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for

Biocatalytic Treatment of Cresols in Aqueous Solution with Soybean Peroxidase

by

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***Pro forma* cost analysis related to SBP production**

In previous work (Steevensz et al.(2013), *Industrial Crops and Products* 48, 13– 18; http://dx.doi.org/10.1016/j.indcrop.2013.03.030), we found that several varieties of 'high-activity' dried hulls had 60 – 70 U/g of SBP activity, i.e.- 60 – 70 MU/ tonne (metric, 1000 kg) of hulls. Recent data (19 Dec.., 2025) from the USDA (ams_3511.pdf) has a range of hull prices, 115 – 200 USD/ton, i.e. 908 kg. If the average ton cost is taken as 160 USD and pro-rated to a metric tonne, the cost is 176 USD/ tonne. At 65 MU/tonne, this would come out to a hull cost of 2.71 USD/MU of peroxidase. In the important scenario whereby SBP is extracted from hulls at a soybean processing site and the hulls are not purchased but 'rented', say at 20% of the purchase price, this hull cost would come down fivefold, while the moist hulls are returned to the processor for feed formulation as usual. The calculation could go on with the optimized U/mL of any given substrate, eg, if 1.0 U/mL were needed for a 1.0 mM substrate of mol. wt. 108 g/mol (eg, cresol), 1 MU would treat 1000 L, i.e., remove 108 g of that substrate. It would have to be decided if $0.54 for SBP input, plus other processing costs, would be reasonable for the treatment of 1 m^3 of that particular wastewater. Additional costs would include the cost of peroxide (*ca*. $0.10)plus the amortized cost of capital (*ca*. $0.14) for a simple treatment plant (Mukherjee et al. (2019), *Chemosphere* 235, 365--372; https://doi.org/10.1016/j.chemosphere.2019.06.182).

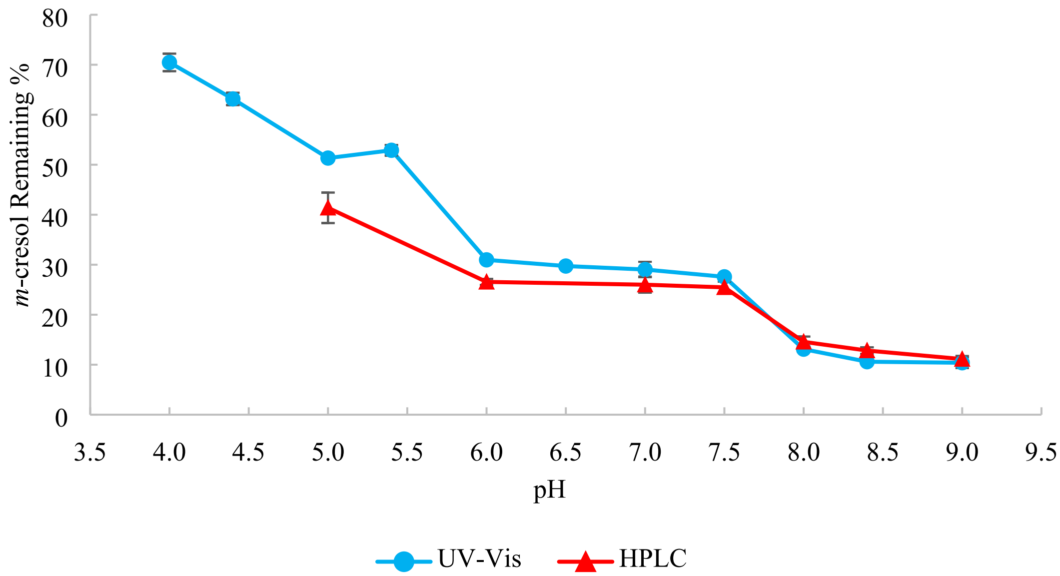

Figure S1 pH dependence of 1mM *m*-cresol concentration after 3 hours. Conditions: 0.6 U/mL SBP, 1.5 mM H2O2, pH 4.0-9.0, 40 mM buffer, room temperature 22±2 °C. Error bars represent standard deviations of triplicate samples.

Table S1 Treatment efficiencies of various substrates with SBP[a] in synthetic wastewater.

Substrate	Substrate concentration (mM)	SBP (U/mL)	Normalized SBP (U/mL/mM)	Reference[b]
o-cresol	1.0	0.70	0.7	This work
m-cresol	1.0	0.80	0.8	This work
p-cresol	1.0	0.30	0.3	This work
p-Anisidine	1.0	0.0018	0.0018	Kaur et al. 2021
CI Methyl Orange	0.5	0.0070	0.014	Kaur et al. 2021
Acid blue 113	1.0	1.52	1.52	Cordova-Villegas et al. 2019
Direct black 38	0.5	2.84	1.42	Cordova-Villegas et al. 2019
Diclofenac	0.1	0.15	1.5	Pishyar et al. 2025
Aceclofenac	0.1	0.6	6	Pishyar et al. 2025
Triclosan[c]	0.010	0.10	10	Li et al. 2016
Triclosan	0.017	0.05	2.9	Mashhadi et al. 2019b
Ioxynil	0.1	0.3	3.0	Zhang 2019
Bromoxynil	0.5	0.9	1.8	Zhang 2019
Nonylphenol	0.023	0.003	0.13	Mashhadi et al. 2019b
Octylphenol	0.024	0.002	0.083	Mashhadi et al. 2019b
Estrone 17β-Estradiol 17α-ethynylestradiol	0.039 each	0.12[d]	3.1	Mashhadi et al. 2019b
3-Aminoquinoline	1.0	4.5	4.5	Mashhadi et al. 2019a
Pyrrole	1.0	5.0	5	Mashhadi et al. 2021
1-Hydroxybenzotriazole	1.0	0.13	0.13	Mashhadi et al. 2021
3-Aminopyrazole	1.0	3.0	3.0	Mashhadi et al. 2021
Sulfamethoxazole	0.20	4.0	20	Mashhadi et al. 2019b; Sharifzadeh et al. 2024
Sulfamethoxazole[e]	0.20	0.10	0.5	Sharifzadeh et al. 2024
Sulfamerazine[e]	0.10	0.05	0.5	Sharifzadeh et al. 2024
4,4-Methylenedianiline	0.5	0.7	1.4	Mukherjee et al. 2019
4,4-Thiodianiline	0.5	0.15	0.3	Mukherjee et al. 2019
3-Hydroxycoumarin	0.5	0.002	0.004	Ziayee Bideh et al. 2021
2-Aminobenzoxazole	0.1	3.5	35	Ziayee Bideh et al. 2021

[a] Except as noted, all substrates were tested with the same preparation of SBP, activity assay by AAP-phenol coupling method; normalized SBP comparison devised by Kaur et al., 2021.
[b] References listed after Table S2.
[c] SBP preparation from Bio-Research Products, activity assay substrate was ABTS.
[d] These compounds treated together at the given concentrations, the SBP requirement is nominally 0.03–0.04 U/mL each.
[e] With mediator, hydroxybenzotriazole at 1.5 molar equivalent.

Table S2 Treatment initial kinetics of various SBP[a] substrates.

Substrate	SBP (U/ mL)	Half-life (min)	Normalized half-life (min.U/mL)	Reference
o-cresol	0.70	8.70 ± 0.07	6.09 ± 0.05	This work
m-cresol	0.80	1.8 ± 0.1	1.45± 0.09	
p-cresol	0.30	2.9 ± 0.3	0.86 ± 0.08	
Diclofenac	0.15	1.43 ± 0.01	0.22 ± 0.001	Pishyar et al. 2025
Aceclofenac	0.60	0.84 ± 0.05	0.49 ± 0.03	
Sulfamethoxazole	4.0	0.804 ± 0.003	3.22 ± 0.01	Sharifzadeh et al. 2024
Sulfamerazine	2.50	1.22 ± 0.01	3.05 ± 0.02	
Pyrrole	5.0	49 ± 3	246 ± 15	Mashhadi et al. 2021
Indole	0.45	26 ± 1	11.3 ± 0.5	
2-Aminothiazole	4.0	33.0 ± 0.6	132 ± 2	
2-Aminobenzothiazole	4.50	720 ± 0.01	3240 ± 0.04	
4-Aminoantipyrine	0.10	61 ± 1	6.1 ± 0.1	
Hydroxybenzotriazole	0.13	42 ± 2	4.97 ± 0.22	
2-Aminoimidazole	1.50	5.1 ± 0.2	7.7 ± 0.3	
2-Amino-benzimidazole	3.0	29.4 ± 0.6	88 ± 2	
3-Aminopyrazole	3.0	37 ± 1	108 ± 4	
3-Hydroxyquinoline	0.10	11.9 ± 0.6	1.19 ± 0.06	Mashhadi et al. 2019a
3-Aminoquinoline	4.50	15.0 ± 0.6	68 ± 3	
4,4'-Methylenebis (2-chlororaniline)	0.10	4.08 ± 0.02	0.408 ± 0.002	Mukherjee et al. 2020
4-Chloro-*o*-toluidine	0.009	11.5 ± 0.0	0.104 ± 0.0	
4,4'-Oxydianiline	0.01	1.80 ± 0.02	0.018 ± 0.0002	Mukherjee et al. 2018
p-Cresidine	0.04	12.4 ± 0.0	0.496 ± 0.0	
4,4’-Thiodianiline	0.15	0.513 ± 0.007	0.077 ± 0.001	Mukherjee et al. 2019
4,4'-Methylenedianiline	0.70	0.58 ± 0.10	0.40 ± 0.07	
Bromoxynil	0.90	3.00 ± 0.02	2.70 ± 0.02	Zhang 2019
Ioxynil	0.30	0.51 ± 0.01	0.153 ± 0.003	
3-Hydroxycoumarin	0.002	12.4 ±0.5	0.0257 ± 0.001	Ziayee Bideh et al. 2021
2-Aminobenzoxazole	3.50	129 ± 4	452 ± 14	
CI Acid Blue 113	1.50	8.8 ± 0.6	13 ± 1	Cordova Villegas et al. 2018
CI Direct Black 38	0.75	2.1 ± 0.2	1.57 ± 0.15	
p-Anisidine	0.0018	5.5 ± 0.8	0.0097 ± 0.0010	Kaur et al. 2021
CI Methyl Orange	0.007	7 ± 2	0.05 ± 0.01	

[a] all substrates tested with the same preparation of SBP; normalized half-life comparison devised by Ziayee Bideh et al., 2021.

References for Tables S1 and S2

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Table S3 Summary of mass spectrometry results for *o*-cresol + ACN filtrate (50:50)

Symbols	Molecular formula	Isotope Value (m/z)	Theoretical Mass (m/z) for H loss	Observed Mass (m/z)	Mass Accuracy (ppm)	Detected
M	C_7H_8O	108.0575	107.0502	107.0501	-1.29	*
M_2 - 2H	$C_{14}H_{14}O_2$	214.0994	213.0921	213.0920	-0.48	*
M_3 - 4H	$C_{21}H_{20}O_3$	320.1425	319.1340	319.1341	0.41	*
M_4 - 6H	$C_{28}H_{26}O_4$	426.1831	425.1758	425.1760	0.39	*
M_5 – 8H	$C_{35}H_{32}O_5$	532.2250	531.2177	531.2170	-1.31	*
M_6 - 10H	$C_{42}H_{38}O_6$	638.2668	637.2596	637.2585	-1.67	*
M_7 - 12H	$C_{49}H_{44}O_7$	744.3087	743.3014	743.3043	3.86	*
M_8 - 14H	$C_{56}H_{50}O_8$	850.3506	849.3433	849.3410	-2.7	*
M_9 - 16H	$C_{63}H_{56}O_9$	956.3924	955.3852	955.3830	-2.26	*
M_{10} - 18H	$C_{70}H_{62}O_{10}$	1062.4343	1061.4265			

Table S4 Summary of mass spectrometry results for *m*-cresol reaction filtrate

Symbols	Molecular formula	Isotope Value (m/z)	Theoretical Mass (m/z) for H loss	Observed Mass (m/z)	Mass Accuracy (ppm)	Detected
M	C_7H_8O	108.0575	107.0502	107.0502	-0.36	*
M_2 - 2H	$C_{14}H_{14}O_2$	214.0994	213.0921	213.0921	-0.02	*
M_3 - 4H	$C_{21}H_{20}O_3$	320.1425	319.1340			
M_4 - 6H	$C_{28}H_{26}O_4$	426.1831	425.1758			
M_5 - 8H	$C_{35}H_{32}O_5$	532.2250	531.2177			
M_6 - 10H	$C_{42}H_{38}O_6$	638.2668	637.2596			
M_7 - 12H	$C_{49}H_{44}O_7$	744.3087	743.3014			
M_8 - 14H	$C_{56}H_{50}O_8$	850.3506	849.3433			
M_9 - 16H	$C_{63}H_{56}O_9$	956.3924	955.3852			
M_{10} – 18H	$C_{70}H_{62}O_{10}$	1062.4343	1061.4265			

Table S5 Summary of mass spectrometry results for *m*-cresol + ACN filtrate (50:50)

Symbols	Molecular formula	Isotope Value (m/z)	Theoretical Mass (m/z) for H loss	Observed Mass (m/z)	Mass Accuracy (ppm)	Detected
M - H	C_7H_8O	108.0575	107.0502	107.0501	-1.29	*
M_2 - 2H	$C_{14}H_{14}O_2$	214.0994	213.0921	213.0921	-0.02	*
M_3 - 4H	$C_{21}H_{20}O_3$	320.1425	319.1340	319.1337	-0.84	*
M_4 - 6H	$C_{28}H_{26}O_4$	426.1831	425.1758	425.1757	-0.31	*
M_5 - 8H	$C_{35}H_{32}O_5$	532.2250	531.2177	531.2173	-0.75	*
M_6 - 10H	$C_{42}H_{38}O_6$	638.2668	637.2596	637.2591	-0.73	*
M_7 - 12H	$C_{49}H_{44}O_7$	744.3087	743.3014	743.3013	-0.17	*
M_8 - 14H	$C_{56}H_{50}O_8$	850.3506	849.3433	849.3422	-1.29	*
M_9 - 16H	$C_{63}H_{56}O_9$	956.3924	955.3852			
M_{10} - 18H	$C_{70}H_{62}O_{10}$	1062.4343	1061.4265			

Table S6 Summary of mass spectrometry results for *p*-cresol reaction filtrate

Symbols	Molecular formula	Isotope Value (m/z)	Theoretical Mass (m/z) for H loss	Observed Mass (m/z)	Mass Accuracy (ppm)	Detected
M	C_7H_8O	108.0575	107.0502	107.0500	-2.23	*
M_2 - 2H	$C_{14}H_{14}O_2$	214.0994	213.0921	213.0917	-1.89	*
M_3 - 4H	$C_{21}H_{20}O_3$	320.1425	319.1340	319.1338	-0.53	*
M_4 - 6H	$C_{28}H_{26}O_4$	426.1831	425.1758	425.1756	-0.55	*
M_5 - 8H	$C_{35}H_{32}O_5$	532.2250	531.2177	531.2177	0	*
M_6 - 10H	$C_{42}H_{38}O_6$	638.2668	637.2596	637.2589	-1.04	*
M_7 - 12H	$C_{49}H_{44}O_7$	744.3087	743.3014	743.3008	-0.84	*
M_8 - 14H	$C_{56}H_{50}O_8$	850.3506	849.3433	849.3425	-0.93	*
M_9 - 16H	$C_{63}H_{56}O_9$	956.3924	955.3852			
M_{10} - 18H	$C_{70}H_{62}O_{10}$	1062.4343	1061.4265			

Table S7 Summary of mass spectrometry results for *p*-cresol + ACN filtrate (50:50)

Symbols	Molecular formula	Isotope Value (m/z)	Theoretical Mass (m/z) for H loss	Observed Mass (m/z)	Mass Accuracy (ppm)	Detected
M	C_7H_8O	108.0575	107.0502	107.0502	-0.36	*
M_2 - 2H	$C_{14}H_{14}O_2$	214.0994	213.0921	213.0922	0.45	*
M_3 - 4H	$C_{21}H_{20}O_3$	320.1425	319.1340	319.1342	0.73	*
M_4 - 6H	$C_{28}H_{26}O_4$	426.1831	425.1758	425.1766	1.8	*
M_5 - 8H	$C_{35}H_{32}O_5$	532.2250	531.2177	531.2183	1.13	*
M_6 - 10H	$C_{42}H_{38}O_6$	638.2668	637.2596	637.2593	-0.41	*
M_7 - 12H	$C_{49}H_{44}O_7$	744.3087	743.3014	743.3007	-0.94	*
M_8 - 14H	$C_{56}H_{50}O_8$	850.3506	849.3433	849.3423	-1.17	*
M_9 - 16H	$C_{63}H_{56}O_9$	956.3924	955.3852	955.3836	-1.63	*
M_{10} - 18H	$C_{70}H_{62}O_{10}$	1062.4343	1061.4265			