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SUPPLEMENTARY MATERIAL TO

Examination and optimization of lignocellulolytic activity of *Stereum gausapatum* F28 on beechwood sawdust supplemented with molasses stillage

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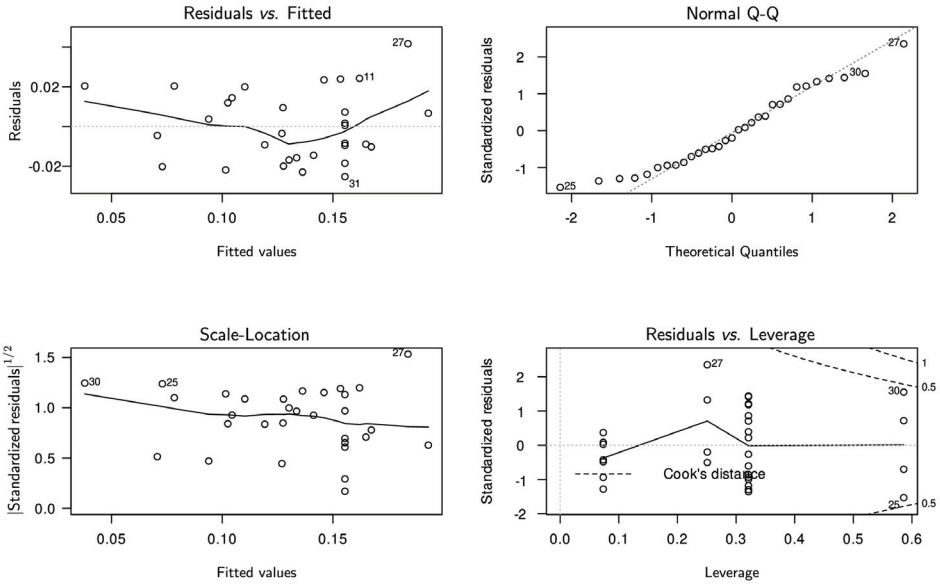

Fig. S-1. Diagnostic plots for examination of the generated laccase activity model.

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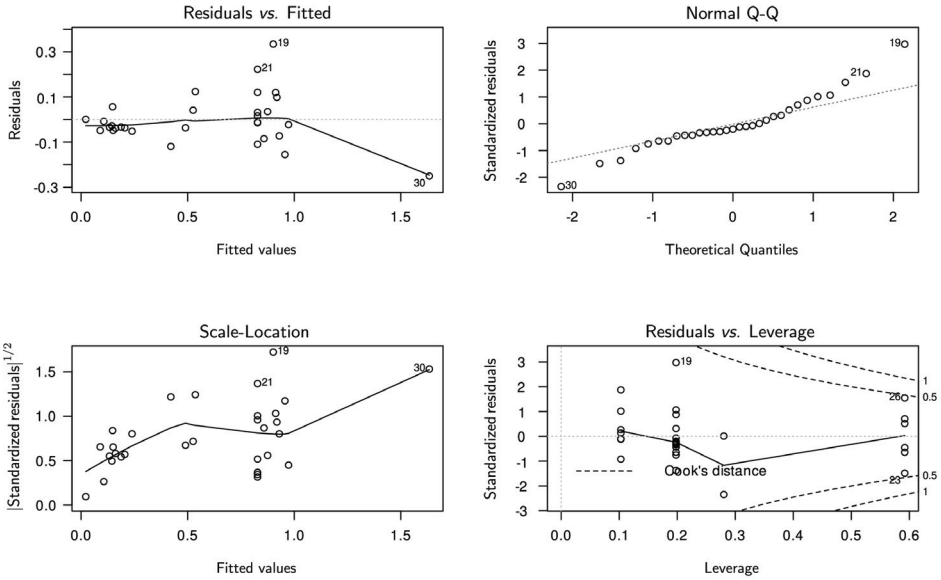

Fig. S-2. Diagnostic plots for examination of the generated MnP activity model.

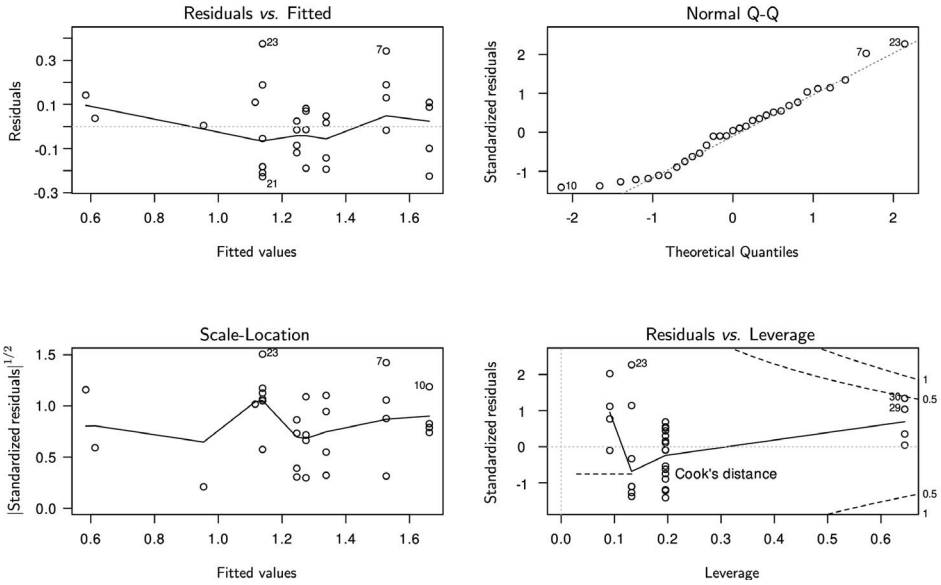

Fig. S-3. Diagnostic plots for examination of the generated cellulase activity model.

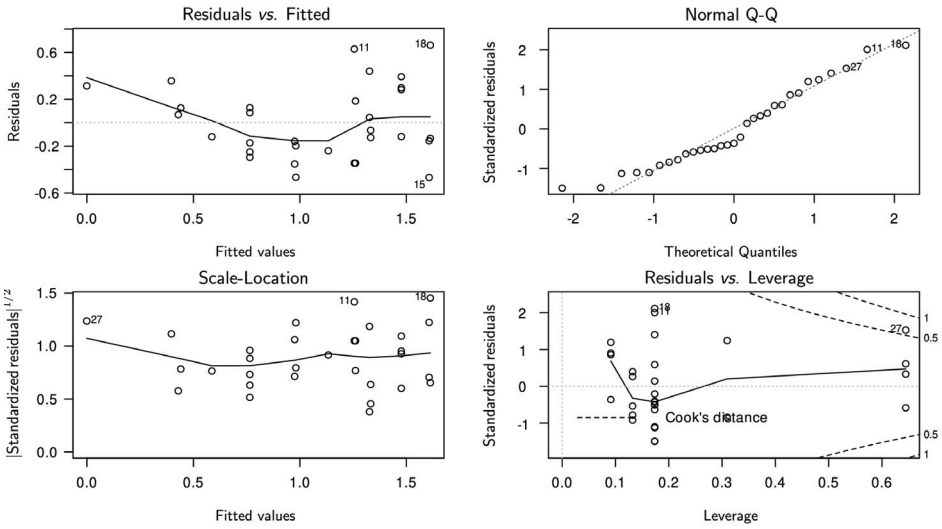

Fig. S-4. Diagnostic plots for examination of the generated xylanase activity model

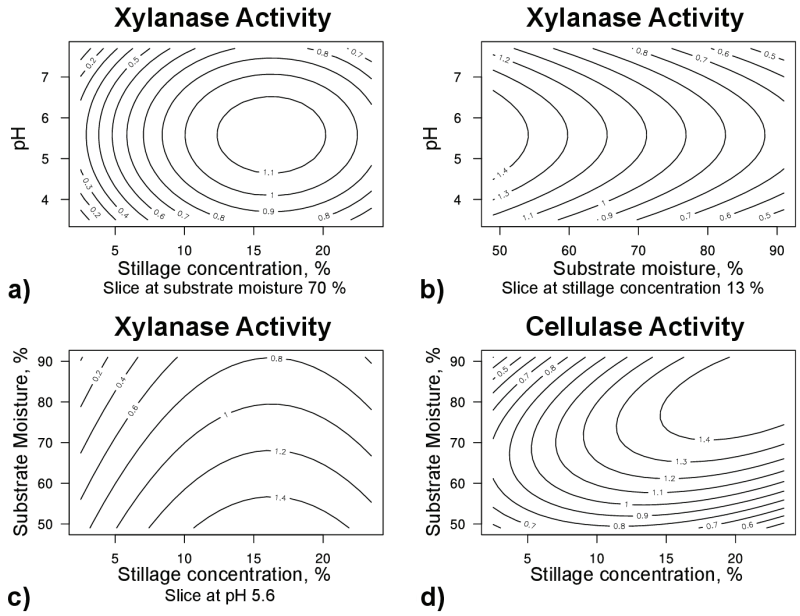

Fig. S-5. Contour plots showing the influence of a) pH and molasses stillage concentration (at a constant substrate moisture of 70 %), b) pH and substrate moisture (at a constant molasses stillage concentration of 13 %), and c) substrate moisture and MS concentration (at a constant temperature of 27 °C and pH 5.6) on the xylanase activity and the influence of substrate moisture and MS concentration on celluase activity (at constant temperature of 27 °C and pH 5.6).

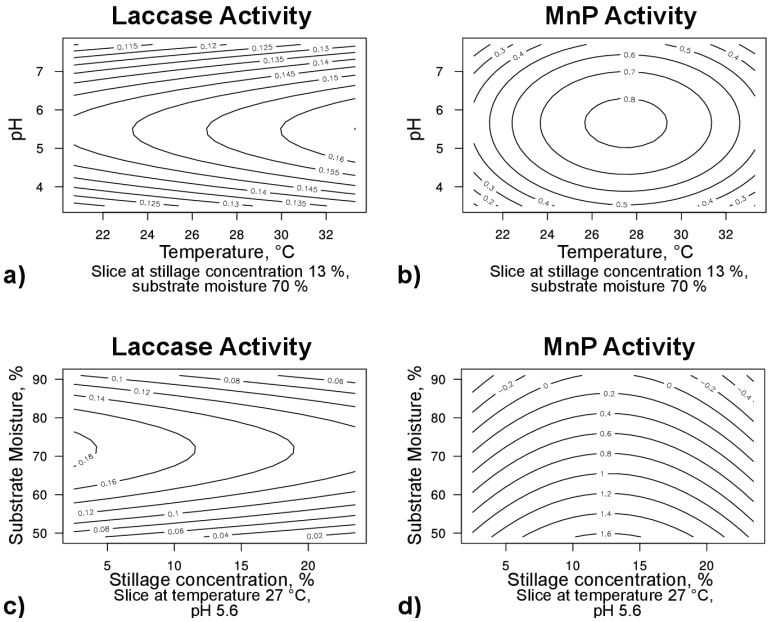

Fig. S-6. Influence of pH and temperature on a) laccase and b) MnP activity at a constant molasses stillage concentration of 13 % and a constant substrate moisture of 70 %, and influence of substrate moisture and MS concentration on c) laccase, d) MnP at a constant temperature and pH of 27 °C and 5.6, respectively.

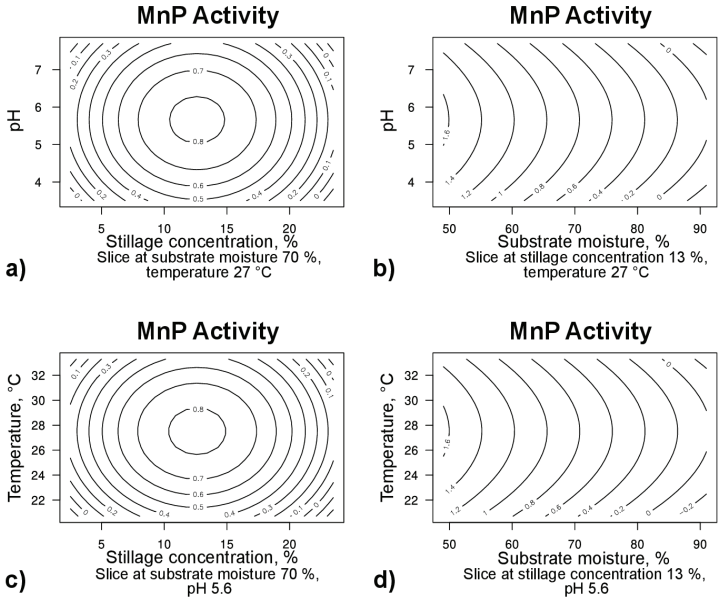

Fig. S-7. Contour plots showing the influence of a) pH and molasses stillage concentration, b) pH and substrate moisture, c) temperature and molasses stillage concentration, and d) temperature and substrate moisture on the laccase activity of *S. gausapatum* F28.

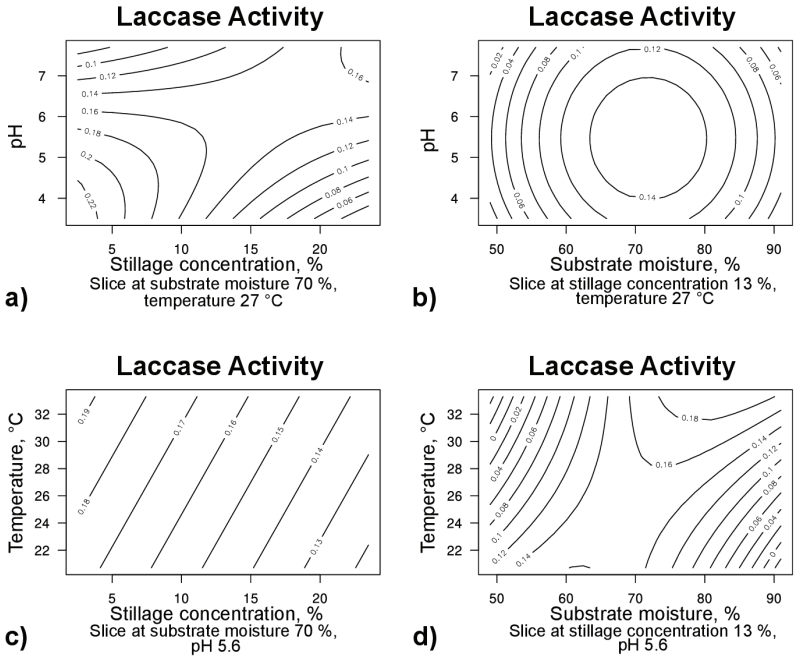

Fig. S-8. Contour plots showing the influence of a) temperature and molasses stillage concentration, b) pH and molasses stillage concentration, c) temperature and substrate moisture, and d) pH and substrate moisture on the MnP activity of *S. gausapatum* F28.

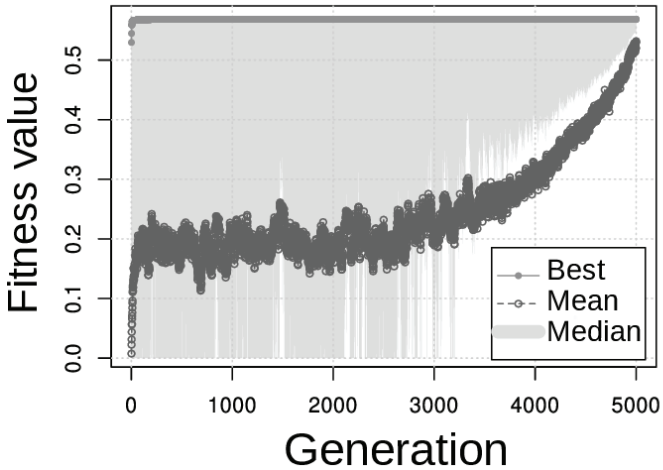

Fig. S-9. Genetic algorithm simulation.

TABLE S-I. Actual and coded values of variables.

Variable	Coded values of variables				
	-α	-1	0	1	α
	Real values of variables				
MS concentration, % (*A*)	2.5	8	13	18	23.5
Substrate moisture, % (*B*)	49	60	70	80	91
Temperature, °C (*C*)	21	24	27	30	33
pH (*D*)	3.5	4.6	5.6	6.6	7.7

TABLE S-II. Central composite design matrix with coded factor values and predicted and measured response values of laccase activity (LA) and MnP activity (MA) per dry substrate mass. The data are presented in standard order of experiments. Independent variables (factors) examined in this research were molasses stillage concentration (*A*), substrate moisture (*B*), incubation temperature (*C*), and pH value (*D*). (The corresponding general enzyme activities are given in Table S-IIA)

Runs	Factor				Experimental		Predicted	
	A	*B*	*C*	*D*	LA, U g^{-1}	MA, U g^{-1}	LA, U g^{-1}	MA, U g^{-1}
1	-1	-1	-1	-1	0.16	1.24	0.17	0.90
2	-2.098	0	0	0	0.23	0.19	0.18	0.24
3	1	-1	-1	-1	0.12	0.77	0.10	0.86
4	2.098	0	0	0	0.12	0.20	0.13	0.15
5	-1	1	-1	-1	0.13	0.10	0.14	0.13
6	0	-2.098	0	0	0.06	1.38	0.04	1.63
7	1	1	-1	-1	0.10	0.04	0.08	0.09
8	0	2.098	0	0	0.05	0.02	0.07	0.02
9	-1	-1	1	-1	0.12	0.80	0.13	0.96
10	0	0	-2.098	0	0.17	0.30	0.15	0.42
11	1	-1	1	-1	0.07	1.03	0.07	0.91
12	0	0	2.098	0	0.16	0.66	0.16	0.54
13	-1	1	1	-1	0.20	0.15	0.19	0.19
14	0	0	0	-2.098	0.14	0.45	0.13	0.49
15	1	1	1	-1	0.11	0.12	0.13	0.14
16	0	0	0	2.098	0.11	0.57	0.12	0.53
17	-1	-1	-1	1	0.11	1.02	0.13	0.92
18	0	0	0	0	0.13	0.86	0.16	0.83
19	1	-1	-1	1	0.11	0.91	0.14	0.87
20	0	0	0	0	0.16	1.05	0.16	0.83
21	-1	1	-1	1	0.08	0.10	0.10	0.15
22	0	0	0	0	0.14	0.95	0.16	0.83
23	1	1	-1	1	0.13	0.10	0.11	0.11
24	-1	-1	1	1	0.10	0.95	0.09	0.97
25	1	-1	1	1	0.11	0.86	0.10	0.93
26	-1	1	1	1	0.18	0.17	0.15	0.20
27	1	1	1	1	0.19	0.12	0.16	0.16
28	0	0	0	0	0.16	0.82	0.16	0.83
29	0	0	0	0	0.15	0.84	0.16	0.83
30	0	0	0	0	0.15	0.81	0.16	0.83
31	0	0	0	0	0.16	0.72	0.16	0.83

TABLE S-IIA. Central composite design matrix with coded factor values and predicted and measured response values of general laccase activity (LA) and MnP activity (MA). The data are presented in standard order of experiments. Independent variables (factors) examined in this research were molasses stillage concentration (A), substrate moisture (B), incubation temperature (C), and pH value (D).

Runs	Factors				Experimental		Predicted	
	A	*B*	*C*	*D*	LA, U L^{-1}	MA, U L^{-1}	LA, U L^{-1}	MA, U L^{-1}
1	-1	-1	-1	-1	14	110.1	14.91	80.33
2	-2.098	0	0	0	20.1	16.7	16.39	21.3
3	1	-1	-1	-1	10.6	68.9	9.31	76.45
4	2.098	0	0	0	11	18.2	11.31	13.17
5	-1	1	-1	-1	11.3	8.82	12.59	11.87
6	0	-2.098	0	0	5.2	123.3	3.38	145.57
7	1	1	-1	-1	8.8	3.68	6.98	7.99
8	0	2.098	0	0	4.7	2.04	6.5	1.96
9	-1	-1	1	-1	10.5	71.4	11.9	85.21
10	0	0	-2.098	0	15.1	26.9	13	37.52
11	1	-1	1	-1	5.9	92	6.3	81.33
12	0	0	2.098	0	13.9	58.8	14.69	47.75
13	-1	1	1	-1	17.8	13.8	17.21	16.75
14	0	0	0	-2.098	12.2	40.4	11.36	43.65
15	1	1	1	-1	10.1	10.4	11.6	12.87
16	0	0	0	2.098	9.8	50.5	10.62	46.82
17	-1	-1	-1	1	9.6	90.6	11.38	81.84
18	0	0	0	0	11.6	76.6	13.85	73.76
19	1	-1	-1	1	10.1	81.1	12.14	77.97
20	0	0	0	0	13.9	93.6	13.85	73.76
21	-1	1	-1	1	7.1	9.1	9.05	13.38
22	0	0	0	0	12.2	84.5	13.85	73.76
23	1	1	-1	1	11.6	8.8	9.82	9.5
24	-1	-1	1	1	8.7	84.7	8.37	86.73
25	1	-1	1	1	10.2	76.4	9.14	82.85
26	-1	1	1	1	15.8	15	13.67	18.26
27	1	1	1	1	16.6	11	14.44	14.38
28	0	0	0	0	14.5	72.7	13.85	73.76
29	0	0	0	0	13.1	75.2	13.85	73.76
30	0	0	0	0	13	72.5	13.85	73.76
31	0	0	0	0	14	64	13.85	73.76

TABLE S-III. Central composite design matrix with coded factor values and predicted and measured response values of cellulase (CMCase) enzyme activity (CA) and xylanase activity (XA) per dry substrate mass. The data are presented in standard order of the experiments. Independent variables (factors) examined in this research were molasses stillage concentration (A), substrate moisture (B), incubation temperature (C), and pH value (D). (The corresponding general enzyme activities are given in Table S-IIIA)

Runs	Factors				Experimental		Predicted	
	A	*B*	*C*	*D*	CA, U g^{-1}	XA, U g^{-1}	CA, U g^{-1}	XA, U g^{-1}
1	-1	-1	-1	-1	1.27	1.27	1.25	1.33
2	-2.098	0	0	0	0.65	0.31	0.61	0.00
3	1	-1	-1	-1	1.14	1.48	1.34	1.61
4	2.098	0	0	0	1.23	0.47	1.12	0.59
5	-1	1	-1	-1	1.09	0.51	1.27	0.98
6	0	-2.098	0	0	0.73	0.89	0.58	1.13
7	1	1	-1	-1	1.44	0.92	1.66	1.26
8	0	2.098	0	0	0.96	0.75	0.95	0.40
9	-1	-1	1	-1	1.13	1.21	1.25	1.33
10	0	0	-2.098	0	1.51	0.59	1.14	0.77
11	1	-1	1	-1	1.35	2.27	1.34	1.61
12	0	0	2.098	0	1.33	0.47	1.14	0.77
13	-1	1	1	-1	1.34	0.78	1.27	0.98
14	0	0	0	-2.098	0.96	0.57	1.14	0.44
15	1	1	1	-1	1.77	1.45	1.66	1.26
16	0	0	0	2.098	1.08	0.50	1.14	0.43
17	-1	-1	-1	1	1.16	1.37	1.25	1.33
18	0	0	0	0	0.96	0.89	1.14	0.77
19	1	-1	-1	1	1.20	1.45	1.34	1.61
20	0	0	0	0	0.91	0.51	1.14	0.77
21	-1	1	-1	1	1.26	0.62	1.27	0.98
22	0	0	0	0	0.93	0.85	1.14	0.77
23	1	1	-1	1	1.56	0.91	1.66	1.26
24	-1	-1	1	1	1.23	1.77	1.25	1.33
25	1	-1	1	1	1.39	1.14	1.34	1.61
26	-1	1	1	1	1.36	0.82	1.27	0.98
27	1	1	1	1	1.75	1.88	1.66	1.26
28	0	0	0	0	1.51	1.76	1.53	1.48
29	0	0	0	0	1.72	1.77	1.53	1.48
30	0	0	0	0	1.66	1.36	1.53	1.48
31	0	0	0	0	1.87	1.87	1.53	1.48

TABLE S-IIIA. Central composite design matrix with coded factor values and predicted and measured response values of general cellulase (CMCase) enzyme activity (CA) and xylanase activity (XA). The data are presented in standard order of experiments. Independent variables (factors) examined in this research were molasses stillage concentration (A), substrate moisture (B), incubation temperature (C), and pH value (D).

Runs	Factors				Experimental		Predicted	
	A	*B*	*C*	*D*	CA, U L^{-1}	XA, U L^{-1}	CA, U L^{-1}	XA, U L^{-1}
1	-1	-1	-1	-1	113.21	112.96	111.04	118.73
2	-2.098	0	0	0	57.95	27.92	54.65	0.061
3	1	-1	-1	-1	102.02	131.76	119.25	143.69
4	2.098	0	0	0	109.19	41.61	99.42	52.28
5	-1	1	-1	-1	96.77	45.89	113.61	87.42
6	0	-2.098	0	0	64.71	79.63	52.06	101.02
7	1	1	-1	-1	128.07	81.62	148.09	112.38
8	0	2.098	0	0	85.41	67.1	84.99	35.34
9	-1	-1	1	-1	100.44	107.42	111.04	118.73
10	0	0	-2.098	0	134.89	52.84	101.48	68.18
11	1	-1	1	-1	120.73	202.69	119.25	143.69
12	0	0	2.098	0	118.28	41.87	101.48	68.18
13	-1	1	1	-1	119.85	69.81	113.61	87.42
14	0	0	0	-2.098	85.23	50.49	101.48	39.27
15	1	1	1	-1	157.8	128.84	148.09	112.38
16	0	0	0	2.098	96.6	44.32	101.48	38.23
17	-1	-1	-1	1	103.42	122.25	111.04	118.24
18	0	0	0	0	85.23	79.53	101.48	68.18
19	1	-1	-1	1	106.56	129.36	119.25	143.19
20	0	0	0	0	81.21	45.89	101.48	68.18
21	-1	1	-1	1	112.34	55.5	113.61	86.93
22	0	0	0	0	82.78	75.77	101.48	68.18
23	1	1	-1	1	139.26	81.1	148.09	111.88
24	-1	-1	1	1	109.71	157.46	111.04	118.24
25	1	-1	1	1	123.53	101.47	119.25	143.19
26	-1	1	1	1	120.9	72.74	113.61	86.93
27	1	1	1	1	155.88	167.91	148.09	111.88
28	0	0	0	0	134.54	156.73	136.04	131.56
29	0	0	0	0	152.9	158.09	136.04	131.56
30	0	0	0	0	147.66	121.002	136.04	131.56
31	0	0	0	0	166.54	166.55	136.04	131.56

TABLE S-IV. Test of significance of regression coefficients and ANOVA analysis of laccase activity model

Laccase activity model ($\hat{y}Lacc$)
Test of significance of regression coefficients

	Estimate	Std. error	*t* value	Pr (>|t|)
(Intercept)	0.1554265	0.0055548	27.9804	$< 2.2\times10^{-16}$ ***
A	-0.0135700	0.0041145	-3.2981	0.0032768 **
B	0.0083493	0.0041145	2.0293	0.0547024 ★★
C	0.0045173	0.0041145	1.0979	0.2841154 ★
D	-0.0019613	0.0041145	-0.4767	0.6382893 ★★
A:D	0.0178854	0.0051225	3.4915	0.0020656 **
B:C	0.0213924	0.0051225	4.1762	0.0003924 ***
B^2	-0.0227264	0.0034906	-6.5107	1.504×10^{-6} ***
D^2	-0.0072959	0.0034906	-2.0902	0.0483768 *

Significant codes: *** – 0.001; ** – 0.01; * – 0.05; ★★ – 0.1; ★ – 1
Multiple $R^2 = 0.8042$, Adjusted $R^2 = 0.733$
F-statistic: 11.29 on 8 and 22 DF, *p*-value = 3.264×10^{-6}

Analysis of variance table

	Df	Sum square	Mean sqare	*F* value	Pr(>F)
FO (*A*, *B*, *C*, *D*)	4	0.0068971	0.0017243	4.1070	0.0123479
TWI (*A*, *D*)	1	0.0051182	0.0051182	12.1909	0.0020656
TWI (*B*, *C*)	1	0.0073221	0.0073221	17.4403	0.0003924
PQ (*B*, *D*)	2	0.0185985	0.0092992	22.1495	5.371×10^{-6}
Residuals	22	0.0092364	0.0004198		
Lack of fit	16	0.0084268	0.0005267	3.9032	0.0505638
Pure error	6	0.0008096	0.0001349		

TABLE S-V: Test of significance of regression coefficients and ANOVA analysis of the MnP activity model

Manganese peroxidase activity model ($\hat{y}MnP$)
Test of significance of regression coefficients

	Estimate	Std. error	*t* value	Pr(>|t|)
(Intercept)	0.8277466	0.0402834	20.5481	2.680×10^{-16} ***
A	-0.0217628	0.0252235	-0.8628	0.397156 ★
B	-0.3841607	0.0252235	-15.2303	1.662×10^{-13} ***
C	0.0273831	0.0252235	1.0856	0.288896 ★
D	0.0085008	0.0252235	0.3370	0.739159 ★
A^2	-0.1441678	0.0215645	-6.6854	8.062×10^{-7} ***
C^2	-0.0793849	0.0215645	-3.6813	0.001237 **
D^2	-0.0727536	0.0215645	-3.3738	0.002620 **

Significant codes: *** – 0.001; ** – 0.01; ★ – 1
Multiple $R^2 = 0.927$, Adjusted $R^2 = 0.9048$
F-statistic: 41.73 on 7 and 23 DF, *p*-value = 1.365×10^{-11}

Analysis of variance table

	Df	Sum square	Mean square	*F* value	Pr(>F)
FO(*A*, *B*, *C*, *D*)	4	3.6921	0.92303	58.4993	1.012×10^{-11}
PQ(*A*, *C*, *D*)	3	0.9166	0.30553	19.3638	1.728×10^{-6}
Residuals	23	0.3629	0.01578		
Lack of fit	17	0.2945	0.01733	1.5202	0.3158
Pure error	6	0.0684	0.01140		

TABLE S-VI: Test of significance of regression coefficients and ANOVA analysis of cellulase activity model

Cellulase activity model ($\hat{y}Cell$)
Test of significance of regression coefficients

(Intercept)	Estimate	Std. error	*t* value	Pr(>|t|)
	1.526680	0.053576	28.4957	$< 2.2\times10^{-16}$ ***
Block	-0.387872	0.066581	-5.8255	5.241×10^{-6} ***
A	0.119775	0.035617	3.3629	0.002583**
B	0.088090	0.035617	2.4733	0.020857 *
A:B	0.073709	0.044343	1.6623	0.10947 ★
A^2	-0.062333	0.030216	-2.0629	0.050105 ★★
B^2	-0.084038	0.030216	-2.7812	0.010372 *

Significant codes: *** – 0.001; ** – 0.01; * – 0.05; ★★ – 0.1; ★ – 1
Multiple $R^2 = 0.7303$, Adjusted $R^2 = 0.6629$
F-statistic: 10.83 on 6 and 24 DF, *p*-value = 7.61×10^{-6}

Analysis of variance table

	Df	Sum square	Mean square	*F* value	Pr(>F)
Block	1	1.06767	1.06767	33.9368	5.241×10^{-6}
FO (*A*, *B*)	2	0.54823	0.27412	8.7130	0.00143
TWI (*A*, *B*)	1	0.08693	0.08693	2.7631	0.10947
PQ (*A*, *B*)	2	0.34163	0.17081	5.4294	0.01134
Residuals	24	0.75505	0.03146		
Lack of fit	3	0.18246	0.06082	2.2306	0.11449
Pure error	21	0.57259	0.02727		

TABLE S-VII. Test of significance of regression coefficients and ANOVA analysis of xylanase activity model

Xylanase activity model ($\hat{y}Xyl$)
Test of significance of regression coefficients

	Estimate	Std. error	*t* value	Pr(>|t|)
(Intercept)	1.4763510	0.1040412	14.1901	3.613×10^{-13} ***
Block	-0.7112477	0.1292971	-5.5009	1.176×10^{-5} ***
A	0.1400227	0.0691661	2.0244	0.05419 ★★
B	-0.1756835	0.0691661	-2.5400	0.01797 *
D	-0.0027794	0.0691661	-0.0402	0.96828 ★
A^2	-0.1072887	0.0586785	-1.8284	0.07994 ★★
D^2	-0.0750504	0.0586785	-1.2790	0.21312 ★

Significant codes: *** – 0.001; * – 0.05; ★★ – 0.1; ★ – 1
Multiple $R^2 = 0.6538$, Adjusted $R^2 = 0.5673$
F-statistic: 7.555 on 6 and 24 DF, *p*-value = 0.0001249

Analysis of variance table

	Df	Sum square	Mean square	*F* value	Pr(>F)
Block	1	3.5901	3.5901	30.2596	1.176×10^{-5}
FO (*A*, *B*, *D*)	3	1.2519	0.4173	3.5172	0.03038
PQ (*A*, *D*)	2	0.5361	0.2681	2.2594	0.12617
Residuals	24	2.8474	0.1186		
Lack of fit	9	1.4259	0.1584	1.6719	0.18203
Pure error	15	1.4215	0.0948		

Table S-VIII. R^2-values before and after model reduction.

Model	R^2	
	Full model	Reduced model
Laccase	0.8753	0.8042
MnP	0.9459	0.927
Cellulase	0.8201	0.7303
Xylanase	0.78	0.6538

Table S-IX. Significance, R^2, lack of fit and AP-values of the generated laccase, MnP, cellulose, and xylanase activity models.

Model	R^2	Adjusted R^2	F-statistics	p-value	Lack of fit	AP (>4)
$\hat{y}Lacc$	0.8042	0.733	11.29	3.264×10^{-6}	0.051	14.05
$\hat{y}MnP$	0.927	0.9048	41.73	1.365×10^{-11}	0.3158	25.26
$\hat{y}Cell$	0.7303	0.6629	10.83	7.61×10^{-6}	0.11449	12.79
$\hat{y}Xyl$	0.6538	0.5673	7.555	0.0001249	0.18203	9.85

Second-order polynomial equations obtained for laccase, manganese-dependent peroxidase, cellulase, and xylanase enzyme activity:

$$\hat{y}Lacc = 0.155 - 0.014A + 0.008B + 0.005C - 0.002D + \\ + 0.018AD + 0.021BC - 0.023B^2 - 0.007D^2 \quad \text{(S1)}$$

$$\hat{y}MnP = 0.828 - 0.022A - 0.384B + 0.027C + 0.008D - 0.144A^2 - \\ - 0.079C^2 - 0.073D^2 \quad \text{(S2)}$$

$$\hat{y}Cell = 1.526 - 0.388\ \text{Block} + 0.119A + 0.088B + 0.074AB - \\ - 0.062A^2 - 0.084B^2 \quad \text{(S3)}$$

$$\hat{y}Xyll = 1.476 - 0.711\ \text{Block} + 0.140A - 0.176B - 0.003D - \\ -0.107A^2 - 0.075D^2 \quad \text{(S4)}$$