

Removal of Pharmaceuticals, Toxicity and Natural Fluorescence by Ozonation in Biologically Pre-Treated Municipal Wastewater, in Comparison to Subsequent Polishing Biofilm Reactors

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Table S1. List of Suppliers.

Compound	Supplier	Product Number	Lot Number
Acetyl-Sulfadiazine	Santa Cruz Biotech	SC-207958	E111
Azithromycin	Dr. Ehrenstorfer	C10386000	20626
Ciprofloxacin	Sigma	17850-5G-F	BCBJ9941V
Clarithromycin	Sigma	C9742-100MG	BCBB7751
Clindamycin	European Pharmacopeia	C2250000	ID00DIO0
Erythromycin	Sigma	E-5389	107H06445
Sulfadiazine	Dr. Ehrenstorfer	C16990500	00818
Sulfamethizole	Dr. Ehrenstorfer	C16998000	20324
Sulfamethoxazole	Sigma	S7507-10G	BCBK6637V
Trimethoprim	Sigma	92131	-
Atenolol	Dr. Ehrenstorfer	C10313000	61017
Metoprolol	Dr. Ehrenstorfer	C15176020	50218
Propranolol	Dr. Ehrenstorfer	C1651000	80704
Sotalol	Dr. Ehrenstorfer	C16972630	61211
Carbamazepine	Sigma	550-084-G001	L02061
Diclofenac	Dr. Ehrenstorfer	C12537500	60426
Ibuprofen	Dr. Ehrenstorfer	C14278000	90216
Phenazone	Dr. Ehrenstorfer	C16003100	50314
Tramadol	Sigma	42961-1G-F	BCBK9246V
Citalopram	Sigma	C7861-10MG	SLBC7491V
Venlafaxine	Sigma	V7264-10MG	002M4703
Diatrizoic acid	Dr. Ehrenstorfer	C12207000	10420
Iohexol	Dr. Ehrenstorfer	C14347000	91203
Iopromide	Dr. Ehrenstorfer	C14348600	10629
Iomeprol	Dr. Ehrenstorfer	C14348000	00702
Iopamidol	Dr. Ehrenstorfer	C14348400	10818
N-acetyl sulfadiazine 13C6	Santa Cruz Biotec	sc-219011	
Sulfadiazine-13C6	Sigma-Aldrich	sc-220154	
Ibuprofen d3	TRC	I140002	
Erythromycin-13C,D3	Santa Cruz Biotec	sc-218331	
rac Propranolol-d7	Santa Cruz Biotec	sc-212742	
Citalopram-d6	Santa Cruz Biotec	sc-217924	
Trimethoprim-d3	Santa Cruz Biotec	sc-220337	
Clindamycin-d3 Hydrochloride	Santa Cruz Biotec	sc-217929	
Ciprofloxacin-d8	Santa Cruz Biotec	sc-217901	

Table S2. Common Parameters of Effluent from MBBRs in Herning.

	Temp (°C)	pH	DO (mg/L)	COD (mg/L)	NH ₄ -N (mg/L)	NO ₃ -N (mg/L)
Effluent	8.9 ± 0.6	7.6 ± 0.3	8.9 ± 0.7	30 ± 7	0.2 ± 0.2	9.3 ± 6.2

Table S3. Initial Concentrations of Pharmaceuticals (C₀) in M5 effluent, and the Relevant Limit of Quantification (LOQ) by HPLC-MS/MS from the Pilot and Laboratory Experiments.

	Pilot		Laboratory	
	C ₀ (µg/L)	LOQ	C ₀ (µg/L)	LOQ
Ac-sulfadiazine	0.12	0.27	0.04	0.04
Atenolol	0.05	0.03	0.09	0.02
Azithromycin	0.33	3.08	0.93	10.53
Carbamazepine	0.38	0.05		
Citalopram	1.55	0.14	7.66	0.04
Clarithromycin	0.25	0.06	0.10	0.08
Clindamycin			0.25	0.18
Diatrizoic acid	0.49	0.53	0.40	0.10
Diclofenac	0.47	0.07	0.23	0.02
Ibuprofen	0.03	0.99	2.26	0.15
Iohexol	0.95	0.30	0.75	0.04
Iomeprol	8.09	0.27	6.74	0.08
Iopamidol	0.52	0.25	9.77	0.04
Iopromide	0.08	1.33	0.19	0.08
Metoprolol	1.77	0.04	0.86	0.02
Phenazone	0.05	0.35	0.01	0.08
Propranolol	0.30	0.07		
Sotalol	0.00	0.06	0.03	0.05
Sulfadiazine	0.10	0.05	0.04	0.02
Sulfamethizole	0.13	0.05	0.19	0.07
Sulfamethoxazole	0.06	0.03	0.04	0.01
Tramadol	95.12	1.20	1.20	0.69
Trimethoprim	0.16	0.06	0.13	0.02
Venlafaxine	2.94	0.11	0.59	0.03

Table S4. Correlation between the changes of concentrations of selected pharmaceuticals ($\Delta C/C_0$) and relative changes of excitation emission matrices fluorescence ($\Delta A/A_0$) under different dosages of ozone at laboratory, which was fitted by the straight line.

Compound	R ²					
	Peak B($\lambda_{231/315}$)	Peak T ₂ ($\lambda_{231/360}$)	Peak A($\lambda_{249/450}$)	Peak B($\lambda_{275/310}$)	Peak T ₁ ($\lambda_{275/340}$)	Peak C($\lambda_{335/450}$)
Ac-sulfadiazine	0.5742	0.6695	0.5289	0.6527	0.6794	0.6043
Atenolol	0.1793	0.4314	-1.165	0.5065	0.5313	-0.5303
Citalopram	0.7186	0.5944	0.5438	0.6143	0.5372	0.6625
Clarithromycin	0.9493	0.9423	0.5566	0.9460	0.9246	0.7023
Clindamycin	0.8458	0.8022	0.9551	0.7936	0.7502	0.8847
Diatrizoic acid	-0.05500	0.1075	-0.1229	0.06714	0.1409	-0.01438
Diclofenac	0.8891	0.9761	0.3259	0.9793	0.9922	0.5403
Ibuprofen	0.8468	0.7934	0.7937	0.7695	0.7332	0.9351
Iohexol	0.7733	0.7587	0.6509	0.7543	0.7033	0.8312
Iomeprol	0.4808	0.6372	-0.5501	0.6924	0.6756	0.03399
Iopamidol	0.5345	0.6849	-0.5224	0.7411	0.7277	0.04573
Iopromide	-0.01730	0.1041	-0.7604	0.1404	0.1831	-0.5641
Metoprolol	0.7914	0.7281	0.5382	0.7302	0.6633	0.8491
Sulfadiazine	0.6578	0.5745	0.6367	0.5603	0.5087	0.7988
Sulfamethizole	0.6533	0.5626	0.5139	0.5640	0.5032	0.7360
Sulfamethoxazole	0.2761	0.2191	0.2245	0.2240	0.1704	0.4293
Tramadol	0.6147	0.6613	-0.1357	0.7101	0.6543	0.3330
Trimethoprim	0.8382	0.9486	0.1436	0.9631	0.9774	0.4157
Venlafaxine	0.8678	0.7986	0.5615	0.8073	0.7375	0.8390
Number (Highest)	3	0	1	3	6	6
Average	0.85		0.96	0.71	0.58	0.76
Number (Second Highest)	6	3	0	5	2	3
Average	0.67	0.48		0.71	0.70	0.80

Table S4. Continued.

Compound	Slope					
	Peak B($\lambda_{231/315}$)	Peak T ₂ ($\lambda_{231/360}$)	Peak A($\lambda_{249/450}$)	Peak B($\lambda_{275/310}$)	Peak T ₁ ($\lambda_{275/340}$)	Peak C($\lambda_{335/450}$)
Ac-sulfadiazine	0.1639	0.1594	0.5447	0.1579	0.1482	0.3334
Atenolol	0.8105	0.7811	2.524	0.7814	0.7195	1.625
Citalopram	0.9643	0.8874	3.187	0.8875	0.8007	2.009
Clarithromycin	1.036	0.9813	3.405	0.9772	0.8961	2.124
Clindamycin	0.6657	0.6185	2.337	0.6170	0.5604	1.379
Diatrizoic acid	0.4259	0.4274	1.430	0.4211	0.3961	0.9086
Diclofenac	1.113	1.063	3.626	1.059	0.9744	2.270
Ibuprofen	0.5625	0.5274	1.918	0.5225	0.4768	1.207
Iohexol	0.3765	0.3560	1.273	0.3541	0.3229	0.8015
Iomeprol	0.6797	0.6517	2.151	0.6513	0.5980	1.385
Iopamidol	0.7012	0.6718	2.219	0.6714	0.6167	1.425
Iopromide	0.5011	0.4804	1.406	0.4822	0.4439	0.9312
Metoprolol	0.7833	0.7345	2.584	0.7313	0.6647	1.669
Sulfadiazine	0.3908	0.3605	1.338	0.3571	0.3226	0.8605
Sulfamethizole	0.6090	0.5639	2.025	0.5615	0.5080	1.311
Sulfamethoxazole	0.2039	0.1867	0.6814	0.1864	0.1656	0.4672
Tramadol	1.020	0.9708	3.256	0.9707	0.8878	2.094
Trimethoprim	0.9941	0.9509	3.214	0.9477	0.8723	2.020
Venlafaxine	0.9267	0.8691	3.046	0.8662	0.7880	1.947
Number (Highest)			19			
Average			2.2			
Number (Second Highest)						19
Average						1.4

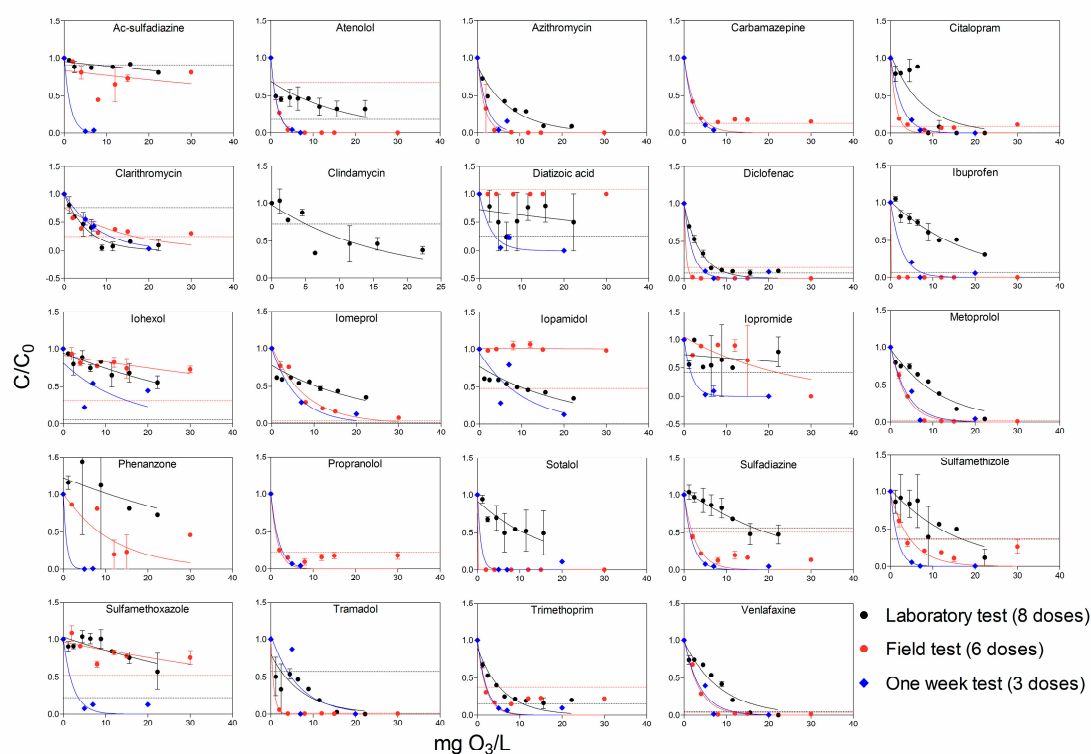


Figure S1. Comparison of pharmaceutical removal by ozone in the effluent of the staged MBBR demonstration plant at Herning municipal wastewater treatment plant using the onsite continuous pilot ozonation system and batch treatment in laboratory. The dash lines in the figure stand for the limit of quantification (LOQ) of pharmaceuticals by HPLC-MS/MS. Error bars represent the standard deviations.

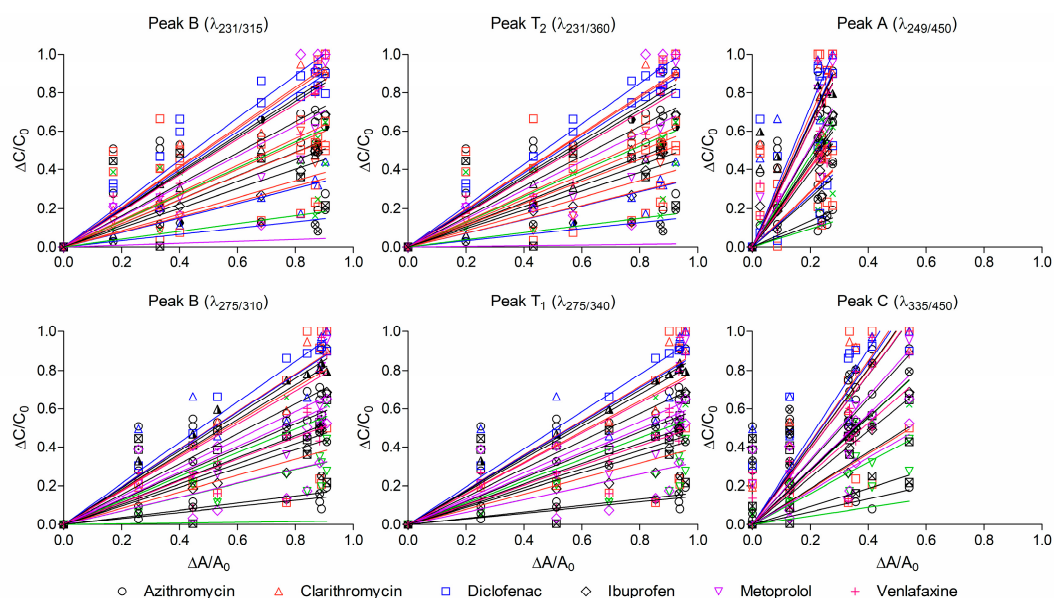


Figure S2. Correlation between the changes of concentrations of pharmaceuticals ($\Delta C/C_0$) and relative changes of excitation emission matrices fluorescence ($\Delta A/A_0$) under different dosages of ozone at laboratory, which was fitted by the straight line.

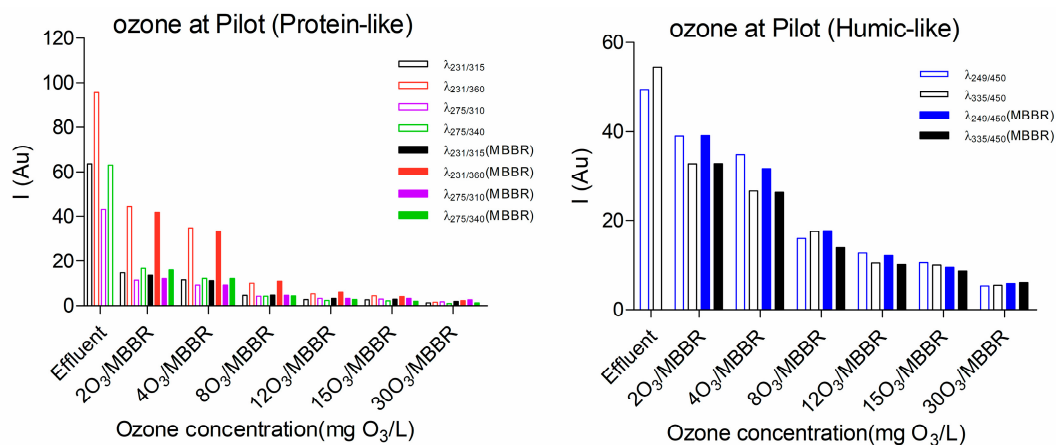


Figure S3. Development of natural fluorescence in M5 effluent treated by six dosages of ozone in pilot with or without a subsequent MBBR polishing.



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