media in which amino acids were omitted, “SynH + 3x Amino Acids_all” refers to synthetic hydrolysate supplemented with amino acids at present at 3X the concentration that was detected in ACSH. Letters at bottom of figure refer to single letter codes for amino acids. Cells were cultured under anaerobic conditions in a microplate reader as described in Materials and Methods. **Lower panel.** Growth of ethanologen in ACSH supplemented with individual amino acids, groups of amino acids, and osmolytes. Concentrations of amino acids were the same as described for upper panel. Fermentations of the ethanologen were carried out in bioreactors (200-400 ml) in ACSH and supplemented ACSH as described in Materials and Methods. Values on graph represent maximum optical density. Single letters listed on graph refer to single letter codes for amino acid.

A



B

Normalized expression levels (\log_2)

Carbohydrate	Transporter gene(s)	MG1655	GLBRC1	GLBRC1	GLBRC1	GLBRC1	GLBRC1	GLBRC1	GLBRC1	GLBRC1
		+O2 GMM_Exp	-O2 GMM_Exp	-O2 GMM_Sta	-O2 SynH_Exp	-O2 SynH_Tran	-O2 SynH_Sta	-O2 ACSH_Exp	-O2 ACSH_Tran	-O2 ACSH_Sta
Glucose	<i>ptsG</i>	12.6	12.6	11.4	13.3	12.8	13.2	13.0	11.4	11.0
	<i>manXYZ</i>	13.2	12.3	12.9	13.7	13.5	12.7	13.9	13.1	12.3
Arabinose	<i>araFGH</i>	9.1	9.0	11.4	10.2	9.4	9.4	9.6	9.1	9.1
	<i>araE</i>	8.6	8.8	9.5	9.7	9.0	9.3	12.5	10.6	9.7
Fructose	<i>fruAB</i>	9.7	11.3	9.7	11.6	12.0	11.1	11.7	12.2	10.7
Xylose	<i>xylE</i>	7.4	7.8	7.7	8.9	9.3	10.6	7.8	7.8	8.0
	<i>xylFGH</i>	7.9	8.1	9.0	10.9	11.3	10.3	8.5	8.3	8.6
Maltose	<i>malX</i>	8.6	8.8	9.3	8.8	8.5	9.2	8.4	8.3	8.4
	<i>galP</i>	9.8	9.1	9.4	8.5	9.1	9.4	11.8	9.8	8.8
Galactose	<i>gal(yjtQRT/yjiF)</i>	8.4	8.5	12.0	9.7	8.3	8.5	8.4	8.2	8.6
	<i>mgIABC</i>	9.6	8.0	13.1	8.2	7.6	7.9	7.5	7.5	7.6
Trehalose	<i>treB</i>	8.2	8.2	9.0	9.8	8.1	7.8	8.4	8.0	7.9
	<i>gltT</i>	9.2	8.6	11.0	9.8	8.9	8.9	11.3	8.7	8.5
Gluconate	<i>gltP</i>	7.6	8.0	8.8	9.1	7.9	7.9	8.4	7.8	8.3
	<i>gltU</i>	8.1	7.8	8.1	8.5	7.9	7.8	11.5	8.1	8.3
	<i>idnT</i>	8.2	8.6	8.5	9.7	8.2	8.4	8.1	8.2	8.3

FIG. S3. Expression of genes necessary for glycolysis, mixed acid fermentation, engineered ethanol production, and sugar transport during aerobic growth in GMM, and during different phases of anaerobic growth in ACSH. Expression values from cells growth in ACSH, SynH, and GMM were determined as described in Materials and Methods. **Panel A.** Expression values of glycolytic genes during growth in ACSH and GMM. Shaded disks represent normalized expression of cells in exponential phase cells in GMM, and exponential, transition and stationary phase cells in ACSH. **Panel B.** Expression levels of genes necessary for transport of glucose, arabinose, fructose, xylose, maltose, galactose, trehalose, and gluconate. Aerobic expression values in GMM were gathered from an independent experiment involving strain MG1655. Shading represents relative \log_2 expression values, with green shading indicative of higher level expression.

TABLE S1. Composition of ACSH, SynH, and GMM.

Media Component	ACSH		SynH	GMM
Carbohydrates (mM)	GC-MS	NMR		
Glucose	328 ± 22	358	333	56
Xylose	210 ± 15	199	200	-
Arabinose	27.3 ± 1.3	33.7	- ^(b)	-
Galactose	8.7 ± 0.5	6.4	-	-
Mannose	4.4 ± 0.2	2.6	-	-
Rhamnose	0.9 ± 0.1	-	-	-
Fucose	0.2 ± 0.01	-	-	-
Fructose	n.d. ^(a)	8.2	-	-
Additional compounds (mM)	HPLC-RID	NMR		
Lactate	< 0.2	0.5	-	-
Formate	10.5 ± 1.8	11.8	-	-
Malate	- ^(c)	9.3	-	-
Succinate	1.4 ± 0.5	0.3	-	-
Acetate	38.6 ± 5.4	33	-	-
Acetamide	-	76	-	-
Glycerol	5.5 ± 1.2	n.d.	-	-
Glycine betaine	-	0.7	-	-
Choline	-	0.7	-	-
Carnitine	-	0.2	-	-
Salts (mM)				
KH ₂ PO ₄	- ^(d)	-	22 ^(e)	22
K ₂ HPO ₄	-	-	42	42
KCl	-	-	45	-
NaCl	-	-	25	9
(NH ₄) ₂ SO ₄	-	-	30	-
NH ₄ Cl	-	-	-	19
MgCl ₂	-	-	1	-
MgSO ₄	-	-	-	1
CaCl ₂	-	-	0.09	0.09
Amino Acids (μM)	Acid hydrolysis		NMR	
Alanine	717	800	700	-
Arginine	370	-	400	-
Asparagine	163	-	200	-
Aspartate	379	-	350	-
Cysteine	n.d.	-	50	-
Glutamine	102	-	100	-
Glutamate	459	-	450	-
Glycine	484	600	400	-

Histidine	56	-	80	-
Isoleucine	0	-	250	-
Leucine	519	400	360	-
Lysine	187	-	200	-
Methionine	n.d.	-	100	-
Phenylalanine	189	-	200	-
Proline	214	-	225	-
Serine	272	-	275	-
Threonine	216	-	225	-
Tryptophan	n.d.	-	50	-
Tyrosine	171	-	175	-
Valine	239	300	225	-
Nucleobases (µM)				
Adenine	- ^(f)	-	100	-
Cytosine	-	-	100	-
Uracil	-	-	100	-
Guanine	-	-	100	-
Vitamins & Micronutrients (µM)				
Thiamine-HCL	- ^(g)	-	10	0.33
Pantothenate	-	-	10	-
p-Aminobenzoic acid	-	-	10	-
p-Hydroxybenzoic acid	-	-	10	-
2,3-di-Hydroxybenzoic acid	-	-	10	-
CuCl ₂	-	-	0.010	0.010
CoCl ₂ ·6H ₂ O	-	-	0.025	0.030
H ₃ BO ₄	-	-	0.400	0.400
(NH ₄) ₆ Mo ₇ O ₂ ·4H ₂ O	-	-	0.003	0.003
FeCl ₃	-	-	16.6	-
FeSO ₄	-	-	-	1
ZnCl ₂	-	-	12	-
ZnSO ₄	-	-	-	0.01
MnCl ₂ ·4H ₂ O	-	-	100	0.08
NiCl ₂	-	-	-	1
Na ₂ SeO ₃	-	-	-	1

(a) Not detectable by methods used.

(b) For ACSH and GMM, was not added to the synthetic medium

(c) Malate, acetamide, glycine betaine, choline, carnitine not detectable via the methods used for endproduct analysis.

(d) Not determined. Concentrations of ions metals available in Table 2.

(e) Salts were added to medium during preparation

(f) Concentrations of nucleosides not determined. Concentration in SynH was added during medium preparation

(g) Concentrations of vitamins and micronutrients not determined in ACSH. Concentration in SynH was added during medium preparation

TABLE S2. Ions and metals present in ACSH and SynH, and changes in concentration with respect to time and pH.

Component (mM)	ACSH pH 5 ^(b)	ACSH pH 7 0 hours ^(b, d)	ACSH pH 7 124 hours ^(e)	SynH pH 7 0 hours	SynH pH 7 53 hours
K	86.0	119.0	74.9	64.1	54.9
P	59.6	52.2	40.4	39.4	32.5
Na	25.4	91.7	96.7	15.9	44.5
Mg	4.6	0.23	0.001	0.62	0.25
Ca	4.2	0.72	0.31	0.32	0.21
S	2.1	2.2	1.5	17.9	14.9
Mn	0.062	0.001	2.5 x 10 ⁻⁵	0.008	0.004
Fe	0.019	0.020	0.009	< 0.001	< 0.001
Cr	0.018	0.019	0.007	0.001	0.001
Zn	0.009	0.010	0.004	<0.001	<0.001
Al	0.007	0.011	<0.200	<0.050	<0.050
B	0.007	0.045	0.060	0.015	0.032
Ni	0.002	0.003	0.001	1.4 x 10 ⁻⁴	1.3 x 10 ⁻⁴
Pb	0.001	0.001	4.8 x 10 ⁻⁴	0.001	4.7 x 10 ⁻⁴
Cu	3.1 x 10 ⁻⁴	0.001	<0.020	<0.005	<0.005
Li	2.9 x 10 ⁻⁴	0.001	0.005	0.001	0.004
Mo	2.1 x 10 ⁻⁴	3.1 x 10 ⁻⁴	2.7 x 10 ⁻⁴	<0.004	<0.004
Cd	1.8 x 10 ⁻⁴	2.7 x 10 ⁻⁴	<0.020	<0.004	<0.004
Co	1.7 x 10 ⁻⁴	3.4 x 10 ⁻⁴	3.1 x 10 ⁻⁴	<0.003	<0.003
Cl	120.5	111.5	105.9	101.1	94.9
NH ₄	67.8	54.1	50.1 ^a	55.1 ^a	44.3 ^a
NO ₃	0.103	0.269	< 0.010	-	-
PO ₄	n.d.	50.8	32.4	58.9 ^a	43.9 ^a

- a Mean of multiple replicates. Error of estimate less than 5%
- b Composition immediately after hydrolysis.
- c Composition after adjustment of pH to 7.
- d Composition at beginning of fermentation
- e Composition at conclusion of fermentation
- n.d. No data

TABLE S3. Extracellular concentrations of carbon sources and metabolic endproducts during fermentation in SynH, and SynH supplemented with amino acids.

Synthetic Hydrolysate										
Hours	Glucose (mM)	Xylose (mM)	Pyruvate (mM)	Xylitol (mM)	Succinate (mM)	Lactate (mM)	Glycerol (mM)	Formate (mM)	Acetate (mM)	Ethanol (mM)
0	338 ± 3	199 ± 3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
3	327 ± 2	193 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	15 ± 0
4	321 ± 7	193 ± 5	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	23 ± 0
10	277 ± 3	186 ± 1	0 ± 0	0 ± 0	3 ± 1.6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	80 ± 2.1
14	238 ± 8	186 ± 0	0 ± 0	0 ± 0	8 ± 4.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	145 ± 8.6
24	144 ± 17	179 ± 5	2 ± 0	0 ± 0	16 ± 9.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	282 ± 26
31	77 ± 27	166 ± 6	3 ± 0	0 ± 0	22 ± 11.8	0 ± 0	0 ± 0	0 ± 0	0 ± 0	347 ± 28
48	0 ± 0	159 ± 5	13 ± 7	0 ± 0	31 ± 14.3	0 ± 0	0 ± 0	0 ± 0	1 ± 0.5	477 ± 15
53	0 ± 0	153 ± 9	20 ± 9	0 ± 0	31 ± 14.3	0 ± 0	0 ± 0	0 ± 0	1 ± 0.5	455 ± 19
56	0 ± 0	159 ± 10	23 ± 7	0 ± 0	32 ± 14.3	0 ± 0	0 ± 0	0 ± 0	1 ± 0.5	455 ± 17
75	0 ± 0	146 ± 13	38 ± 5	0 ± 0	29 ± 11.8	0 ± 0	0 ± 0	0 ± 0	1 ± 0.6	390 ± 10
129	0 ± 0	113 ± 16	46 ± 3	0 ± 0	37 ± 14.3	0 ± 0	0 ± 0	0 ± 0	3 ± 1.1	303 ± 8
144	0 ± 0	106 ± 18	46 ± 4	0 ± 0	37 ± 14.3	0 ± 0	0 ± 0	0 ± 0	1 ± 1.1	282 ± 13
Synthetic Hydrolysate + 3X Amino Acids										
Hours	Glucose (mM)	Xylose (mM)	Pyruvate (mM)	Xylitol (mM)	Succinate (mM)	Lactate (mM)	Glycerol (mM)	Formate (mM)	Acetate (mM)	Ethanol (mM)
0	349 ± 29	193 ± 16	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
3	338 ± 32	193 ± 16	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	17 ± 0
6	310 ± 38	193 ± 17	0 ± 0	0 ± 0	4 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	56 ± 0
8	271 ± 46	186 ± 16	0 ± 0	0 ± 0	11 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	108 ± 23
10	233 ± 55	179 ± 19	0 ± 0	0 ± 0	17 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	164 ± 30
12	183 ± 61	179 ± 19	0 ± 0	0 ± 0	24 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	217 ± 36
14	127 ± 55	173 ± 13	1 ± 2	0 ± 0	32 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	282 ± 43
23	0 ± 0	153 ± 7	17 ± 0	0 ± 0	51 ± 6	0 ± 0	0 ± 0	0 ± 0	1 ± 0	477 ± 58
28	0 ± 0	146 ± 5	30 ± 4	0 ± 0	53 ± 7	0 ± 0	0 ± 0	0 ± 0	3 ± 0	434 ± 58
49	0 ± 0	119 ± 3	48 ± 10	0 ± 0	56 ± 7	0 ± 0	0 ± 0	0 ± 0	3 ± 0	347 ± 52
96	0 ± 0	59 ± 3	29 ± 4	0 ± 0	61 ± 6	0 ± 0	0 ± 0	0 ± 0	5 ± 0	260 ± 36
120	0 ± 0	33 ± 3	15 ± 4	0 ± 0	63 ± 5	0 ± 0	0 ± 0	0 ± 0	5 ± 0	217 ± 30

TABLE S4. Extracellular concentrations of carbon sources and metabolic endproducts during fermentation in ACSH, and ACSH supplemented with amino acids.

AFEX-pretreated Corn Stover Hydrolysate										
Hours	Glucose (mM)	Xylose (mM)	Pyruvate (mM)	Xylitol (mM)	Succinate (mM)	Lactate (mM)	Glycerol (mM)	Formate (mM)	Acetate (mM)	Ethanol (mM)
0	321 ± 22	206 ± 17	32 ± 8	0 ± 0	1.4 ± 0.5	0 ± 0	5.5 ± 1.2	10.5 ± 1.8	38.6 ± 5.4	2.7 ± 3.9
3	318 ± 23	209 ± 19	32 ± 9	0 ± 0	2.2 ± 0.7	0 ± 0	5.5 ± 1.3	8.3 ± 1.5	39.1 ± 5.2	17.4 ± 3.5
5	316 ± 14	208 ± 14	31 ± 8	0 ± 0	3.0 ± 0.3	0 ± 0	5.5 ± 1.6	8.5 ± 2.5	41.1 ± 6.6	22.6 ± 1.4
6	314 ± 16	210 ± 13	31 ± 7	0 ± 0	3.5 ± 1.1	0 ± 0	5.5 ± 1.4	6.9 ± 1.8	40 ± 4	30.4 ± 0
8	308 ± 23	214 ± 25	45 ± 29	0 ± 0	5.3 ± 1.4	0 ± 0	5.5 ± 1.5	5.2 ± 1.9	41.4 ± 4.2	44.5 ± 2.2
10	301 ± 21	213 ± 22	45 ± 28	0 ± 0	7.5 ± 2.0	0.4 ± 0.5	5.8 ± 2.0	2.4 ± 3.5	42.2 ± 4.3	60.5 ± 2.4
12	287 ± 26	210 ± 25	45 ± 28	0 ± 0	10.8 ± 2.3	0.4 ± 0.6	5.9 ± 1.7	0.8 ± 1.2	42.4 ± 4.9	79.8 ± 3.4
14	272 ± 20	204 ± 18	42 ± 24	0 ± 0	14.4 ± 2.8	0.4 ± 0.6	5.7 ± 1.6	0 ± 0	42.9 ± 4.9	96.2 ± 2.9
16	264 ± 23	205 ± 22	45 ± 27	0 ± 0	16.8 ± 2.7	0.3 ± 0.5	5.6 ± 1.5	0 ± 0	42.3 ± 3.3	113.1 ± 0.1
23	226 ± 24	200 ± 20	45 ± 26	0 ± 0	24.2 ± 3.2	0.4 ± 0.5	5.5 ± 1.5	0 ± 0	43.3 ± 4.5	162 ± 3
28	197 ± 24	200 ± 19	45 ± 25	0 ± 0	30.5 ± 3.7	0.4 ± 0.6	5.5 ± 1.5	0 ± 0	44.1 ± 4.3	202 ± 8
52	76 ± 24	190 ± 10	40 ± 15	0 ± 0	52.7 ± 5.5	0.5 ± 0.7	5.1 ± 1.5	0 ± 0	45 ± 4.8	316 ± 39
72	14 ± 19	191 ± 10	44 ± 16	0 ± 0	66.1 ± 7.2	0.6 ± 0.8	4.9 ± 1.4	0 ± 0	45 ± 4.3	370 ± 54
99	0 ± 0	192 ± 12	49 ± 20	0 ± 0	73.3 ± 3.1	0.7 ± 0.5	4.4 ± 0.5	0 ± 0	45.6 ± 4.1	321 ± 115
111	0 ± 0	192 ± 12	49 ± 20	0 ± 0	74.3 ± 3	0.6 ± 0.7	4 ± 0.8	0 ± 0	46 ± 4.1	286 ± 117
124	0 ± 0	193 ± 11	50 ± 20	0 ± 0	75.8 ± 2.3	0.7 ± 0.6	3.8 ± 0.7	0 ± 0	46.4 ± 3.6	252 ± 124
AFEX-pretreated Corn Stover Hydrolysate + 3X Amino Acids										
Hours	Glucose (mM)	Xylose (mM)	Pyruvate (mM)	Xylitol (mM)	Succinate (mM)	Lactate (mM)	Glycerol (mM)	Formate (mM)	Acetate (mM)	Ethanol (mM)
0	271 ± 0	399 ± 0	42 ± 0	0 ± 0	0 ± 0	52.1 ± 0	3.2 ± 0	0 ± 0	27 ± 0	0 ± 0
2	266 ± 0	399 ± 0	34 ± 0	0 ± 0	0.8 ± 0	52.1 ± 0	3.2 ± 0	0 ± 0	27 ± 0	6.5 ± 0
4	266 ± 1	419 ± 1	45.4 ± 0	0 ± 0	1.6 ± 0	52.1 ± 0	3.2 ± 0	0 ± 0	28.7 ± 0	15.1 ± 0
6	249 ± 1	392 ± 2	34 ± 0	0 ± 0	3.3 ± 0	51 ± 0	3.2 ± 0	0 ± 0	28.7 ± 0	30.3 ± 0
8	249 ± 0	412 ± 0	45.4 ± 0	0 ± 0	5 ± 0	51 ± 0	4.3 ± 0	0 ± 0	30.4 ± 0	43.4 ± 0
11	233 ± 2	412 ± 4	45.4 ± 1	0 ± 0	8.4 ± 0	51 ± 1	4.3 ± 0	0 ± 0	30.4 ± 0	80.3 ± 0
13	216 ± 2	399 ± 3	45.4 ± 1	0 ± 0	11.8 ± 0	49.9 ± 0	3.2 ± 0	0 ± 0	30.4 ± 0	115 ± 0
24	77 ± 0	359 ± 0	34 ± 0	0 ± 0	38.1 ± 0	46.6 ± 2	3.2 ± 0	0 ± 0	32.1 ± 0	347.2 ± 0
27	55 ± 0	366 ± 1	22.7 ± 0	0 ± 0	42.3 ± 0	43.2 ± 0	3.2 ± 0	0 ± 0	32.1 ± 0	390.7 ± 2
32	11 ± 0	366 ± 1	22.7 ± 0	0 ± 0	53.3 ± 0	43.2 ± 0	3.2 ± 0	0 ± 0	32.1 ± 0	455.8 ± 0
37	0 ± 0	366 ± 0	22.7 ± 0	0 ± 0	57.5 ± 0	41 ± 0	3.2 ± 0	0 ± 0	32.1 ± 0	477.5 ± 2
49	0 ± 0	353 ± 0	22.7 ± 0	0 ± 0	59.2 ± 0	39.9 ± 0	2.1 ± 0	0 ± 0	32.1 ± 0	455.8 ± 0
56	0 ± 0	346 ± 0	22.7 ± 0	0 ± 0	60.1 ± 0	39.9 ± 0	1 ± 0	0 ± 0	33.8 ± 0	434.1 ± 2
73	0 ± 0	339 ± 1	22.7 ± 0	0 ± 0	63.5 ± 0	38.8 ± 0	0 ± 0	0 ± 0	33.8 ± 0	390.7 ± 2
78	0 ± 0	333 ± 0	22.7 ± 0	0 ± 0	63.5 ± 0	38.8 ± 0	0 ± 0	0 ± 0	33.8 ± 0	390.7 ± 0
86	0 ± 0	326 ± 1	22.7 ± 0	0 ± 0	66.8 ± 0	37.7 ± 0	0 ± 0	0 ± 0	33.8 ± 0	347.2 ± 2
122	0 ± 0	306 ± 2	22.7 ± 0	0 ± 0	66.8 ± 0	36.6 ± 0	0 ± 0	0 ± 0	33.8 ± 0	325.5 ± 0
146	0 ± 0	299 ± 0	34 ± 0	0 ± 0	68.5 ± 0	35.5 ± 0	0 ± 0	0 ± 0	35.5 ± 0	303.8 ± 2

Table S5. NMR analysis of ACSH at beginning and end of fermentation.

Compound (mM)	ACSH 0 hours	ACSH 124 hours
Glucose	358.2	17.6
Xylose	198.5	176.4
Arabinose	33.7	16.4
Fructose	8.2	0.0
Galactose	6.4	5.0
Mannose	2.6	0.0
Malate	9.3	0.0
Acetamide	75.8	70.4
Acetate	33.3	41.5
Succinate	0.3	62.0
Ethanol	55.0	273.0
Betaine	0.7	0.6
Choline	0.7	0.7
Carnitine	0.2	0.1
Alanine	0.8	1.1