Comprehensive Evaluation of Non-Catalytic Wet Air Oxidation as a Pretreatment to Remove Pharmaceuticals from Hospital Effluents

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Electronic Supplementary Information

Section S1. Experimental methods

Table S1. Multiple reaction monitoring transitions and optimized parameters for quantitative analyses by LC-QqQMS (Waters Micromass Quattro Premier XE Mass Spectrometer).

Compound	RT[a] (min)	Precursor ion (*m/z*)	CV[b] (V)	Quantification Product ion (*m/z*)	CE[c] (V)	Confirmation Product ion (*m/z*)	CE[c] (V)
Acetaminophen	2.76	151.7	30	93.2	20	110.2	15
Baclofen	4.03	214.1	20	115.1	35	150.9	20
Carbamazepine	10.13	236.9	30	178.9	40	193.9	15
Cetirizine	11.30	389.1	30	165.9	40	200.9	20
Diclofenac	13.91	295.9	20	214.9	20	249.9	15
Gabapentin	3.66	171.9	25	137.3	15	154.4	15
Pregabalin	3.47	159.9	20	142.3	10	97.4	15
Quetiapine	8.74	383.9	40	253.3	25	221.3	35
Sulfamethoxazole	5.49	254.0	25	155.8	15	91.9	25
Trimethoprim	4.81	291.1	45	122.9	30	230.0	25

[a] Retention time; [b] Cone voltage; [c] Collision energy

Table S2. Multiple reaction monitoring transitions and optimized parameters for quantitative analyses by LC-QqQMS (Xevo TQ-S micro mass spectrometer).

Compound	RT[a] (min)	Precursor ion (*m/z*)	CV[b] (V)	Quantification Product ion (*m/z*)	CE[c] (V)	Confirmation Product ion (*m/z*)	CE[c] (V)
Acetaminophen	2.72	152.0	35	82.0	22	92.8	22
Baclofen	4.02	214.0	10	115.7	32	151.0	17
Carbamazepine	9.98	237.1	30	193.6	16	179.0	34
Cetirizine	11.16	389.1	5	165.9	43	201.0	19
Diclofenac	13.69	296.0	5	250.0	12	214.9	19
Gabapentin	3.62	172.1	20	137.0	15	95.0	22
Pregabalin	3.45	160.0	10	142.0	10	97.1	14
Quetiapine	8.63	384.1	10	253.0	22	158.1	22
Sulfamethoxazole	5.40	254.0	10	92.0	28	107.9	24
Trimethoprim	4.75	291.1	10	123.0	23	230.0	22

[a] Retention time; [b] Cone voltage; [c] Collision energy

Section S1.1. LC-QqQMS method performance

Since three types of samples with different sample preparation or mass spectrometers were analyzed (deionized water, untreated hospital wastewater, treated hospital water), method performance figures of merit such as limits of quantification, linearity, precision, and trueness were measured in each case. They are found in Tables S3, S4 and S5.

Table S3. Method performance for the analysis of spiked deionized water. This method was used for the optimization of WAO using spiked concentrations of target compounds at 1500 µg L^{-1}.

Compound	Linearity	LOQ [a] (µg L^{-1})	Precision [b] (%)	Trueness [b] (%)
Acetaminophen	0.9925	7.8	2.4	24.2
Baclofen	0.9972	16.6	6.9	10.7
Carbamazepine	0.9977	8.7	1.4	9.6
Cetirizine	0.9999	1.2	1.0	7.3
Diclofenac	0.9976	0.8	5.6	10.6
Gabapentin	0.9936	5.4	11.3	9.3
Pregabalin	0.9998	2.4	3.6	17.4
Quetiapine	0.9973	28.3	4.2	2.2
Sulfamethoxazole	0.9999	3.2	5.4	8.9
Trimethoprim	0.9945	12.2	3.9	7.9

[a] Determined using the standard deviation of the concentration of 10 replicates (5µg L^{-1}, except quetiapine and trimethoprim for which 10µg L^{-1} was used) multiplied by 10. [b] Determined using a quality control sample spiked at 40 µg L^{-1} (n=5).

Table S4. Method performance for the analysis of untreated hospital wastewater. This method was used for the quantification of pharmaceuticals shown in Figure 2 of the manuscript.

	Untreated hospital wastewater			
Compound	**Linearity**	**LOQ [a] (ng L^{-1})**	**Precision [b] (%)**	**Trueness [b] (%)**
Acetaminophen	N.A.	N.A.	N.A.	N.A.
Baclofen	0.9950	5.4	1.8	7.8
Carbamazepine	0.9985	1.7	0.8	6.1
Cetirizine	0.9891	0.12	2.3	14
Diclofenac	0.9996	0.22	2.8	12
Gabapentin	0.9847	6.4	1.3	8.8
Pregabalin	0.9688	26	1.5	15
Quetiapine	0.9988	4.6	2.0	12
Sulfamethoxazole	0.9840	0.18	1.6	2.7
Trimethoprim	0.9958	0.74	3.1	4.9

[b] Determined according to a S/N=10. [b] Determined using a quality control sample spiked at 150 ng L^{-1} (n=5). N.A.: Not available. Acetaminophen could not be quantified in the untreated hospital wastewater samples.

Table S5. Method performance for the analysis of WAO treated hospital wastewater. This method was employed to obtain the results shown in Table 4.

	WAO treated hospital wastewater			
Compound	**Linearity**	**LOQ [a] (ng L^{-1})**	**Precision [b] (%)**	**Trueness [b] (%)**
Acetaminophen	0.9978	83	2.2	-1.1
Baclofen	0.9972	58	9.6	24
Carbamazepine	0.9973	4.3	14	12
Cetirizine	0.9970	16	45	7.1
Diclofenac	0.9976	9.0	17	13
Gabapentin	0.9972	147	2.2	9.7
Pregabalin	0.9970	26	13	18
Quetiapine	0.9928	10.0	11	12
Sulfamethoxazole	0.9978	5.2	12	3.2
Trimethoprim	0.9963	10	8.0	14

[a] Determined using 10× the standard deviation of 10 blank samples divided by the slope of the calibration curve, except for acetaminophen, carbamazepine, quetiapine and sulfamethoxazole (10× standard error of the calibration curve divided by the slope) [b] Determined using a quality control sample spiked at 80 ng L^{-1}, except for gabapentin (2000 ng L^{-1}).

Section S1.2. Preparation of QC samples

For tests using batch reactor model Cellule 2646 1000, a volume of 0.5 mL of the test sample introduced in the reactor was pipetted in an amber vial and then diluted with 1.5 mL of deionized water was used as quality control (QC) sample. This dilution is the same as the sample will undergo in the reactor. During WAO tests, the QC sample is left at room temperature under the same conditions as the samples. At the end of the test, the QC sample is refrigerated at the same time as the test samples. Concentration values determined by LC-QqQMS should be the same for QC samples and tests samples collected immediately after dilution in the reactor. If there was a difference of more than 20% between these two values, the WAO test was considered invalid.

Section S1.3 *Daphnia magna* acute toxicity bioassay protocol and quality control

The culture medium used is standard freshwater prepared with the following salts in deionized water: $NaHCO_3$ (64.75 mg L^{-1}), $CaCl_2.2H_2O$ (294 mg L^{-1}), $MgSO_4.7H_2O$ (123.25 mg L^{-1}) and KCl (5.75 mg L^{-1}). The ephippia are rinsed and then transferred to a petri dish with 50 mL of the standard freshwater solution previously bubbled with air for 15 min. The hatching lasts 72 hours, at 20-22 ° C under lighting of 2000 ± 70 Lux. Five dilutions (C1 to C5) of the test sample with standard freshwater are evaluated during a test. Two hours before the test, daphnids were fed with spirulina powder in order to avoid high mortality (>10% of daphnids). The test plate consists of 30 wells: 6 rows (control, C1, C2, C3, C4 and C5) and 5 columns (one for transferring daphnids and four replicate exposure tests). Exactly five daphnids are then placed in each well of the test plate with 10 mL of standard freshwater (control) or the corresponding effluent dilutions. A piece of sealing film (Parafilm M) is placed on the test plate. After 24 and 48 hours of incubation (20 ±°C, in darkness), the number of immobilized daphnids is counted. Daphnids not moving after gentle agitation are considered immobilized. These results allow the calculate the median lethal concentration (LC_{50}).

The quality control sample was a solution of potassium dichromate ($K_2Cr_2O_7$), a toxic reference substance. The following series of dilutions were used 3.2, 1.8, 1.0, 0.56 and 0.32 mg L^{-1}. The median lethal concentration (LC_{50}) obtained with QC samples at 24 h must be located within the limits mentioned in the technical sheet of each Daphtoxkit (between 0.6 mg L^{-1} and 2.1 mg L^{-1}). Also, the mortality rate in control daphnids must not exceed 10%. If so, the test is considered

invalid because it means that part of the immobilization can be explained by something other than exposure to contaminants. LC_{50} values were obtaining after fitting the data on number of immobilized daphnids as a function of volume percentage of test sample using a dose-response model and the Levenberg Marquardt iteration algorithm in OriginPro 2021 developped by OriginLab (Northampton, MA). Quality of the fit was evaluated by the adjusted coefficient of determination (R'^2). In all cases R'^2 was > 0.9 except one set of data (exposure to untreated hospital wastewater). For one sample (hospital wastewater treated by WAO for 15 min) the fitting failed and no LC_{50} could be calculated.

Section S1.4 *Aliivibrio fischeri* acute toxicity bioassay protocol and quality control

Microtox bioassays were completed using standard kits purchased from EBPI and the Microtox M500 system. The testing kit included lyophilized *Aliivibrio fischeri*, reagent diluent, osmotic adjustment solution (OAS), and sample diluent. For each test, one vial of *Aliivibrio fischeri* was rehydrated with 1 mL of reagent diluent at 4 °C for 30 minutes. Before the assay, the reagent (*A. fischeri)* was incubated in the Microtox M500 at 15 °C for 30 minutes. To prepare the sample for the test, the pH was measured to ensure the test sample was between 6-8.5. All samples fell within this range, so no adjustment was required. Next, the salinity of the sample was adjusted by adding 1/10 of the sample volume of OAS. Then, the sample was diluted serially with sample diluent at a dilution factor of 1.5. Eight dilutions of the test sample were used for the analysis. After the reagent had properly incubated at 15 °C, 10 µL of reagent stock was pipetted into cuvettes with 500 µL of sample diluent also at 15°C. After stabilization for 15 minutes, initial light intensity readings of each cuvette (I_0) were taken. Next, 500 µL of each dilution of the test sample was transferred into the corresponding reagent cuvette. After five minutes, light intensity readings were taken again (I_5). EC_{20} values were then calculated by the Microtox Omni Software. If the tested sample was not toxic enough to cause a measurable light inhibition, the sample was retested for confirmation of the results.

Two blanks were analyzed in each run. The blank consisted of 500 µL of sample diluent. When calculating the EC_{20}, all light readings were compared to the blanks. The blanks account for the natural death of the *A. fischeri*. If the blanks had an inadequate light reading at any point in the

testing procedure, the results were not considered, and the test was restarted. As suggested by the EBPI kit, a positive control of phenol at a concentration of 45 mg L^{-1} was also used in each run. After 5 minutes, around 80% of light inhibition was observed in the positive control.

Section S2. Results

Table S6. Organic pharmaceuticals consumed in the local hospital.

Pharmaceutical	Mass (kg)
Acetaminophen	1.05×10^{2}
Metformin	1.87×10
Docusate	6.28
Lidocaine	4.98
Sodium divalproex	4.55
Amoxicillin	2.63
Cefazoline	2.57
Acetylsalicylic acid	2.32
Pantoprazole	1.86
Gabapentin	1.84
Quetiapine	1.70
Naproxen	1.50
Pregabalin	1.48
Ciprofloxacin	1.30
Levetiracetam	1.22
Levodopa	1.20
Furosemide	1.04
Moxifloxacin	1.03
Venlafaxine	1.01
Phenytoin	9.00×10^{-1}
Dexlansoprazole	8.57×10^{-1}
Clopidogrel	6.75×10^{-1}
Amiodarone	5.20×10^{-1}
Clozapine	4.80×10^{-1}
Tetracaine	4.08×10^{-1}
Thiamine	3.60×10^{-1}
Oxazepam	3.57×10^{-1}
Sennosides	3.43×10^{-1}
Carbidopa	3.00×10^{-1}
Dimenhydrinate	2.59×10^{-1}
Citalopram	2.57×10^{-1}
Allopurinol	2.50×10^{-1}
Trazodone	2.25×10^{-1}
Isosorbide-5-mononitrate	2.10×10^{-1}
Atorvastatin	1.75×10^{-1}
Metoprolol	1.75×10^{-1}
Gliclazide	1.68×10^{-1}
Mirtazapine	1.08×10^{-1}
Methylprednisolone	1.04×10^{-1}
Amlodipine	9.73×10^{-2}
Domperidone	9.00×10^{-2}
Phenobarbital	9.00×10^{-2}
Salbutamol	7.44×10^{-2}

Morphine	7.35×10^{-2}
Donepezil	6.70×10^{-2}
Rosuvastatin	6.50×10^{-2}
Prednisone	6.00×10^{-2}
Hydromorphone	5.25×10^{-2}
Bisoprolol	4.83×10^{-2}
Baclofen	4.50×10^{-2}
Olanzapine	4.16×10^{-2}
Hydralazine	4.00×10^{-2}
Cetirizine	3.90×10^{-2}
Buspirone	3.70×10^{-2}
Metoclopramide	3.15×10^{-2}
Midodrine	2.90×10^{-2}
Procyclidine	2.65×10^{-2}
Perindopril	2.64×10^{-2}
Apixaban	2.52×10^{-2}
Lorazepam	2.20×10^{-2}
Betamethasone	1.95×10^{-2}
Dexamethasone	1.20×10^{-2}
Loperamide	1.04×10^{-2}
Risperidone	8.50×10^{-3}
Methadone	4.00×10^{-3}
Tamsulosin	3.28×10^{-3}
Clonazepam	2.95×10^{-3}
Levothyroxine	2.03×10^{-3}
Fentanyl	3.00×10^{-4}
Tiotropium	1.67×10^{-4}
TOTAL	**170.71**

Table S7. Estimated amounts of the 25 top pharmaceuticals rejected in the hospital effluent using a conservative daily water consumption of 420.8 L/bed (number of beds = 166).

Pharmaceutical	Estimated concentration in the effluent ($\mu g\ L^{-1}$)
Metformin	979.23
Acetaminophen	165.08
Cefazoline	134.24
Amoxicillin	107.49
Gabapentin	96.22
Pregabalin	75.62
Ciprofloxacin	52.14
Furosemide	48.99
Levetiracetam	42.11
Lidocaine	26.06
Moxifloxacin	23.65
Acetylsalicylic acid	12.13
Divalproex	7.14
Levodopa	6.28
Carbidopa	5.49
Quetiapine	4.44
Rosuvastatin	3.06
Venlafaxine	2.63
Phenytoin	2.35
Baclofen	2.00
Thiamine	1.88
Citalopram	1.61
Allopurinol	1.31
Bisoprolol	1.26
Cetirizine	1.22
TOTAL	1803.64

Table S8. Energy balance for a WAO unit of 86 L min^{-1}.

Power Consumption		
	Installed Power kW	**Power Consumption MWh/year**
Compressor	10	75
Pump	23	183
Cooling System	100	791
Total Power Consumption (MWh)		1049
Heat Consumption		
	Flow Nm^3/h	**Heat Consumption GJ/year**
Natural gas	33.8	5344
Total Heat Consumption (MWh)		1484
Power and Heat		
Energy balance (MWh)		2533
Proportion		
Electricity		41%
Heat		59%

Table S9. Cases for WAO unit sensitivity analysis.

Inlet Flow rate (L/min)	**Concentration factor**	**COD (mg O_2/L)**
5	17	23800
10	9	12600
25	3.5	4900
50	1.7	2380
75	1.2	1680
86	1	1400