

Supplementary Information for

Manganese Peroxidase-Catalyzed Treatment of Aqueous Chlorophenols

by S. Narimannejad, N. Biswas, E.E. Hood and K.E. Taylor

Table S1 Treatment efficiencies of various substrates with SBP^a in synthetic wastewater. (Narimannejad, 2025 and Haghghatnama et al., 2026).

Substrate	Substrate concentration (mM)	SBP (U/mL)	Normalized SBP (U/mL/mM)	Reference ^b
<i>o</i> -cresol	1.0	0.70	0.7	
<i>m</i> -cresol	1.0	0.80	0.8	Haghghatnama et al. 2026
<i>p</i> -cresol	1.0	0.30	0.3	
Phenol	1.0	0.90	0.9	
2-Chlorophenol	1.0	0.23	0.23	
3-Chlorophenol	1.0	0.65	0.65	(Caza et al., 1999)
4-Chlorophenol	1.0	0.20	0.2	
2,4-Dichlorophenol	1.0	0.08	0.08	
Bisphenol A	0.5	0.90	0.45	
<i>p</i> -Anisidine	1.0	0.0018	0.0018	
CI Methyl Orange	0.5	0.0070	0.014	Kaur et al. 2021
Acid blue 113	1.0	1.52	1.52	
Direct black 38	0.5	2.84	1.42	Cordova-Villegas et al. 2019
Diclofenac	0.1	0.15	1.5	
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	
Bromoxynil	0.5	0.9	1.8	Zhang 2019
Nonylphenol	0.023	0.003	0.13	
Octylphenol	0.024	0.002	0.083	
Estrone				Mashhadi et al. 2019b
17β-Estradiol	0.039 each	0.12 ^d	3.1	
17α-ethynylestradiol				
3-Aminoquinoline	1.0	4.5	4.5	Mashhadi et al. 2019a
Pyrrole	1.0	5.0	5	
1-Hydroxybenzotriazole	1.0	0.13	0.13	Mashhadi et al. 2021
3-Aminopyrazole	1.0	3.0	3.0	
Sulfamethoxazole	0.20	4.0	20	Mashhadi et al. 2019b; Sharifzadeh et al. 2024
Sulfamethoxazole ^c	0.20	0.10	0.5	Sharifzadeh et al. 2024
Sulfamerazine ^c	0.10	0.05	0.5	Sharifzadeh et al. 2024
4,4-Methylenedianiline	0.5	0.7	1.4	
4,4-Thiodianiline	0.5	0.15	0.3	Mukherjee et al. 2019
3-Hydroxycoumarin	0.5	0.002	0.004	
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. (Haghighatnama et al., 2026).

Substrate	SBP (U/mL)	Half-life (min)	Normalized half-life (min.U/mL)	Reference	
<i>o</i> -cresol	0.70	8.70 ± 0.07	6.09 ± 0.05	Haghighatnama et al. 2026	
<i>m</i> -cresol	0.80	1.8 ± 0.1	1.45 ± 0.09		
<i>p</i> -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	
Acceclofenac	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- <i>o</i> -toluidine	0.009	11.5 ± 0.0	0.104 ± 0.0	Mukherjee et al. 2018	
4,4'-Oxydianiline	0.01	1.80 ± 0.02	0.018 ± 0.0002		
<i>p</i> -Cresidine	0.04	12.4 ± 0.0	0.496 ± 0.0	Mukherjee et al. 2019	
4,4'-Thiodianiline	0.15	0.513 ± 0.007	0.077 ± 0.001		
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	Ziayee Bideh et al. 2021	
3-Hydroxycoumarin	0.002	12.4 ± 0.5	0.0257 ± 0.001		
2-Aminobenzoxazole	3.50	129 ± 4	452 ± 14	Cordova Villegas et al. 2018	
CI Acid Blue 113	1.50	8.8 ± 0.6	13 ± 1		
CI Direct Black 38	0.75	2.1 ± 0.2	1.57 ± 0.15		
<i>p</i> -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

- Caza, N., Bewtra, J., Biswas, N., & Taylor, K. (1999). Removal of phenolic compounds from synthetic wastewater using soybean peroxidase. *Water Research*, 33(13), 3012-3018.
- Cordova Villegas LG, Mazloun S, Taylor KE, Biswas N (2018) Soybean peroxidase-catalyzed treatment of azo dyes with or without Fe^o pretreatment. *Water Environ Res* 90(8):675–684. <https://doi.org/10.2175/106143017X15131012153149>
- Cordova-Villegas LG, Cordova-Villegas AY, Taylor KE, Biswas N (2019) Response surface methodology for optimization of enzyme-catalyzed azo dye decolorization. *J Environ Eng* 145(5):04019026. [https://doi.org/10.1061/\(ASCE\)EE.1943-7870.00015](https://doi.org/10.1061/(ASCE)EE.1943-7870.00015)
- Haghighatnama M, Narimannejad S, Biswas N, Taylor KE (2026) Biocatalytic treatment of cresols in aqueous solution with soybean peroxidase, *RSC Adv.*, 16, pp. 5079–5087. <https://doi.org/10.1039/d5ra08019c>
- Kaur A, Taylor KE, Biswas N (2021) Soybean peroxidase-catalyzed degradation of a sulfonated dye and its azo-cleavage product. *J Chem Technol Biotechnol* 96(2):423-430. <https://doi.org/10.1002/jctb.6555>
- Li J, Peng J, Zhang Y, Ji Y, Shi H, Mao L, Gao S (2016) Removal of triclosan via peroxidases-mediated reactions in water: reaction kinetics, products and detoxification. *J Hazard Mater* 310:152–160. <https://doi.org/10.1016/j.jhazmat.2016.02.037>
- Mashhadi N, Taylor KE, Biswas N, Meister P, Gauld JW (2019a) Oligomerization of 3-substituted quinolines by catalytic activity of soybean peroxidase as a wastewater treatment. Product formation and computational studies. *Chem Eng J* 364:340–348. <https://doi.org/10.1016/J.CEJ.2019.01.184>
- Mashhadi N, Taylor KE, Jimenez N, Varghese ST, Levi Y, Lonergan C, Lebeau E, Lamé M, Lard E, Biswas N (2019b) Removal of Selected Pharmaceuticals and Personal Care Products from Wastewater using Soybean Peroxidase. *Environ Manage* 63:408–415. doi: 10.1007/s00267-018-01132-9
- Mashhadi N, Taylor KE, Biswas N, Meister P, Gauld JW (2021) Biocatalytic oligomerization of azoles; experimental and computational studies. *Environ Sci: Water Res Technol* 7(6):1103-1113. <https://doi.org/10.1039/D1EW00079A>
- Mukherjee D, Taylor KE, Biswas N (2018) Soybean peroxidase-induced treatment of dye-derived arylamines in water. *Water Air Soil Pollut* 229:283. <https://doi.org/10.1007/s11270-018-3936-5>
- Mukherjee D, Bhattacharya S, Taylor KE, Biswas N (2019) Enzymatic treatment for removal of hazardous aqueous arylamines, 4,4'-methylenedianiline and 4,4'-thiodianiline. *Chemosphere* 235:365-372. <https://doi.org/10.1016/j.chemosphere.2019.06.182>
- Mukherjee D, Taylor KE, Biswas N (2020) Soybean peroxidase-catalyzed oligomerization of arylamines in water: optimization, kinetics, products and cost. *J Environ Chem Eng* 8(4):103871. <https://doi.org/10.1016/j.jece.2020.103871>
- Narimannejad S (2025). Treatment of Synthetic Wastewater Catalyzed by Manganese Peroxidase and Soybean Peroxidase University of Windsor (Canada).
- Pishyar S, Narimannejad S, Taylor KE, Biswas N (2025) Enzymatic removal of diclofenac and aceclofenac from water by soybean peroxidase. *Molecules* 30(8):1817. <https://doi.org/10.3390/molecules30081817>
- Sharifzadeh M, Narimannejad S, Taylor KE, Biswas N (2024) Enzymatic removal of sulfa drugs sulfamethoxazole and sulfamerazine from synthetic wastewater by soybean peroxidase. *Environ Sci Pollut Res* 31(56):64760–64771. <https://doi.org/10.1007/s11356-024-35578-8>

Zhang X (2019) Enzymatic treatment of selected pesticides in aqueous system. MASc thesis, University of Windsor, Windsor. <https://scholar.uwindsor.ca/etd/7857>

Ziayee Bideh N, Mashhadi N, Taylor KE, Biswas N (2021) Elimination of selected heterocyclic aromatic emerging contaminants from water using soybean peroxidase. *Environ Sci Pollut Res* 28:37570-37579. <https://doi.org/10.1007/s11356-021-13403-w>

Table S3 Relative area ratios (%) for oligomers in enzymatic reaction mixture, directly or in aqueous-organic extract

	2-CP, areas as % of total		3-CP, areas as % of total		4-CP, areas as % of total		2,4-DCP, areas as % of total	
	Aqueous	Aq-organic	Aqueous	Aq-organic	Aqueous	Aq-organic	Aqueous	Aq-organic
dimer	54.7	7.8	27.6	16	77.5	11	-	73.6
dimer(-Cl-H)	-	-	4.9	3.3	11.7	0.1	-	0.1
dimer(-Cl+H)	5.8	-	1.1	-	7.8	0.1	-	1.5
trimer	39.4	38.7	8.4	18.3	-	25	-	16.8
tetramer	-	22.4	18.3	32.2	2.9	41.7	-	5.7
pentamer	-	17.8	18.6	14.8	-	17.8	-	0.55
hexamer	-	11.9	11.5	8.5	-	3.4	-	-
heptamer	-	1.3	6.2	4.1	-	0.35	-	-
octamer	-	-	3.2	2	-	0.4	-	-
nonamer	-	-	-	0.5	-	-	-	-
decamer	-	-	-	0.2	-	-	-	-

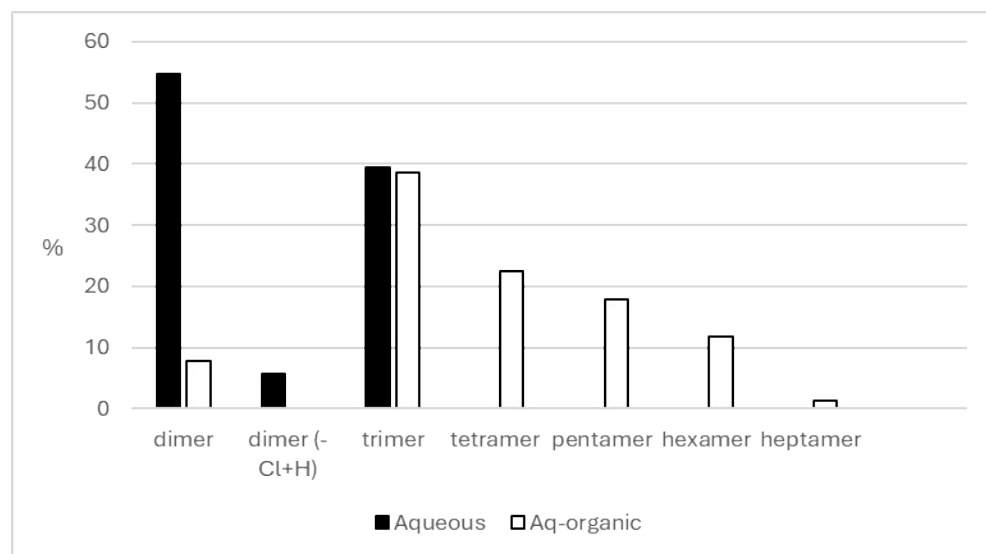


Figure S1 Oligomer distribution for 2-CP in aqueous reaction mixture and in aqueous-organic extract. The enzymatic reaction mixture was filtered directly for the aqueous sample; for the extract, the reaction mixture was diluted 4-fold with acetonitrile, vortexed for an hour, then filtered. MS peak areas (all isomers) are expressed as a % of the total set of areas for that solvent (Table S3).

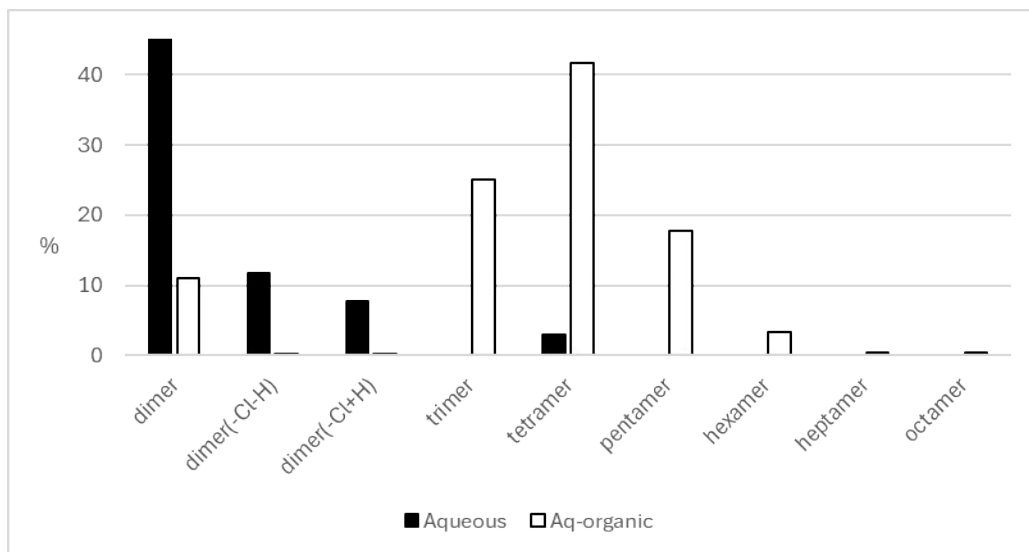


Figure S2 Oligomer distribution for 4-CP in aqueous reaction mixture and in aqueous-organic extract. The enzymatic reaction mixture was filtered directly for the aqueous sample; for the extract, the reaction mixture was diluted 4-fold with acetonitrile, vortexed for an hour, then filtered. MS peak areas (all isomers) are expressed as a % of the total set of areas for that solvent (Table S3).

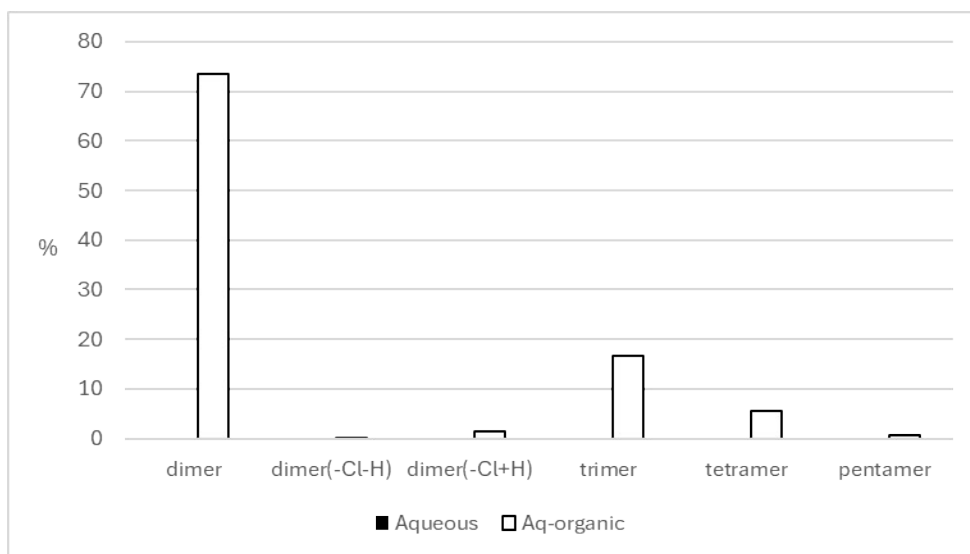


Figure S3 Oligomer distribution for 2,4-CP in aqueous reaction mixture and in aqueous-organic extract. The enzymatic reaction mixture was filtered directly for the aqueous sample; for the extract, the reaction mixture was diluted 4-fold with acetonitrile, vortexed for an hour, then filtered. MS peak areas (all isomers) are expressed as a % of the total set of areas for that solvent (Table S3).