Supplementary information to Municipal solid waste as carbon and energy source for *Escherichia coli*

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Acid-catalyzed hydrolysis of DSMSW

Solid-to-liquid ratio in acid-catalyzed hydrolysis

In order to select a solid-to-liquid ratio (w/v) to be used further in acid-catalyzed hydrolysis, the effect of DSMSW load on yield was investigated. Here, where the solid-to-liquid ratio was varied (Table SI-1). For D-glucose, the yield remained constant at approximately 0.30 g D-glucose/g DSMSW up to a solid-to-liquid load of 1:10 (w/v, ratio tested from 1:250 w/v). Increasing the load above 1:10 (w/v, ratio tested to 1:3 w/v) resulted in a drop in yield to approximately 0.23 g D-glucose/g DSMSW. For D-xylose, no significant change in yield was seen.

Table 1: Acid-catalyzed hydrolysis[a] of DSMSW carbohydrate content at different solid-to-liquid ratios. The corresponding yields (g sugar/g DSMSW) and concentrations (g L^{-1}) of produced D-glucose and D-xylose are shown.

		Glucose		**Xylose**	
DSMSW (mg)	**Ratio (g:mL)**	**Conc. (g L^{-1})**	**Yield (g/g)**	**Conc. (g L^{-1})**	**Yield (g/g)**
4	1:250	1.2	0.29	0.19	0.048
11	1:90	3.0	0.28	0.74	0.067
23	1:43	7.4	0.32	1.5	0.064
70	1:14	22	0.32	4.0	0.057
103	1:10	31	0.30	6.6	0.064
200	1:5	45	0.23	13	0.063
299	1:3	69	0.23	18	0.058

[a] Reaction conditions: 6 % sulfuric acid, 155 °C, 30 minutes.

Theoretical model for parameters temperature (121, 138 and 155 °C), concentration of sulfuric acid (2, 4, 6 % w/w) and time (10, 20, 30 minutes) varied in a full factorial design

Acid-catalyzed hydrolysis was performed according to the generated set-up from DOE where temperature (121, 138 and 155 °C), concentration of sulfuric acid (2, 4 6 % w/w) and time (10, 20, 30 minutes) were varied in a full factorial design, resulting in a total of 30 experiments (Table SI-2).

Table 2: Design of experiments for acid-catalyzed hydrolysis with the parameters temperature (121, 138 and 155 °C), concentration of sulfuric acid (2, 4, 6 % w/w) and time (10, 20, 30 minutes) varied in a full factorial design together with response values for concentration of D-glucose, D-xylose, HMF and furfural

Temp. (°C)	**Acid conc. (%)**	**Time (min)**	**Glucose (g L^{-1})**	**Xylose (g L^{-1})**	**HMF (mg L^{-1})**	**Furfural (mg L^{-1})**
121	2	10	3.2	2.5	50.5	1.6
138	2	10	9.0	2.9	206.1	2.1
155	2	10	14.3	4.6	346.1	2.4
121	4	10	6.5	2.8	123.1	1.8
138	4	10	14.1	3.7	309.1	2.6
155	4	10	22.6	7.2	711.9	4.7
121	6	10	10.9	3.7	201.8	2.1
138	6	10	20.6	5.3	545.4	3.8
155	6	10	24.0	6.8	998.3	10.6
121	2	20	7.3	3.2	160.6	2.0
138	2	20	13.4	4.2	297.9	2.5
155	2	20	23.9	6.2	1011.4	7.1
121	4	20	13.9	4.1	261.3	2.6
138	4	20	21.9	5.4	769.7	5.3
155	4	20	24.0	6.1	1106.7	14.2
121	6	20	10.1	3.4	179.2	1.9
138	6	20	21.4	6.0	823.8	8.4
155	6	20	25.4	6.7	942.9	17.0
121	2	30	9.7	3.6	239.7	2.6
138	2	30	22.1	5.5	805.8	4.6
155	2	30	22.8	6.0	940.0	8.0
121	4	30	17.2	4.6	367.8	2.8
138	4	30	27.7	7.8	911.7	4.7
155	4	30	24.7	7.2	1066.4	8.7
121	6	30	18.5	5.3	347.9	2.4
138	6	30	25.3	5.9	921.6	13.0
155	6	30	24.3	6.7	738.1	27.9
138	4	20	22.2	5.7	771.4	5.0
138	4	20	22.7	5.4	714.7	4.0
138	4	20	22.4	5.6	705.7	4.4

Within the first tested factor boundaries (i), the models (Equations SI-1-SI-4, T=tempe rature, C=concentration, t=time) show a considerable increase of the concentration of D-glucose and D-xylose at high acid concentration and at an increased time. The model indicates that increasing the parameter setting above the modelled parameter values (i), might further increase the concentration of both D-glucose and D-xylose. However, the models should not be used to predict a response value outside the set parameter settings. For HMF, a complex relationship is seen, where the highest concentrations are located at the far right center where high temperatures and a medium acid concentration gives the highest values. High concentration of sulfuric acid decreases the concentration of produced HMF. The chemical explanation for this could be that the HMF decomposes at high acid concentrations to levulinic acid and formic acid [1], which were not measured in this study. Leuvulinic acid and formic acid are both weak acids that also affect the growth of the bacterium *E. coli* negatively [2]. The concentration of furfural, increases with increased parameters setting of acid concentration and reaction time. The generated values are however low (approximately 20 mg L^{-1}).

$$\begin{aligned} Y_{[\text{glucose}]} &= 0.0315 * T + 0.0157 * C + 0.0194 * t \\ &-0.0152 * T^2 - 0.0128 * C^2 \ [gL^{-1}] \end{aligned} \tag{1}$$

$$\begin{aligned} Y_{[\text{xylose}]} &= 0.123 * T + 0.0582 * C + 0.0715 * t \\ &-0.0479 * C^2 - 0.0340 * T * t \ [gL^{-1}] \end{aligned} \tag{2}$$

$$\begin{aligned} Y_{[\text{HMF}]} &= 0.323 * T + 0.114 * C + 0.175 * t \\ &-0,156 * T^2 - 0.103302 * C^2 - 0.0758 * T * t \\ &-0.114 * C * t \ [gL^{-1}] \end{aligned} \tag{3}$$

$$\begin{aligned} Y_{[\text{furfural}]} &= 0.255 * T + 0.135 * C + 0.127 * t \\ &+0.114 * T * C + 0.0627 * T * t \ [gL^{-1}] \end{aligned} \tag{4}$$

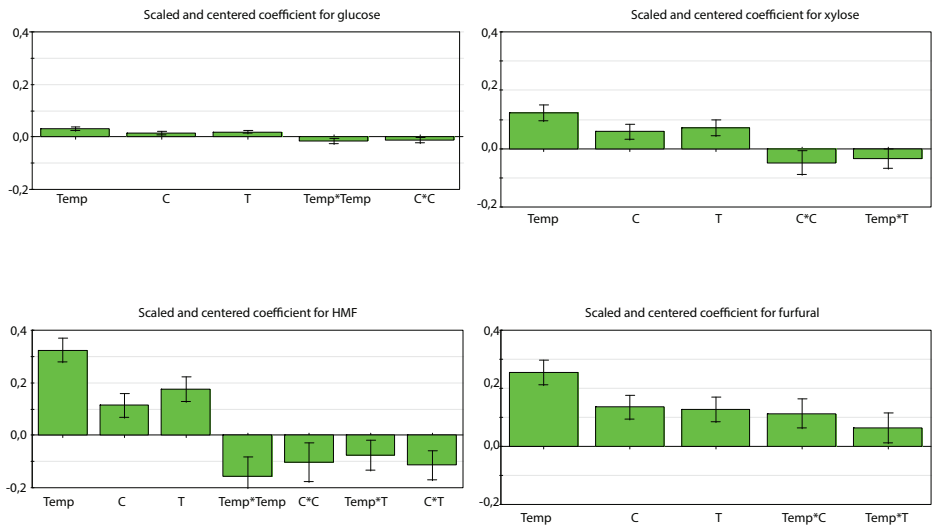


Figure 1: Visualization of the model coefficients together confidence interval (95 %) for DOE with the parameters temperature (121, 138 and 155 °C), concentration of sulfuric acid (2, 4, 6 % w/w) and time (10, 20, 30 minutes) varied in a full factorial design

Model validation

The generated models were evaluated according to the requirements for a good model presented by [3]; difference goodness of fit (R^2) - goodness of prediction (Q^2) <0.2, Q^2 >0.5, model validity >0.25, reproducibility >0.5 was evaluated together with an statistical analysis presented in an Analysis of variance (ANOVA) table. The summary plot, containing R^2, Q^2, Model validity and Reproducibility is visualized in Figure SI-2. The negative and low values for model validity is likely an artifact from very good replicates [3], as displayed in the ANOVA table (pure error, Table SI-3). The high values obtained for R^2 and Q^2 indicate a very good model. Also, the difference between R^2 and Q^2 is less than 0.2, and overfitting of the model is thus avoided.

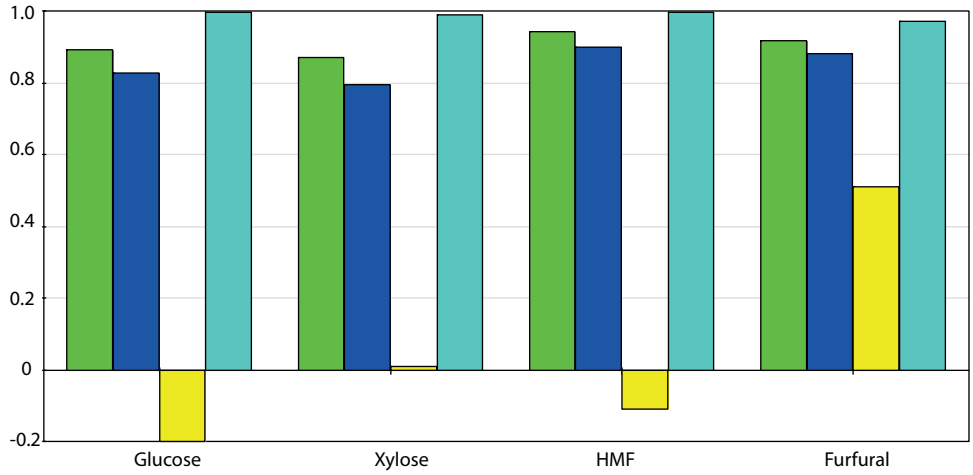


Figure 2: Summary plot describing model quality for the parameters temperature (121, 138 and 155 °C), concentration of sulfuric acid (2, 4, 6 % w/w) and time (10, 20, 30 minutes); goodness of fit (R^2, green), goodness of prediction (Q^2, blue), model validity (yellow) and reproducibility (cyan)

Table 3: Analysis of variance (ANOVA) evaluation for D-glucose, D-xylose, HMF and furfural for the parameters temperature (121, 138 and 155 °C), concentration of sulfuric acid (2, 4 6 % w/w) and time (10, 20, 30 minutes) varied in a full factorial design

Glucose						
	Degrees of freedom	**Sum of squares**	**Mean square**	**F**	**p**	**SD**
Total	29	0.0362	0.0012			0.0353
Corrected						
Regression	5	0.0323	0.0065	394,.176	0.0000	0.0804
Residual	24	0.0039	0.0002			0.0128
Lack of Fit	21	0.0039	0.0002	532.386	0.0040	0.0137
Pure Error	3	1.0526	0.3509			0.0019
	Q2 =	0.8270				
	R2 =	0.8920				
Xylose						
Total	29	0.5231	0.0180			0.1343
Corrected						
Regression	5	0.4543	0.0909	317.006	0.0000	0.3014
Residual	24	0.0688	0.0029			0.0535
Lack of Fit	21	0.0682	0.0032	170.094	0.0190	0.0570
Pure Error	3	0.0006	0.0002			0.0138
	Q2 =	0.7940				
	R2 =	0.8680				
HMF						
Total	29	337.946	0.1165			0.3414
Corrected						
Regression	7	318.676	0.4553	519.747	0.0000	0.6747
Residual	22	0.1927	0.0088			0.0936
Lack of Fit	19	0.1914	0.0101	237.927	0.0120	0.1004
Pure Error	3	0.0013	0.0004			0.0206
	Q2 =	0.8980				
	R2 =	0.9430				
Furfural						
Total	30	94.127	313.757			
Constant	1	919.604	919.604			
Total	29	216.659	0.0747			0.2733
Corrected						
Regression	5	198.954	0.3979	539.392	0.0000	0.6308
Residual	24	0.177047	0.0074			0.0859
Lack of Fit	21	0.170859	0.0081	394.455	0.1420	0.0902
Pure Error	3	0.0062	0.0021			0.0454
	Q2 =	0.8800				
	R2 =	0.9180				

Theoretical model for parameters for concentration of sulfuric acid (4, 6, 8 % w/w) and time (30, 40, 60 minutes) varied in a full factorial design

As the models (Equations SI-1-SI-4, T=temperature, C=concentration, t=time) for the first tested factor boundaries (i) indicated that an increased reaction time and acid concentration could increase the D-glucose and D-xylose levels, a second set of experiments was needed. A second experiment was performed where the parameter settings reaction time and concentration of sulfuric acid were expanded (ii). Within the new set of parameter boundaries, it is clear that the concentration of D-glucose is negatively affected by an increased acid concentration and reaction time (Figure SI-4, Equation SI-5). For D-xylose, only the reaction time has a significant effect, where a increased time results in an decreased concentration (Equation SI-6). The concentration of HMF decreased with an increased reaction time and acid concentration (Equation SI-7) while the opposite was true for furfural where the concentration increases (Equation SI-8). Also, from the raw data (Table SI-2, Table SI-4) it is clear that the overall concentration of furfural has increased within the expanded factor boundaries.

Thus, the first set of experiments (i) and the generated model includes the optimal parameter settings for high D-glucose and D-xylose values while minimizing the formation of by-products.

Table 4: Design of experiments for acid-catalyzed with extended factor boundaries; concentration of sulfuric acid (4, 6 and 8 %) and time (30, 40, 60 minutes) varied in a full factorial design together with response values for concentration of D-glucose, D-xylose, HMF and furfural. The temperature was held constant at 155 °C

Time (min)	**Acid conc. (%)**	**Glucose (g L^{-1})**	**Xylose (g L^{-1})**	**HMF (mg L^{-1})**	**Furfural (mg L^{-1})**
30	4	22.7	5.9	1326	20.2
45	4	20.0	5.6	919	26.0
60	4	19.7	5.2	802	33.7
30	6	20.2	5.9	752	30.9
45	6	18.5	5.5	511	34.6
60	6	18.7	5.1	493	39.4
30	8	20.7	5.9	608	39.8
45	8	17.9	5.0	448	47.1
60	8	15.7	4.1	396	56.0
45	6	20.6	5.7	567	35.0
45	6	20.8	6.4	598	46.9
45	6	21.3	5.4	641	43.4

The models for the second experiments with extended factor boundaries are presented below.

$$Y_{[\text{glucose}]} = -1.584 * t - 1.352 * C \ [gL^{-1}] \tag{5}$$

$$Y_{[\text{xylose}]} = -0.541 * t \ [gL^{-1}] \tag{6}$$

$$Y_{[\text{HMF}]} = -0.0979 * t - 0.159 * C + 0.0681 * C^2 \ [gL^{-1}] \tag{7}$$

$$Y_{[\text{furfural}]} = 6.360 * t + 10.493 * C \ [gL^{-1}] \tag{8}$$

Model validation

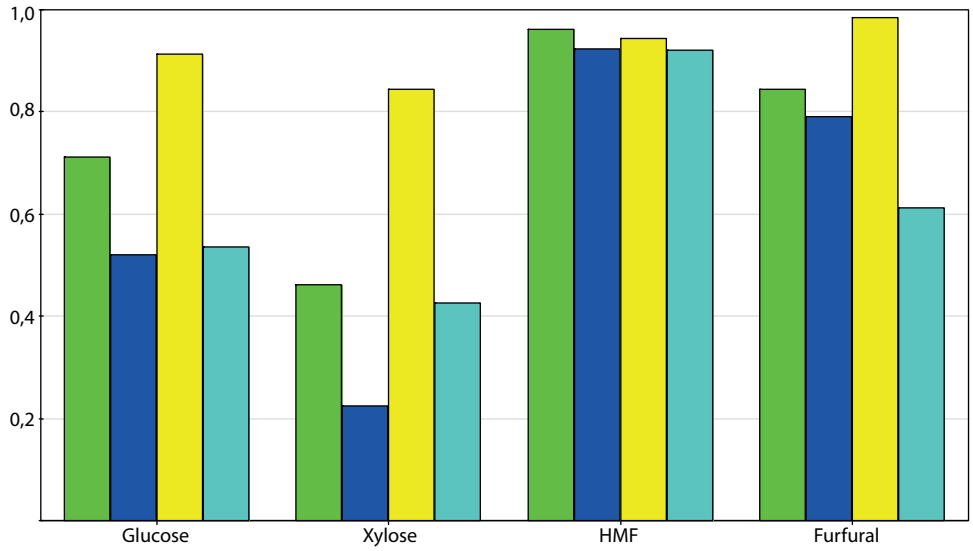


Figure 3: Summary plot describing model quality for the second set of experiments; goodness of fit (R^2. green), goodness of prediction (Q^2, blue), model validity (yellow) and reproducibility (cyan).

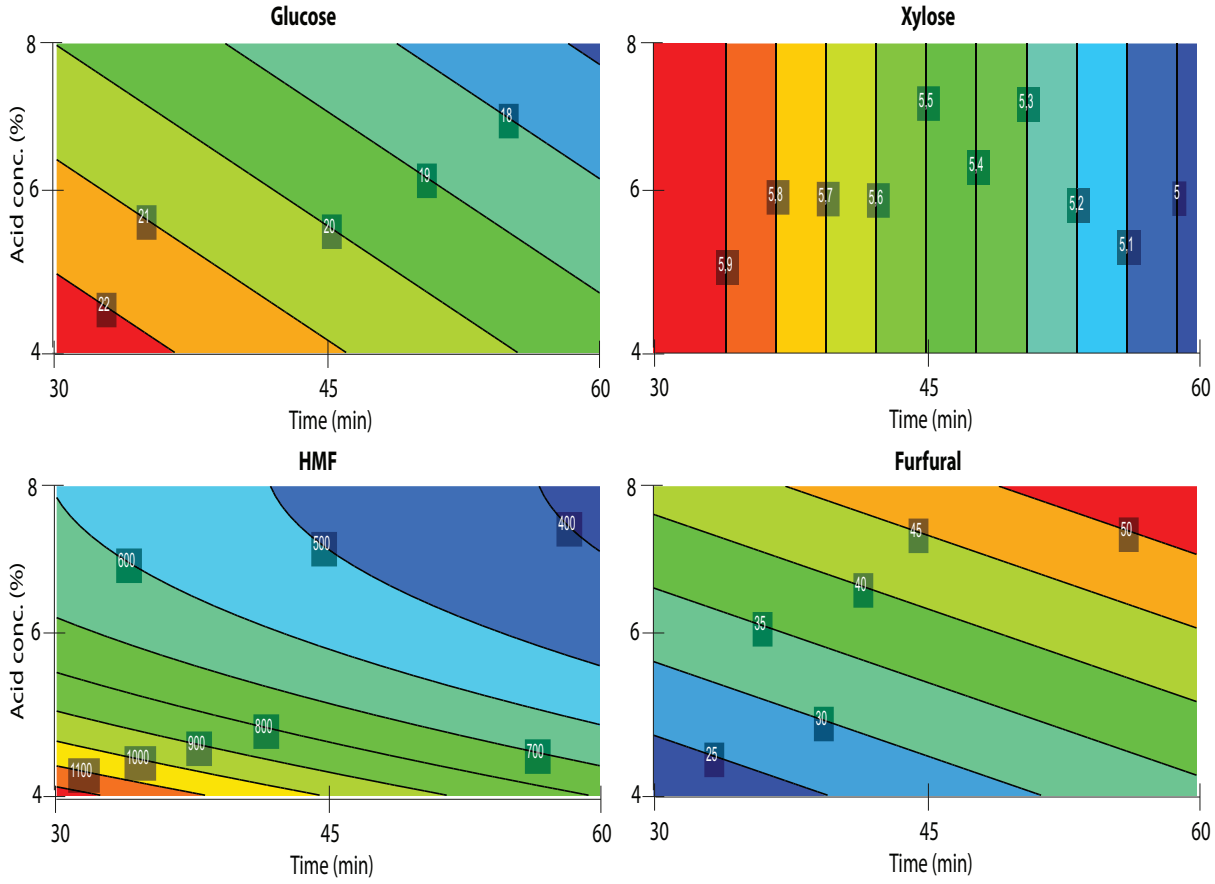
The models for D-glucose, HMF and furfural are within the requirements for a good model although the Q^2 value (0.521) and reproducibility (0.540) value for D-glucose are on the lower limit. For D-xylose, on the other hand, the R^2 and Q^2 was measured to 0.462 and 0.22, respectively. These values do not fulfill the requirements for a good model. The

model validity is however high for both D-glucose and D-xylose (0.913 and 0.842). This probably indicates that the concentration of both D-glucose and D-xylose are sensitive in this parameter setting area. The concentration of both D-glucose and D-xylose decrease with an increased time, which is probably a sign that D-glucose and D-xylose is converted into their decomposing products. A likely reason for the decrease in D-glucose and D-xylose is that the substrate availability is low. The models for HMF and furfural fulfill the requirements for a good model (Figure SI-3).

Table 5: Analysis of variance (ANOVA) evaluation for D-glucose, D-xylose, HMF and furfural in the second set of experiments.

	Degrees of freedom	**Sum of squares**	**Mean square**	**F**	**p**	**SD**
Glucose						
Total Corrected	11	366,118	332,835			182,438
Regression	2	26,029	130,145	11,068	0.004	360,756
Residual	9	105,828	117,587			108,438
Lack of Fit	6	595,034	0.991724	0.642239	0.706	0.995853
Pure Error	3	46,325	154,417			124,264
		Q2 =	0.521			
		R2 =	0.711			
Xylose						
Total Corrected	11	380,719	0.346108			0.58831
Regression	1	175,827	175,827	858,149	0.015	1,326
Residual	10	204,892	0.204892			0.452649
Lack of Fit	7	145,363	0.207662	104,653	0.535	0.455699
Pure Error	3	0.595284	0.198428			0.445453
	Q2 =	0.224				
	R2 =	0.462				
HMF						
Total Corrected	11	0.232826	0.021166			0.145485
Regression	3	0.223954	0.0746514	673,141	0	0.273224
Residual	8	0.00887201	0.001109			0.0333017
Lack of Fit	5	0.00378477	0.000756954	0.446383	0.799	0.0275128
Pure Error	3	0.00508724	0.00169575			0.0411795
	Q2 =	0.924				
	R2 =	0.968				
Furfural						
Total Corrected	11	1069.07	971,884			985,842
Regression	2	903,408	451,704	245,397	0	212,533
Residual	9	165,664	184,071			429,035
Lack of Fit	6	523,697	872,828	0.231123	0.94	295,437
Pure Error	3	113,294	377,647			61,453
	Q2 =	0.791				
	R2 =	0.845				

Figure 4: Representation of modeled response values (g L^{-1}) as contour plots for acid-catalyzed hydrolysis with extended parameter boundaries; concentration of sulfuric acid (4, 6 and 8 %) and time (30, 40, 60 minutes) varied in a full factorial design together with response values for concentration of D-glucose, D-xylose, HMF and furfural. Temperature held constant at 155 °C.



Optimization

To find the optimal parameter settings within the first set of experiments, a built in function in MODDE called Optimizer was used. For this, the results from Table SI-2 were used to set desired response levels, as the response targets must be in a realistic range for the Optimizer to work properly [3]. The response desirabilities were set as follows: D-glucose was maximized and a value of 30 g L^{-1} was desirable and 25 g L^{-1} was acceptable; D-xylose was maximized where 10 g L^{-1} was set as target and 5 g L^{-1} as the acceptable level. Toxic concentrations of HMF and furfural have reported to be 3 g L^{-1} and 2.3 g L^{-1}, receptively [4]. The values correspond to the amount of substance

that reduces bacterial (*Escherichia coli*) growth rate with 25 % when used alone. Here, a combination of the two substances will be present. Therefore, the desired limit was set to 1.15 g L^{-1} for each substance. As no information on a acceptable limit was presented, the target was set close to the maximum level, at a value of 0.8 g L^{-1}.

The Optimizer function works through interpolation to find a parameter combination that fulfills the requested response profile. A so called simplex function (Nelder-Mead function) are created at start values chosen within the investigated design region. The function then, simplified, measures the distance to the targets and tries to optimize the overall desirability. The success of the simplex function, is expressed as log(D), where a negative value indicates that all results are within the specified response limits. At a value of -10, all desirabilities are on the response target values.

Table 6: Simplex values for interpolation for model optimization where the parameters temperature (121, 138 and 155 °C), concentration of sulfuric acid (2, 4 6 % w/w) and time (10, 20, 30 minutes) varied in a full factorial design. The concentration of D-glucose and D-xylose are set to be maximized while HMF and furfural are to be minimized.

No.	**Temperature (°C)**	**Acid concentration (%)**	**Time (min)**	**Glucose (g L^{-1})**	**Xylose (g L^{-1})**	**HMF (mg L^{-1})**	**Furfural (mg L^{-1})**	**iter**	**log(D)**
1	144.79	5.75	29.99	27.37	6.94	980.84	13.54	202	-0.66
2	154.99	5.79	29.99	28.36	7.83	1014.81	25.15	127	-0.78
3	144.76	5.76	29.97	27.35	6.93	978.90	13.53	136	-0.66
4	155.00	6.00	30.00	28.21	7.77	967.62	26.79	25	-0.87
5	154.90	5.90	29.99	28.28	7.79	989.03	25.93	138	-0.83
6	142.18	5.94	29.65	26.58	6.65	891.48	11.84	34	-0.64
7	143.10	6.00	30.00	26.85	6.73	899.53	12.87	9	-0.68

Table 7: Second optimization with new start points centered around row 4 in the first optimizer test.

Temp. (°C)	**Acid conc. (%)**	**Time (min)**	**Glucose (g L^{-1})**	**Xylose (g L^{-1})**	**HMF (mg L^{-1})**	**Furfural (mg L^{-1})**	**iter**	**log(D)**
144.8	6	30	27.12	6.87	928.7	14.31	17	-0.70
143.6	5.98	29.3	26.77	6.72	909.07	12.95	24	-0.66
144.8	6	30	27.12	6.87	928.72	14.32	14	-0.71
155	6	30	28.21	7.77	967.62	26.79	25	-0.87
155	6	30	28.21	7.77	967.62	26.79	25	-0.87

Enzyme-catalyzed hydrolysis

In enzyme-catalyzed hydrolysis of DSMSW different combinations of three enzymes were applied; Cellic® Ctec 2 (A), Viscozyme® L (B) and Spirizyme® Achieve (C) (Figure SI-8).

Table 8: The concentration of D-glucose and D-xylose (g L^{-1}) produced from enzyme-catalyzed carbohydrate hydrolysis[a] after 2.5 and 5 hours using Cellic® Ctec 2 (A), Viscozyme® L (B) and Spirizyme® Achieve (C) in different combinations. The numbers are presented with the sugars from the enzyme solutions subtracted.

No.	**Enzyme addition**		**Glucose (g L^{-1})**		**Xylose (g L^{-1})**	
	0 h	2.5 h	2.5 h	5 h	2.5 h	5 h
1	-	-	1.9	2.2	2.5	2.6
2	A	-	9.6	14	4.9	6.3
3	B	-	17	19	3.7	3.9
4	C	-	23	24	3.1	3.4
5	A + C	-	25	28	5.5	6.1
6	B + C	-	21	22	2.2	2.4
7	A + B + C	-	26	26	5.2	5.4
8	A	C	11	27	6.1	5.8
9	A	B	10	20	5.6	5.6
10	B	A	14	21	2.2	4.5
11	B	C	16	26	3.0	3.8
12	C	A	21	26	2.3	5.6
13	C	B	22	25	3.1	4.7

[a] Reaction conditions: 100 µL Spirizyme® Achieve, 400 µL Viscozyme® and 250 µL Cellic® Ctec 2 in different combinations, solid-to-liquid ratio 1:10 (w/v), reaction volume adjusted to 10 mL with sodium acetate buffer (100 mM, pH 4.5), 50 °C, 150 rpm.

DSMSW/Enzyme load

To ensure that an appropriate amount of enzyme was applied, the enzyme-catalyzed reaction was supplemented with additional enzymes after 2.5 hours and 5 hours, respectively. No significant increase in D-glucose concentration was shown after 5 or 7.5 hours. In contrast, the concentration of D-xylose was increased.

Furthermore, additional amounts of DSMSW was added, to confirm that the enzymes remained active throughout the hydrolysis reaction. The addition of more DSMSW generated almost the double amount of D-glucose, when added after 2.5 and 5 hours. Thus, the enzymes were active throughout the reaction time of 7.5 hours. For D-xylose, an

increase is seen, although the yield seems to decrease. This indicated that the yield of D-xylose might be increased by the addition of more enzyme.

Table 9: Enzyme-catalyzed hydrolysis[a] of DSMSW using Cellic® Ctec 2 (A) and Spirizyme® Achieve (C) and the effect of enzyme or substrate (S) addition on concentration of D-glucose and D-xylose during time course. The numbers are presented with the sugars from the enzyme solutions subtracted.

Addition at			**Glucose (g L^{-1})**			**Xylose (g L^{-1})**		
0 h	2.5 h	5 h	2.5 h	5 h	7.5 h	2.5 h	5 h	7.5 h
S + A + C	A + C	-	26	28	30	5.7	8.3	9.0
S + A + C	-	A + C	26	28	29	5.2	5.5	8.4
S + A + C	S[b]		23	47	46	5.0	7.4	7.3
S + A + C	-	S[b]	26	28	50	5.4	5.8	7.7

[a] Reaction conditions: 125 µL Enzyme preparation A, 50 µL Enzyme preparation C, solid-to-liquid ratio 1:10 (w/v), reaction volume adjusted to 5 mL with sodium acetate buffer (100 mM, pH 4.5), 50 °C, 150 rpm.

[b] S= 0.5 g DSMSW

Exchanging buffer in enzyme-catalyzed hydrolysis

The acetic acid origin from the buffer used in the enzyme-catalyzed hydrolysis showed a negative impact on the growth of *E. coli* PPA652ara. Therefor, it was tested if the acetate buffer could be replaced without affecting the hydrolysis yield. Phosphate buffer (100 mM, pH 5.8) was chosen as the new buffer. The pH value was chosen as close as possible to the acetate buffer, without losing the buffer capacity.

Table 10: Enzyme-catalyzed hydrolysis[a] of DSMSW using actetate or phosphate as buffering agent.

D-Glucose				**D-Xylose**		
Time (h)	2	4	24	2	4	24
Acetate buffer [b]	19.8	20.8	24.0	5.6	5.8	6.9
Phosphate buffer [c]	22.4	22.7	21.6	6.6	6.6	6.7

[a] Reaction conditions: 125 µL Enzyme preparation A, 50 µL Enzyme preparation C, solid-to-liquid ratio 1:10 (w/v), reaction volume adjusted to 5 mL with buffer, 50 °C, 150 rpm.

[b] 100 mM, pH 4.5

[c] 100 mM, pH 5.8

From this, it is evident that the change of buffer did not greatly effect the hydrolysis efficiency. Therefor, phosphate buffer was used when the DSMSW hydrolyzate was to be applied in cultivations of *E.coli* PPA652ara.

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