Supporting Information

Is anaerobic digestion effective for the removal of organic micropollutants and biological activities from sewage sludge?

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S1. Recoveries of OMPs in the solid and liquid phase of raw and digested sludge

Table S1. Absolute recoveries (mean and average error) in the liquid and solid phase of sewage sludge (SS), mesophilic (MAD) and thermophilic (TAD) digestates.

		Sludge matrix	Recovery (%)	
			Liquid	Solid
LC/MS-MS	ERY	SS-MAD-TAD	62.0 ± 7.2	51.4 ± 8.2
	ROX	SS	46.2 ± 0.1	54.6 ± 2.8
		MAD-TAD	16.0 ± 2.8	
	SMX	SS	86.2 ± 3.2	54.7 ± 6.9
		MAD	74.0 ± 0.7	
		TAD	57.3 ± 3.2	
	TMP	SS	70.7 ± 1.0	54.2 ± 3.9
		MAD	60.6 ± 0.9	42.2 ± 5.7
		TAD	45.8 ± 2.4	
	FLX	SS	30.5 ± 2.3	44.4 ± 0.6
		MAD-TAD	8.3 ± 2.3	18.3 ± 3.2
	CTL	SS	18.3 ± 0.4	53.3 ± 1.1
		MAD-TAD	9.3 ± 0.8	36.9 ± 4.7
	CBZ	SS	84.8 ± 3.4	63.8 ± 3.5
		MAD	77.0 ± 1.3	
		TAD	70.2 ± 1.4	
	DZP	SS	22.9 ± 0.5	68.7 ± 7.1
		MAD-TAD	10.3 ± 0.6	
	E1	SS	22.9 ± 0.3	67.8 ± 6.6
		MAD-TAD	13.1 ± 1.3	
	E2	SS	59.8 ± 2.8	63.3 ± 6.3
		MAD-TAD	16.3 ± 4.3	
	EE2	SS	57.3 ± 0.6	70.1 ± 7.7
		MAD	34.3 ± 1.5	
		TAD	17.6 ± 2.8	
GC/MS	ADBI	SS-MAD-TAD	11.9 ± 3.3	168 ± 28
	HHCB	SS-MAD-TAD	22.8 ± 13.1	159 ± 26
	AHTN	SS-MAD-TAD	35.1 ± 14.9	189 ± 8
	IBP	SS-MAD-TAD	163 ± 17	178 ± 11
	NPX	SS	194 ± 9	133 ± 1
		MAD-TAD		88.8 ± 8.2
	DCF	SS-MAD-TAD	141 ± 19	134 ± 10
	OP	SS-MAD-TAD	11.1 ± 0.8	135 ± 32
	NP	SS	8.4 ± 4.5	146 ± 11
		MAD-TAD		80.2 ± 13.8
	TCS	SS-MAD-TAD	12.1 ± 1.5	151 ± 20

In general, the recoveries in the solid phase were quite similar regardless the matrix and, hence, average values were used in the calculations (Table S1). Nevertheless, FLX, CTL, NP, NPX, TMP presented a higher recovery in the mixed sewage sludge than in the digested. Ternes et al. (2005), whose USE extraction protocol was adapted in the present study, also observed this tendency for some OMPs. Sludge recoveries were generally above 50%, although some OMPs measured by GC-MS presented recoveries higher than 100%, due to the presence of interfering substances in the chromatogram. In comparison with the methodology of Ternes et al. (2005), the recoveries of CBZ and DZP were improved, while those of IBP, DCF, AHTN, and HHCB were lower.

The recoveries of some OMPs in the liquid phase were quite low, especially in the case of hydrophobic compounds. This fact could be related to the presence of colloids in the liquid phase, which hinders enormously the filtration process (Bivins and Novak, 2001) and hence decreased the recovery of OMPs. Likewise, the different concentration of colloids in the matrixes (TAD > MAD >> SS) (Bivins and Novak, 2001) could explain the general tendency observed in liquid recoveries: TAD < MAD << SS. In fact, FLX, CTL, ROX, DZP, E1 and E2 presented higher recoveries in the liquid SS matrix than in the digestates, while for SMX, TMP, CBZ and EE2 the differences between the three matrixes were significant.

The relative standard deviations (RSD) of the solid phase recoveries were under 20% for all compounds, except for OP (24%), which indicates that the overall precision and repeatability of the method was quite good and comparable to other studies (Malmborg and Magnér, 2015; Radjenović et al., 2009; Ternes et al., 2005). Besides, the RSD of most OMPs in the liquid phase were inside this variation range, only some compounds (musk fragrances, NP, FLX, and E2) showed higher RSD due to their low recoveries, suggesting that the determination of these OMPs in the liquid phase is semiquantitative.

S2. Estrogenic Activity

Figure S2 represents an example (first sampling campaign) of the calibration curve of the estrogenic assay, between RLU (Relative Light Units) normalized towards protein content and estradiol equivalent concentration (EEQ). The calibration curve shows a typical dose-response pattern (R^2=0.92) between the estrogenic activity (response) and the E2 concentration (dose), whose main parameters are presented in Table S2. The consistency of cell responsiveness can be highlighted: the estrogenicity increased from a concentration of 100 fM-E2 up to 1 nM-E2 (representing the upper asymptote).

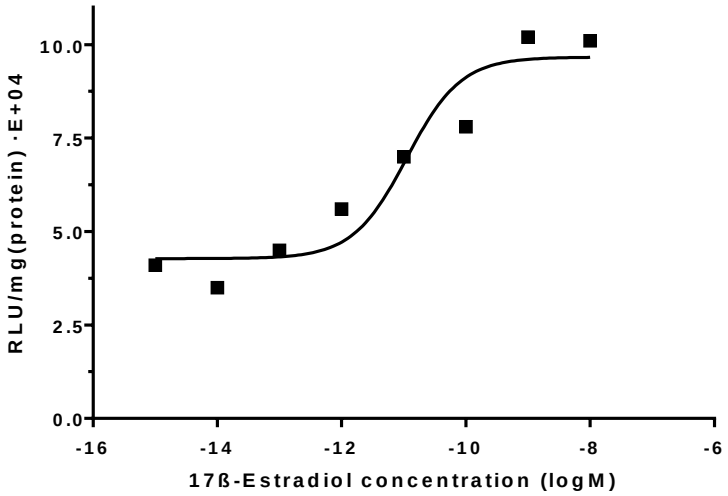

Figure S2. Calibration curve for the estrogenic biological assay performed for the sampling campaign of Period I.

Table S2. Main parameters derived from cell responsivity tests of the first sampling campaign (Period I).

		RESPONSE Luciferase value (RLU/mg protein·10^4)	*DOSE* Estradiol concentration (ng E2/$L_{bioassay}$)
Control (MCF7 cells alone)		3.1	–
LOQ	Lower	4.3	0.016
	Upper	9.7	≈ 1,000
EC_{50} (95% confidence interval)		–	3.1 (0.5, 18.9)

LOQ, limit of quantification; EC_{50}, half maximal effective concentration

S3. Concentration of OMPs in sludge

Table S3a. Total (Ct), liquid (Cw) and solid (Cs) phase average concentrations of OMPs in the mixed sewage sludge and in the digestates of the mesophilic (MAD) and thermophilic (TAD) digesters during Period I.

	Sewage Sludge				MAD				TAD			
	Cw (µg/L)	Cs (ng/g)	Cs (µg/L)	**Ct (µg/L)**	Cw (µg/L)	Cs (ng/g)	Cs (µg/L)	**Ct (µg/L)**	Cw (µg/L)	Cs (ng/g)	Cs (µg/L)	**Ct (µg/L)**
HHCB	9.20 ± 0.62	4055 ± 275	131 ± 9	**141 ± 9**	6.91 ± 0.44	3392 ± 216	86.5 ± 5.5	**93.4 ± 5.5**	9.79 ± 2.26	3701 ± 714	113 ± 23	**122 ± 23**
AHTN	<LOQ	2814 ± 330	91.3 ± 10.7	**91.3 ± 10.7**	<LOQ	2472 ± 168	63.0 ± 4.3	**63.0 ± 4.3**	<LOQ	2882 ± 183	88.0 ± 5.8	**88.0 ± 5.8**
CBZ	0.176 ± 0.004	7.62 ± 0.79	0.247 ± 0.026	**0.423 ± 0.026**	0.140 ± 0.004	5.49 ± 2.53	0.140 ± 0.065	**0.280 ± 0.065**	0.179 ± 0.017	7.47 ± 0.76	0.190 ± 0.024	**0.369 ± 0.029**
DZP	<LOQ	<LOQ	<LOQ	**<LOQ**	<LOQ	<LOQ	<LOQ	**<LOQ**	<LOQ	<LOQ	<LOQ	**<LOQ**
CTL	0.719 ± 0.070	55.2 ± 8.6	1.79 ± 0.28	**2.51 ± 0.29**	0.033 ± 0.001	28.9 ± 1.3	0.737 ± 0.032	**0.770 ± 0.032**	0.057 ± 0.034	39.0 ± 5.4	1.19 ± 0.17	**1.25 ± 0.18**
FLX	0.126 ± 0.012	74.3 ± 6.9	2.41 ± 0.22	**2.53 ± 0.22**	0.031 ± 0.005	31.6 ± 5.7	0.806 ± 0.144	**0.837 ± 0.145**	0.114 ± 0.021	81.4 ± 12.0	1.02 ± 0.38	**1.13 ± 0.38**
IBP	9.84 ± 0.96	487 ± 12	15.8 ± 0.4	**25.6 ± 1.0**	8.67 ± 0.69	388 ± 7	9.90 ± 0.17	**18.6 ± 0.7**	7.71 ± 0.19	448 ± 3	13.7 ± 0.1	**21.4 ± 0.2**
NPX	<LOQ	<LOQ	<LOQ	**<LOQ**	<LOQ	<LOQ	<LOQ	**<LOQ**	<LOQ	<LOQ	<LOQ	**<LOQ**
ROX	0.006 ± 0.002	1.81 ± 0.46	0.059 ± 0.015	**0.065 ± 0.015**	0.012 ± 0.007	3.86 ± 0.44	0.098 ± 0.011	**0.110 ± 0.013**	0.023 ± 0.005	9.95 ± 0.00	0.092 ± 0.000	**0.116 ± 0.005**
SMX	<LOQ	<LOQ	<LOQ	**<LOQ**	<LOQ	<LOQ	<LOQ	**<LOQ**	<LOQ	<LOQ	<LOQ	**<LOQ**
TMP	0.264 ± 0.016	12.2 ± 2.4	0.397 ± 0.078	**0.661 ± 0.079**	0.025 ± 0.001	2.14 ± 0.74	0.055 ± 0.019	**0.080 ± 0.019**	0.027 ± 0.009	2.45 ± 0.68	0.074 ± 0.022	**0.101 ± 0.023**
TCS	<LOQ	n.a.	n.a.	**n.a.**	<LOQ	549 ± 32	14.7 ± 0.8	**14.7 ± 0.8**	<LOQ	838 ± 61	25.8 ± 1.9	**25.8 ± 1.9**
E1	1.04 ± 0.02	230 ± 144	7.46 ± 4.67	**8.50 ± 4.67**	5.89 ± 0.03	338 ± 80	8.6 ± 0.0	**14.5 ± 2.0**	2.44 ± 2.36	210 ± 45	6.24 ± 1.42	**8.46 ± 2.75**
E2	0.056 ± 0.011	18.0 ± 4.3	0.583 ± 0.140	**0.640 ± 0.140**	0.897 ± 0.126	220 ± 31	5.61 ± 0.79	**6.51 ± 0.18**	0.500 ± 0.107	83.9 ± 2.8	2.65 ± 0.09	**3.15 ± 0.15**
EE2	0.617 ± 0.095	14 ± 1	0.45 ± 0.05	**1.07 ± 0.11**	0.288 ± 0.090	<LOQ	<LOQ	**0.288 ± 0.090**	0.073 ± 0.009	<LOQ	<LOQ	**0.073 ± 0.009**

LOQ, limit of quantification; n.a., not available.

Table S3b. Total (Ct), liquid (Cw) and solid (Cs) phase average concentrations of OMPs in the mixed sewage sludge and in the digestates of the mesophilic (MAD) and thermophilic (TAD) digesters during Period II.

	Sewage Sludge				**MAD**				**TAD**			
	Cw (µg/L)	Cs (ng/g)	Cs (µg/L)	**Ct (µg/L)**	Cw (µg/L)	Cs (ng/g)	Cs (µg/L)	**Ct (µg/L)**	Cw (µg/L)	Cs (ng/g)	Cs (µg/L)	**Ct (µg/L)**
HHCB	<LOQ	4975 ± 574	141 ± 16	**141 ± 16**	<LOQ	13922 ± 1782	192 ± 25	**192 ± 25**	<LOQ	6814 ± 1253	110 ± 20	**110 ± 20**
AHTN	<LOQ	1272 ± 87	36.1 ± 2.5	**36.1 ± 2.5**	<LOQ	7318 ± 796	101 ± 11	**101 ± 11**	<LOQ	3661 ± 661	59.3 ± 10.7	**59.3 ± 10.7**
CBZ	0.318 ± 0.039	158 ± 24	4.48 ± 0.67	**4.80 ± 0.67**	0.728 ± 0.326	136 ± 8	1.87 ± 0.10	**2.60 ± 0.34**	0.611 ± 0.116	114 ± 10	1.85 ± 0.15	**2.47 ± 0.19**
DZP	0.434 ± 0.054	131 ± 105	3.72 ± 2.99	**4.16 ± 2.99**	1.05 ± 0.21	79.9 ± 8.2	1.10 ± 0.11	**2.15 ± 0.24**	0.965 ± 0.140	68.1 ± 4.6	1.10 ± 0.07	**2.07 ± 0.16**
CTL	<LOQ	122 ± 13	3.48 ± 0.38	**3.48 ± 0.38**	<LOQ	137 ± 2	1.89 ± 0.03	**1.89 ± 0.03**	<LOQ	105 ± 4	1.69 ± 0.07	**1.69 ± 0.07**
FLX	0.062 ± 0.024	426 ± 318	12.1 ± 9.0	**12.2 ± 9.0**	0.101 ± 0.031	279 ± 99	3.85 ± 1.36	**3.95 ± 1.36**	0.414 ± 0.140	114 ± 10	1.85 ± 0.16	**2.26 ± 0.25**
IBP	4.33 ± 0.29	264 ± 97	7.50 ± 2.75	**11.8 ± 2.8**	6.15 ± 1.62	133 ± 32	1.83 ± 0.44	**7.98 ± 1.68**	6.09 ± 0.48	64.7 ± 16.7	1.05 ± 0.27	**7.14 ± 0.55**
NPX	0.586 ± 0.191	<LOQ	<LOQ	**0.586 ± 0.191**	<LOQ	<LOQ	<LOQ	**<LOQ**	<LOQ	<LOQ	<LOQ	**<LOQ**
ROX	<LOQ	64.8 ± 35.6	1.84 ± 1.01	**1.84 ± 1.01**	<LOQ	18.7 ± 2.1	0.258 ± 0.028	**0.258 ± 0.028**	<LOQ	9.01 ± 1.98	0.15 ± 0.03	**0.146 ± 0.032**
SMX	<LOQ	626 ± 160	17.8 ± 4.6	**17.8 ± 4.6**	<LOQ	273 ± 31	3.77 ± 0.43	**3.77 ± 0.43**	<LOQ	95.9 ± 24.0	1.55 ± 0.39	**1.55 ± 0.39**
TMP	0.273 ± 0.004	235 ± 15	6.66 ± 0.42	**6.94 ± 0.42**	0.242 ± 0.112	174 ± 21	2.40 ± 0.30	**2.64 ± 0.32**	0.297 ± 0.056	161 ± 9	2.61 ± 0.14	**2.91 ± 0.15**
TCS	<LOQ	1418 ± 181	38.1 ± 5.1	**38.1 ± 5.1**	<LOQ	2227 ± 216	32.3 ± 3.0	**32.3 ± 3.0**	<LOQ	1886 ± 471	30.9 ± 7.6	**30.9 ± 7.6**
E1	0.934 ± 0.116	128 ± 2	3.64 ± 0.05	**4.57 ± 0.16**	0.604 ± 0.331	488 ± 25	6.7 ± 0.3	**7.3 ± 0.5**	0.521 ± 0.026	274 ± 22	4.44 ± 0.36	**4.96 ± 0.36**
E2	0.097 ± 0.004	40.1 ± 20.1	1.14 ± 0.57	**1.24 ± 0.57**	0.252 ± 0.017	549 ± 5	7.57 ± 0.07	**7.82 ± 0.07**	0.103 ± 0.014	36.4 ± 2.2	0.590 ± 0.035	**0.694 ± 0.038**
EE2	0.602 ± 0.007	38.1 ± 1.6	1.08 ± 0.05	**1.69 ± 0.05**	0.602 ± 0.019	9.75 ± 1.62	0.135 ± 0.019	**0.737 ± 0.030**	0.215 ± 0.083	5.44 ± 1.85	0.088 ± 0.083	**0.303 ± 0.088**

LOQ, limit of quantification.

S4. Anaerobic digesters performance

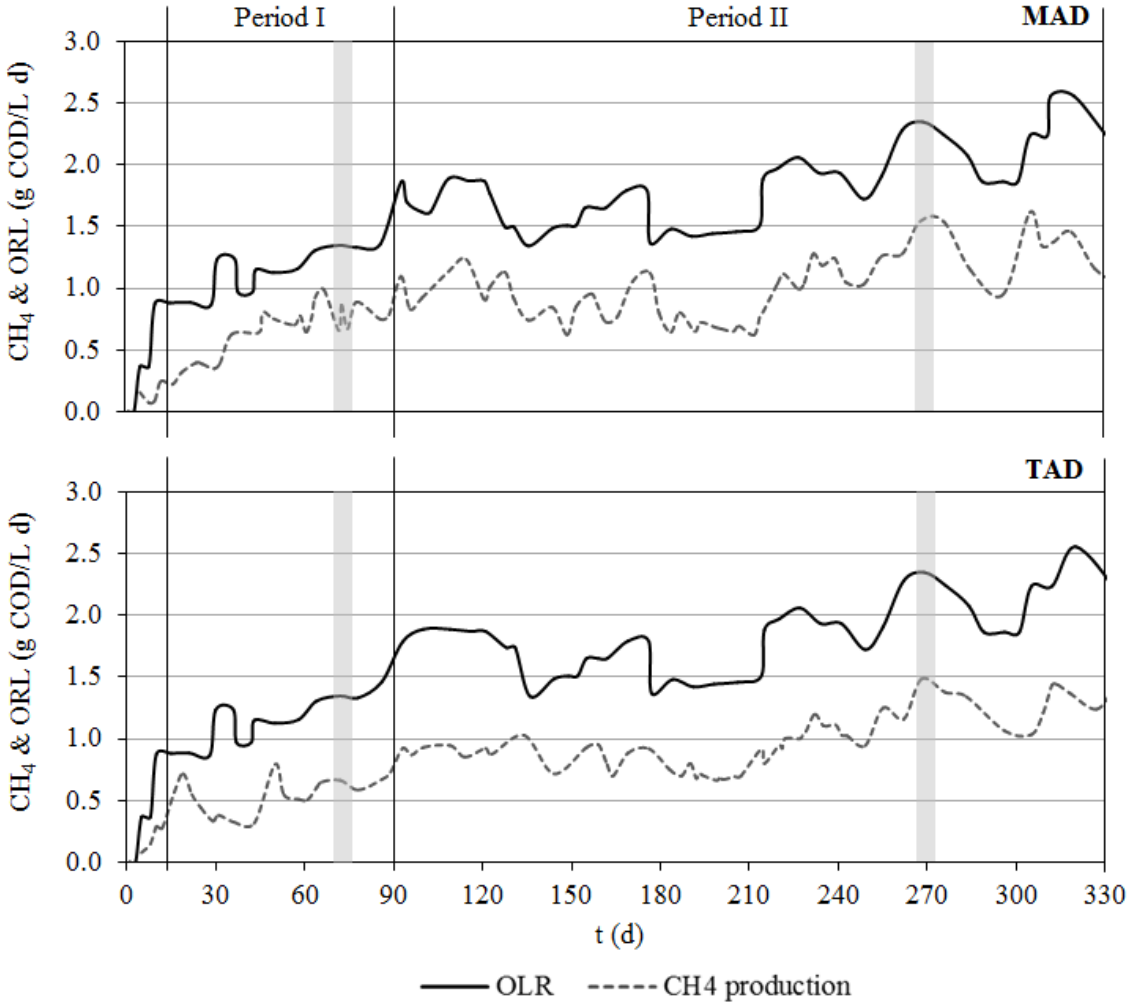

Figure S4. Organic loading rate (OLR) and methane production rate in the mesophilic (MAD) and thermophilic (TAD) digester. The grey sections represent the operation of the digesters during the monitoring campaigns of Period I and Period II.

S5. Influence of operational parameters on the biotransformation of OMPs

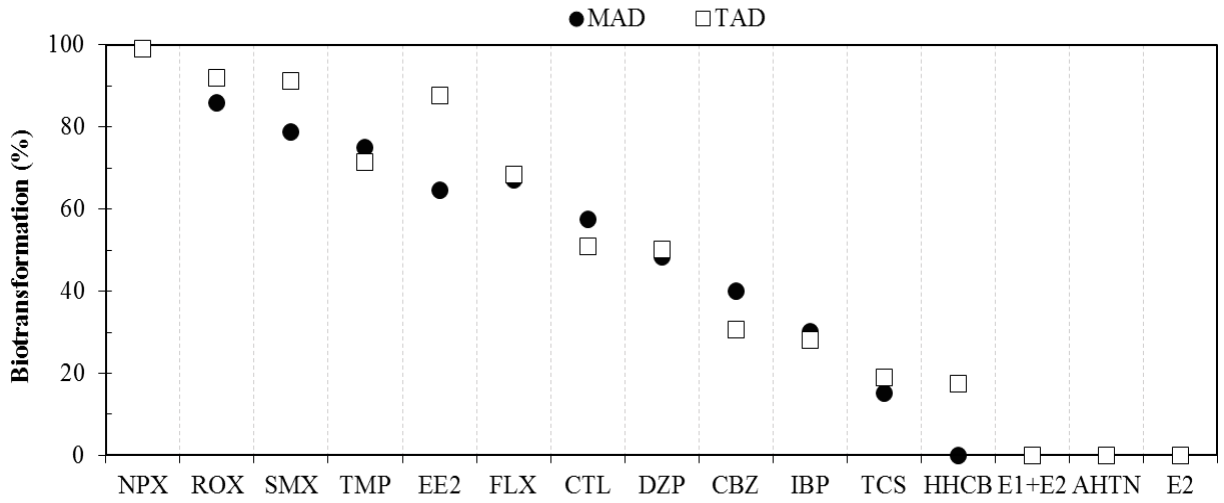

Figure S5a. Average biotransformation (Period I and Period II) of OMPs in the mesophilic (MAD) and thermophilic (TAD) digesters.

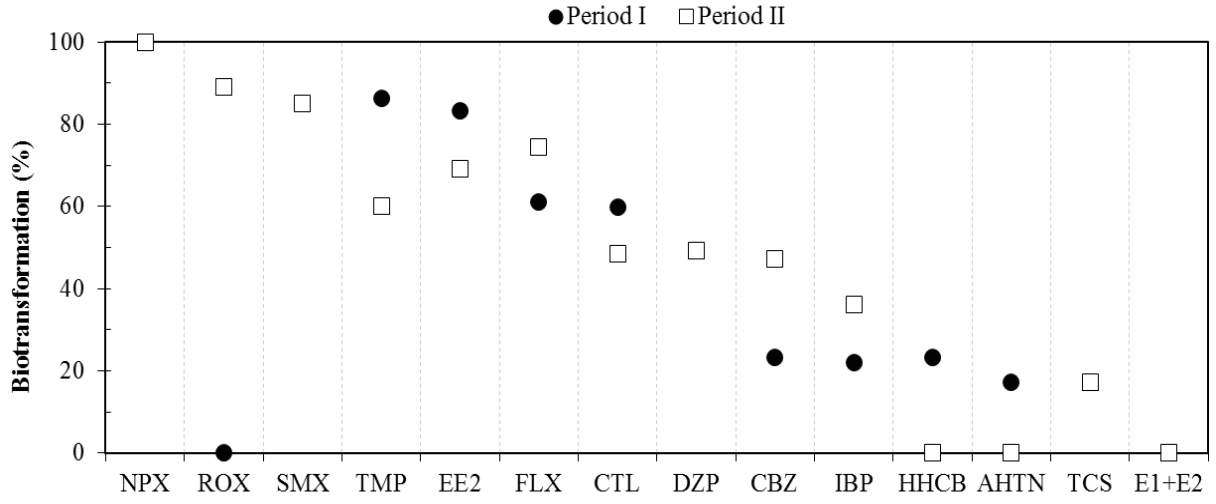

Figure S5b. Average biotransformation (MAD and TAD) during the first (Period I) and the second (Period II) monitoring campaigns.

S6. Distribution of OMPs between the liquid and solid phases of sludge

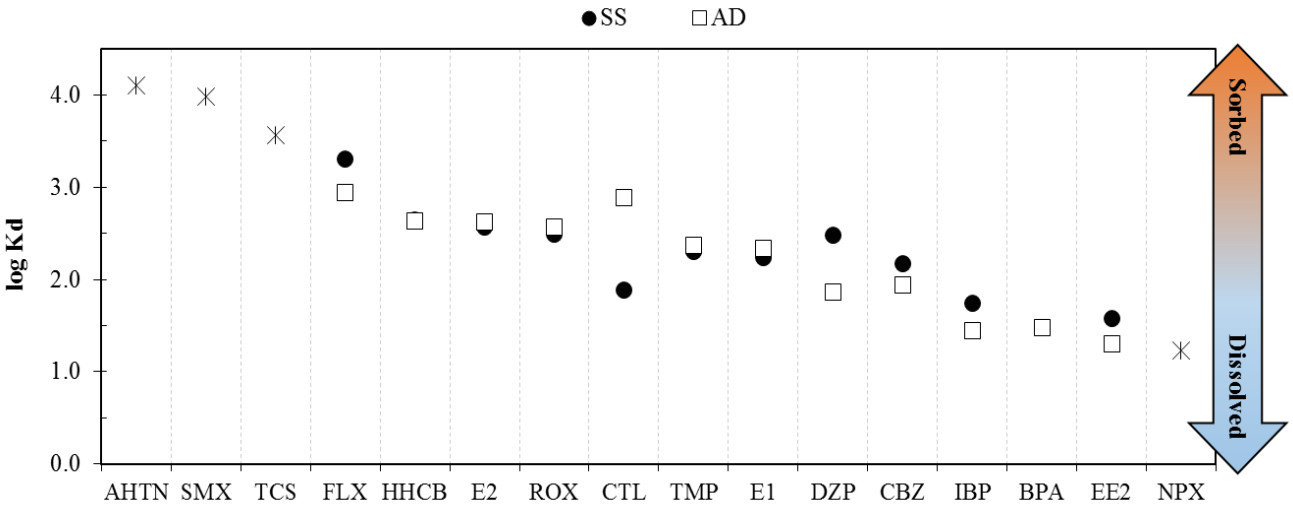

Figure S6. Representation of OMPs partition between solid and liquid phase, through log K_d values, in the mixed sewage sludge (SS) and in the digested sludge by the average of MAD and TAD (AD). Asterisks refer the minimum values of log K_d calculated with the LOQ (AHTN, TCS, and SMX were only measured in solid phase; NPX was entirely in the liquid phase).

S7. Estrogenic activity of sludge

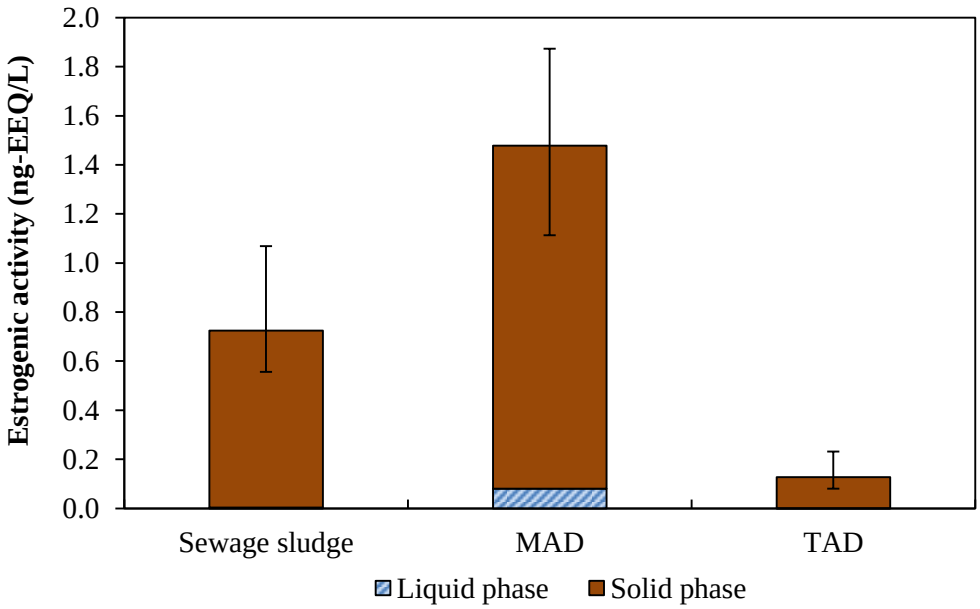

Figure S7. Estrogenic activity exerted by sludge samples in the second sampling campaign (Period II), expressed as estradiol equivalent concentration. Values report the mean of 3 replicates, together with the maximum and minimum value, split up by phase (solid and liquid).

S8. Ames test

Table S8a. Ames test: mutagenicity expressed as revertants/plate (mean and standard deviation) and mutagenicity ratio (revertants number in the sample/revertants number in negative control) for solid phase samples referring to both monitoring campaigns.

Sample	Dose (mg/plate)	Period I				Period II			
		TA98-S9		TA98+S9		TA98-S9		TA98+S9	
		Revertants/plate	Mutagenicity ratio	Revertants/plate	Mutagenicity ratio	Revertants/plate	Mutagenicity ratio	Revertants/plate	Mutagenicity ratio
Sewage Sludge	1	18.5 ± 6.4	0.98	21.5 ± 7.8	0.67	16.5 ± 2.1	1.06	25.0 ± 0.0	0.90
	10	21.5 ± 3.5	1.14	20.0 ± 1.4	0.62	21.5 ± 4.9	1.39	23.3 ± 0.8	0.83
	25	20.5 ± 3.5	1.09	22.0 ± 1.4	0.68	15.0 ± 0.0	1.97	27.0 ± 0.0	0.97
	50	22.0 ± 5.7	1.17	18.5 ± 4.9	0.58	n.a.	n.a.	27.0 ± 0.0	0.97
MAD	1	16.5 ± 2.1	0.88	27.5 ± 2.1	0.85	16.0±7.1	1.03	28.0±4.2	1.01
	10	19.0 ± 4.2	1.01	24.0 ± 1.4	0.75	16.0±0.0	1.03	27.5±0.7	0.99
	25	Tox	Tox	25.5 ± 3.5	0.79	14.0±0.0	0.90	25.0±0.0	0.90
	50	Tox	Tox	21.0 ± 0.0	0.65	n.a.	n.a.	19.0±0.0	0.68
TAD	1	13.0 ± 0.0	0.7	29.5 ± 2.1	0.92	22.0±4.2	1.42	29.5±7.8	1.06
	10	21.0 ± 4.2	1.1	30.0 ± 4.2	0.93	13.0±4.2	0.84	28.0±4.2	1.01
	25	15.0 ± 5.7	0.8	20.5 ± 4.9	0.64	23.0±0.0	1.48	32.0±0.0	1.15
	50	Tox	Tox	25.0 ± 1.4	0.78	n.a.	n.a.	26.0±0.0	0.94
Control -		18.8 ± 5.3		32.3 ± 5.2		15.5 ± 1.9		27.8 ± 2.5	
Control +		667 ± 26.9		934.9 ± 98.9		>1000		>1000	

Negative control: 100 µl/plate DMSO; Positive control: 10 µg/plate 2-nitrofluorene for TA98-S9 and 10 µg/plate 2-aminofluorene for TA98+S9.
Tox = toxic to bacteria; n.a. = not available

Table S8b. Ames test: mutagenicity expressed as revertants/plate (mean and standard deviation) and mutagenicity ratio (revertants number in the sample/revertants number in negative control) for liquid phase samples, referring to the monitoring campaign of Period I.

Sample	Dose (mL/plate)	TA98-S9		TA98+S9	
		Revertants/plate	Mutagenicity ratio	Revertants/plate	Mutagenicity ratio
Sewage Sludge	1	18.5 ± 0.7	1.09	23.0 ± 0.0	0.85
	5	25.0 ± 4.2	1.47	28.5 ± 2.1	1.06
	10	22.0 ± 1.4	1.29	29.5 ± 2.1	1.09
	25	15.0 ± 0.0	0.88	32.0 ± 7.1	1.19
	50	Tox	Tox	26.0 ± 0.0	0.96
MAD	1	17.0 ± 4.2	1.00	26.0 ± 5.7	0.96
	5	19.5 ± 6.4	1.15	32.5 ± 0.7	1.20
	10	20.5 ± 4.9	1.21	19.0 ± 4.2	0.70
	25	18.0 ± 2.8	1.06	28.0 ± 5.7	1.04
	50	16.0 ± 0.0	0.94	30.0 ± 0.0	1.11
TAD	1	22.0 ± 1.4	1.29	28.5 ± 4.9	1.06
	5	13.5 ± 0.7	0.79	24.0 ± 5.7	0.89
	10	19.0 ± 0.0	1.12	25.5 ± 3.5	0.94
	25	18.0 ± 0.0	1.06	19.5 ± 0.7	0.72
	50	Tox	Tox	14.0 ± 0.0	0.52
Control -		17.0 ± 2.9		27.0 ± 6.4	
Control +		>1000		>1000	

Negative control: 100 µl/plate DMSO; Positive control: 10 µg/plate 2-nitrofluorene for TA98-S9 and 10 µg/plate 2-aminofluorene for TA98+S9.
Tox = toxic to bacteria

S9. Comet test

Table S9. Comet test results on solid phase samples for the two monitoring campaigns expressed as average values of the visual score and tail intensity and their corresponding standard deviations.

Sample	Dose (mg_{eq})	Period I		Period II	
		Visual score	Tail Intensity (%)	Visual score	Tail Intensity (%)
Sewage sludge	0.5	148 ± 8**	3.99 ± 1.80	147 ± 4*	3.83 ± 0.76
	1	159 ± 12***	5.13 ± 2.09**	171 ± 13***	7.58 ± 2.28***
	5	Tox	Tox	Tox	Tox
	10	Tox	Tox	Tox	Tox
	25	Tox	Tox	Tox	Tox
	50	Tox	Tox	Tox	Tox
MAD	0.5	126 ± 6	1.54 ± 0.57	127 ± 1	1.37 ± 0.70
	1	143 ± 11**	2.81 ± 2.45	145 ± 7*	2.86 ± 1.47
	5	159 ± 7***	5.29 ± 0.58*	206 ± 6***	12.2 ± 1.2 ***
	10	188 ± 8***	8.62 ± 1.98***	273 ± 19 ***	22.5 ± 2.0 ***
	25	Tox	Tox	Tox	Tox
	50	Tox	Tox	Tox	Tox
TAD	0.5	122 ± 3	2.36 ± 0.35	128 ± 6	1.41 ± 0.75
	1	145 ± 7	3.18 ± 1.42	141 ± 7***	2.77 ± 1.30**
	5	159 ± 1	5.36 ± 1.65	166 ± 0***	5.23 ± 1.25***
	10	247 ± 54***	19.7 ± 8.1***	247 ± 4***	16.2 ± 1.7***
	25	275 ± 10 ***	24.2 ± 5.7***	309 ± 0***	29.9 ± 0.0***
	50	328 ± 20***	29.2 ± 4.2***	Tox	Tox
Negative control		117±4	0.80 ± 0.00	118 ± 0	0.86 ± 0.22

* statistical significance vs negative control: $p < 0.05$ in accordance with Dunnett test
** statistical significance vs negative control: $p < 0.01$ in accordance with Dunnett test
*** statistical significance vs negative control: $p < 0.001$ in accordance with Dunnett test

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REFERENCES

Bivins, J.L., Novak, J.T., 2001. Changes in Dewatering Properties between the Thermophilic and Mesophilic Stages in Temperature-Phased Anaerobic Digestion Systems. Water Environ. Res. 73, 444–449.

Malmborg, J., Magnér, J., 2015. Pharmaceutical residues in sewage sludge: Effect of sanitization and anaerobic digestion. J. Environ. Manage. 153, 1–10.

Radjenović, J., Petrović, M., Barceló, D., 2009. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Res. 43, 831–841.

Ternes, T.A., Bonerz, M., Herrmann, N., Löffler, D., Keller, E., Lacida, B.B., Alder, A.C., 2005. Determination of pharmaceuticals, iodinated contrast media and musk fragrances in sludge by LC tandem MS and GC/MS. J. Chromatogr. A 1067, 213–223.