

September 17, 2010

Supplementary Tables for Banerjee et al. “Rapid optimization of enzyme mixtures for deconstruction of diverse pretreatment/biomass feedstock combinations”

Supplementary Table S1 Optimized proportions of the core set for a 1:1 yield of Glc and Xyl. MP, model prediction. Exptl., experimental results with the model prediction proportions.

Feedstock	Pre-treatment	Optimized enzyme proportions (%)						Glc yield (%)		Xyl yield (%)	
		CBH1	BG	EG1	BX	EX3	CBH2	MP	Exptl.	MP	Exptl.
corn stover	AFEX	30	4	29	4	29	4	41.7	42.0 ± 0.5	27.9	28.0 ± 1.1
	0.25% NaOH	46	4	38	4	4	4	41.7	41.2 ± 0.3	25.2	27.0 ± 0.5
	Alk. peroxide	41	4	23	4	24	4	58.5	58.5 ± 0.9	36.9	35.3 ± 1.5
switchgrass	AFEX	28	4	20	4	40	4	22.9	24.2 ± 1.0	25.7	27.0 ± 0.5
	0.25% NaOH	40	4	24	4	24	4	25.6	26.0 ± 0.5	25.1	26.2 ± 0.1
	Alk. peroxide	45	4	4	4	39	4	36.7	36.8 ± 0.5	38.2	37.0 ± 0.5
Miscanthus	AFEX	39	4	41	4	8	4	23.3	25.0 ± 1.2	30.1	32.0 ± 1.4
	0.25% NaOH	34	4	34	4	20	4	16.5	18.8 ± 1.3	21.6	21.0 ± 1.0
	Alk. peroxide	37	4	28	4	23	4	28.9	26.0 ± 0.5	39.9	42.0 ± 2.1
DDGS	AFEX	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	0.25% NaOH	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Alk. peroxide	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
poplar	AFEX	37	4	29	4	21	5	13.4	14.0 ± 0.2	18.4	18.0 ± 0.2
	0.25% NaOH	42	4	28	4	18	4	8.5	10.0 ± 0.5	20.6	22.0 ± 0.5
	Alk. peroxide	25	4	26	4	37	4	8.5	9.50 ± 0.2	24.8	22.9 ± 1.3

Supplementary Table S2 Monosaccharide and lignin composition of feedstocks used in this paper. Analyses were done prior to pretreatments. Data are % of dry weight. N.D., not determined.

Feedstock	Glc		Xyl	Ara	Man	Gal	Uronic acid	Lignin	total
	total Glc	starch							
corn stover	34.4	<1%	22.4	4.2	0.6	1.4	2.0	11.0	76
switchgrass	35	N.D.	25.3	2.0	0.3	1.0	2.0	16.7	82.3
Miscanthus	44	N.D.	19.0	1.8	0.1	0.4	1.8	24.1	90.9
DDGS	22	5.0	8.0	6.3	2.5	3.0	3.0	< 5	49.8
poplar	43.8	N.D.	14.9	0.6	3.9	1.0	N.D.	29.1	93.3

Supplementary Table S3 Experimental results for optimization of digestion of AP-treated DDGS with mixtures of four commercial enzyme preparations. Loading was fixed at 15 mg protein/g glucan.

Enzyme Proportion				% Glc yield	% Xyl yield
Accellerase 1000	Multifect-Xylanase	Multifect-Pectinase	Novozyme 188		
1.00	0.00	0.00	0.00	38.5 ± 0.0	8.6 ± 0.8
0.00	1.00	0.00	0.00	24.6 ± 0.0	17.7 ± 0.6
0.00	0.00	1.00	0.00	34.6 ± 0.1	26.5 ± 0.9
0.00	0.00	0.00	1.00	28.7 ± 0.0	8.1 ± 0.1
0.50	0.50	0.00	0.00	40.2 ± 0.0	17.6 ± 2.3
0.50	0.00	0.50	0.00	51.7 ± 0.1	28.5 ± 0.0
0.50	0.00	0.00	0.50	50.9 ± 0.2	16.5 ± 2.1
0.00	0.50	0.50	0.00	40.7 ± 0.1	26.0 ± 0.7
0.00	0.50	0.00	0.50	35.8 ± 0.0	15.3 ± 0.4
0.00	0.00	0.50	0.50	34.1 ± 0.1	22.6 ± 0.5
0.63	0.13	0.13	0.13	51.9 ± 0.2	21.3 ± 0.6
0.13	0.63	0.13	0.13	51.7 ± 0.1	23.7 ± 0.4
0.13	0.13	0.63	0.13	53.2 ± 0.3	29.4 ± 1.4
0.13	0.13	0.13	0.63	51.7 ± 0.2	24.5 ± 0.4
0.25	0.25	0.25	0.25	52.9 ± 0.2	25.9 ± 1.1

Supplementary Table S4 Proteomics analysis of the commercial enzyme product Novozyme 188. The JGI Protein ID's are from the Department of Energy Joint Genome Institute (<http://genome.jgi-psf.org/Aspni5/Aspni5.home.html>). All hits with more than two peptides and a probability >95% as calculated by Scaffold are shown. No proteins from species other than *Aspergillus niger* were identified.

JGI Protein ID	Annotation	Spectral counts
213597	Amyloglucosidase (Glucoamylase) (GH15)	448
56782	β -glucosidase (GH3)	90
47911	α -amylase	31
211032	Hypothetical tripeptidyl peptidase	21
56553	Hypothetical cell wall protein	20
201655	Aspergillopepsin A-like	15
205361	Hypothetical protein	12
44517	Cell wall glucanase (GH17)	11
50599	Hypothetical protein	10
35378	Hypothetical protein	8
54398	β -N-acetylhexosaminidase (GH20)	8
46979	Carboxypeptidase CpdS	8
214786	Hypothetical protein	7
52703	Hypothetical protein	7
55270	Exo- β -1,3-glucanase	6
57436	Endo- β -1,4-xylanase	6
196122	Cell wall glucanase Crf1 (GH16)	6
53033	1,3- β -glucanosyltransferase	5
37736	α -galactosidase A	3
214608	Endoglucanase A (GH5)	2
54865	Hypothetical protein	2

Supplementary Table S5 Statistical analysis for Glc optimization from pretreatment/substrate combinations (see Tables 2, 3, and Figures 1, 2, 3).

Feedstock	Pre-treatment	No. of components	p-value	F-value	R ²	Adjusted R ²	Predicted R ²	Difference between Adj and Pred R ²	Adequate Precision
corn stover	AFEX	6	<0.0001	21.6	0.93	0.88	0.84	0.04	17.8
		16	<0.0001	12.4	0.60	0.55	0.43	0.12	17.6
	Mild Base	6	<0.0001	15.6	0.90	0.84	0.76	0.08	14.5
	Alk. peroxide	6	<0.0001	13.6	0.89	0.82	0.74	0.08	14.5
16		<0.0001	13.9	0.60	0.55	0.43	0.12	19.4	
switchgrass	AFEX	6	<0.0001	13.0	0.88	0.82	0.80	0.02	14.3
	Mild Base	6	<0.0001	11.6	0.87	0.80	0.70	0.10	12.3
	Alk. peroxide	6	<0.0001	11.0	0.86	0.78	0.72	0.06	12.2
Miscanthus	AFEX	6	<0.0001	11.7	0.87	0.80	0.70	0.10	13.9
	Mild Base	6	<0.0001	9.0	0.84	0.74	0.70	0.04	11.1
	Alk. peroxide	6	<0.0001	8.2	0.83	0.73	0.60	0.13	11.1
DDGS	AFEX	6	<0.0001	14.0	0.90	0.83	0.75	0.08	15.0
		16	<0.0001	16.8	0.66	0.62	0.52	0.10	18.8
	Mild Base	6	<0.0001	19.0	0.92	0.87	0.82	0.05	14.7
	Alk. peroxide	6	<0.0001	11.6	0.87	0.80	0.67	0.13	15.1
poplar	AFEX	6	<0.0001	15.8	0.90	0.85	0.76	0.09	16.7
	Mild Base	6	<0.0001	6.0	0.77	0.63	0.60	0.03	9.2
	Alk. peroxide	6	<0.0001	22.6	0.77	0.73	0.72	0.01	17.4

Supplementary Table S6 Statistical analysis for Xyl optimization from pretreatment/substrate combinations (see Tables 2, 3, and Figures 1, 2, 3).

Feedstock	Pre-treatment	No. of components	p-value	F-value	R ²	Adjusted R ²	Predicted R ²	Difference between Adj and Pred R ²	Adequate Precision
corn stover	AFEX	6	<0.0001	17.0	0.91	0.86	0.75	0.11	15.5
		16	<0.0001	9.9	0.55	0.50	0.45	0.05	14.5
	Mild Base	6	<0.0001	26.3	0.94	0.90	0.88	0.02	24.9
	Alk. peroxide	6	<0.0001	16.2	0.90	0.85	0.78	0.07	16.5
		16	<0.0001	10.0	0.51	0.46	0.31	0.15	14.5
switchgrass	AFEX	6	<0.0001	17.1	0.91	0.86	0.81	0.05	14.5
	Mild Base	6	<0.0001	9.5	0.85	0.76	0.61	0.15	11.6
	Alk. peroxide	6	<0.0001	19.7	0.92	0.87	0.83	0.04	17.6
Miscanthus	AFEX	6	<0.0001	13.0	0.88	0.82	0.74	0.08	15.4
	Mild Base	6	<0.0001	7.2	0.81	0.70	0.68	0.02	10.3
	Alk. peroxide	6	<0.0001	9.7	0.85	0.76	0.66	0.10	12.0
DDGS	AFEX	6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		16	<0.0001	10.7	0.57	0.52	0.37	0.15	21.7
	Mild Base	6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Alk. peroxide	6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
poplar	AFEX	6	<0.0001	7.6	0.82	0.71	0.57	0.14	11.0
	Mild Base	6	<0.0001	32.3	0.95	0.92	0.88	0.04	18.7
	Alk. peroxide	6	<0.0001	28.4	0.94	0.91	0.87	0.04	19.4