SUPPLEMENTARY DATA

Role of methanogenesis on the biotransformation of organic micropollutants during anaerobic digestion

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S1. Physicochemical characteristics of selected OMPs

Table S1. Application and main physicochemical properties of the selected OMPs.

OMP	Application	MW (g/mol)	s (mg/L)	H (atm m^3/mol)	pKa	log K_{ow}
ERY	Antibiotic	733.9	1.4	$5.4 \cdot 10^{-29}$	8.9	3.1
ROX	Antibiotic	837.1	0.02	$5.0 \cdot 10^{-31}$	9.1	2.8
SMX	Antibiotic	253.3	610	$6.4 \cdot 10^{-13}$	6.2	0.9
TMP	Antibiotic	290.3	400	$2.4 \cdot 10^{-14}$	7.1	0.9
FLX	Antidepressant	309.3	60	$8.9 \cdot 10^{-8}$	9.8	4.1
CBZ	Anticonvulsant	236.3	112	$1.1 \cdot 10^{-10}$	15.9	2.5
DZP	Anxiolytic	284.7	50	$3.6 \cdot 10^{-9}$	3.4	2.8
E1	Estrogen	270.4	30	$3.8 \cdot 10^{-10}$	10.3	3.1
E2	Estrogen	272.4	3.6	$3.6 \cdot 10^{-11}$	10.3	4.0
EE2	Contraceptive/ estrogen	296.4	11.3	$7.9 \cdot 10^{-12}$	10.3	3.7
ADBI	Fragrance	244.4	0.22	$2.1 \cdot 10^{-4}$	–	5.9
HHCB	Fragrance	258.4	1.8	$1.3 \cdot 10^{-4}$	–	5.9
AHTN	Fragrance	258.4	0.21	$2.6 \cdot 10^{-4}$	–	5.8
IBP	Anti-inflammatory	206.3	21	$1.5 \cdot 10^{-6}$	4.9	4.0
NPX	Anti-inflammatory	230.3	15.9	$3.4 \cdot 10^{-10}$	4.2	3.2
DCF	Anti-inflammatory	296.2	2.4	$4.7 \cdot 10^{-12}$	4.2	4.2
OP	Surfactant	206.3	3.1	$8.5 \cdot 10^{-6}$	≈10	5.5
NP	Surfactant	220.4	7.0	$3.4 \cdot 10^{-5}$	≈10	5.8
TCS	Antiseptic	289.5	10	$5.0 \cdot 10^{-9}$	7.7	4.7
BPA	Fungicide/plasticizer	228.3	120	$1.0 \cdot 10^{-11}$	10.1	3.3

Molecular weight (MW), Henry's law constant (H), solubility at 25 °C (s), acid dissociation constant (pKa), octanol-water coefficient (K_{ow}).
Data obtained from DrugBank and PhysProp databases and from Varhanickova et al. (1995).

S2. Parameter estimation

The pseudo-first order kinetic constant was estimated by least-square fitting of the results of the kinetic experiments to equation S1.

$$k_{biol} = \text{argmin} \sum \left(C_T(t) - C_{T,exp}\right)^2 \qquad \textit{Equation S1}$$

where C_T was obtained according to equation 2 of the manuscript:

$$C_T = C_0 \cdot e^{\left(-\frac{1}{HRT} - k_{biol} \cdot X_{VSS}\right) \cdot t}$$

To obtain a robust estimation of k_{biol} a bootstrap procedure was followed to determine the expected value and the confidence interval of the parameter. The procedure was done as follows:

i) Estimate the residuals (e) of equation S1 by equation S2, which are used to simulate the experimental error in the measurements;

$$e = C_T(t) - C_{T,exp} \qquad \textit{Equation S2}$$

ii) Simulate new experimental results ($C^*_{T,exp}$) by randomly adding some of the residuals to the experimental data;

$$C^*_{T,exp} = C_{T,exp} + e_j \quad \text{where } e_j \text{ is a random variable } e_j \in e \qquad \textit{Equation S3}$$

iii) Carry out the least square estimation of k^*_{biol} for the new simulated data by using equation S1.

iv) Iterate through steps ii) and iii) until convergence (number of iterations = 1000) on a distribution of k_{biol} and use this distribution to find the expected value and its confidence interval.

S3. Analysis of organic micropollutants

Table S2. Limits of quantification (LOQ) and recovery ranges (n=3) of OMPs in the liquid and solid phase of the methanogenic sludge.

	LOQ		Recovery range (%)	
OMP	Liquid (ng/L)	Solid (ng/g)	Liquid	Solid
ERY	3	0.6	90-110	65-80
ROX	3	0.6	80-90	55-65
SMX	15	3	85-90	55-60
TMP	15	3	70-75	55-60
FLX	3	0.6	30-35	45-50
CBZ	15	3	65-75	70-80
DZP	15	3	50-60	60-70
E1	30	6	40-50	60-70
E2	30	6	60-70	60-70
EE2	30	6	60-80	60-75
ADBI	150	30	45-55	120-150
HHCB	150	30	40-60	120-150
AHTN	150	30	35-50	130-160
IBP	60	12	130-150	120-140
NPX	75	15	110-120	100-120
DCF	300	60	120-140	130-140
OP	60	12	60-70	120-140
NP	60	12	60-70	120-140
TCS	150	30	55-65	110-130
BPA	75	15	110-120	70-80

S4. Methanogenic reactors operation

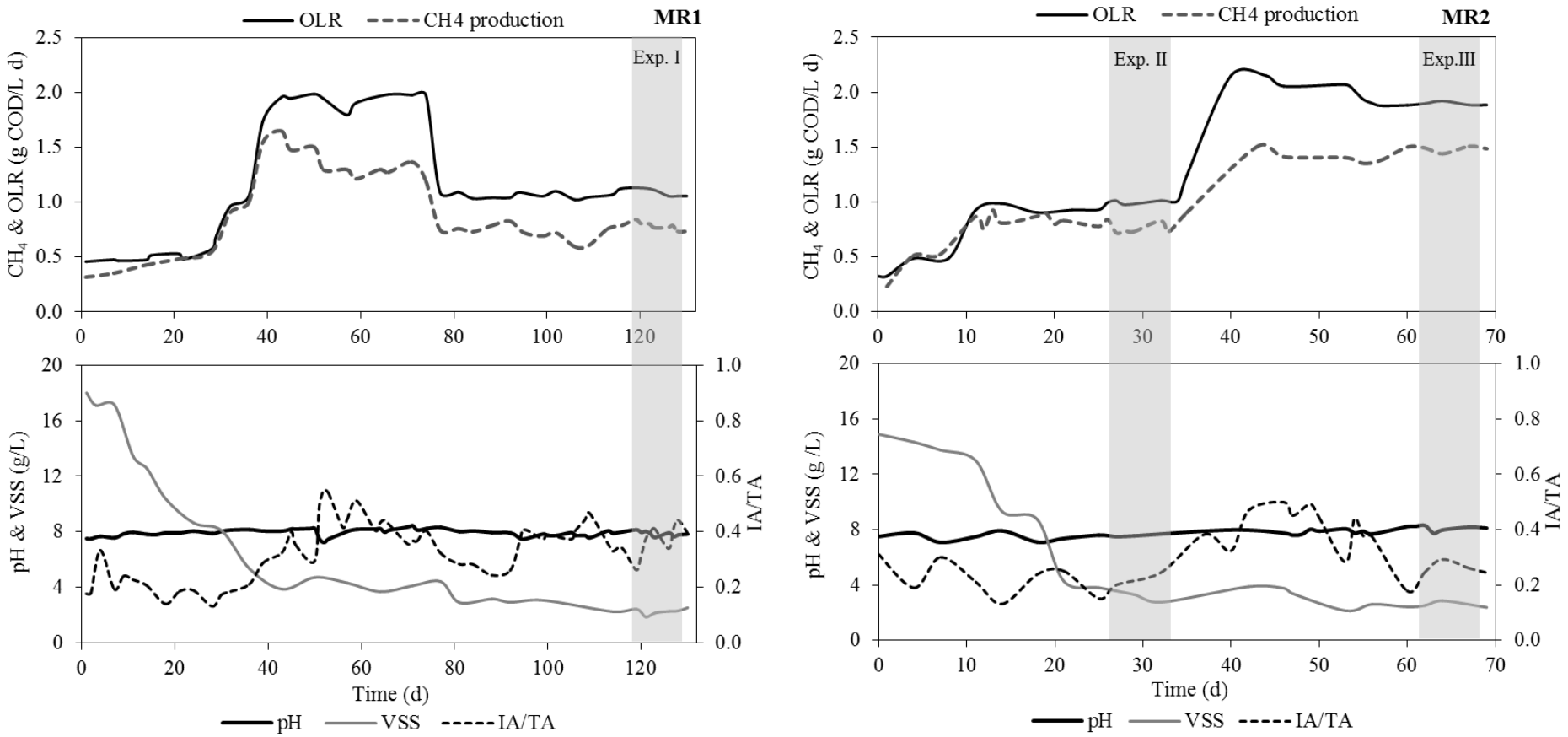

Figure S1. Performance of the methanogenic reactors (MR1 left side and MR2 right side) during the whole operation. Organic loading rate (OLR) and methane production (CH_4) are represented in the upper graphs, while pH, volatile suspended solids (VSS) and intermediate/total alkalinity ratio (IA/TA) are depicted in the bottom graphs. Grey areas highlight the periods when the kinetic experiments with OMPs were conducted.

Table S3. Average feeding characteristics of methanogenic reactors during both steady-state operational stages (OLR=1 and 2 g COD/L d).

	OLR=1 g COD/L d	OLR=2 g COD/L d
pH	6.3 ± 0.7	6.4 ± 0.6
Acetic acid (HAc, g/L)	4.7 ± 0.3	9.5 ± 0.4
Propionic acid (g/L)	1.7 ± 0.1	3.3 ± 0.1
Butyric acid (g/L)	1.4 ± 0.1	2.7 ± 0.1
Total VFA (g HAc_{eq}/L)	7.9 ± 0.7	15.9 ± 0.5
COD (g/L)	9.5 ± 1.0	18.8 ± 1.0
NH_4Cl (g/L)	0.6	0.6
KH_2PO_4 (g/L)	0.4	0.4

Trace concentrations of micro-nutrients (Fe, Ca, Mg, Cr, Co, Cu, Mn, Mo, Ni, Se, Zn, B) were added according to Angelidaki and Sanders (2004).

S5. Sorption of OMPs in digested sludge

Table S4. Partitioning coefficients (log *Kd*) reported for anaerobically digested sludge. The average range represents the minimum and the maximum values from literature.

OMP	log *Kd*					
	Gonzalez-Gil et al. (2016)	Carballa et al. (2007)	Narumiya et al. (2013)	Clara et al. (2011)	Ivashechkin et al. (2004)	**Average range**
TCS	3.56-4.35					**3.56-4.35**
AHTN	4.22-4.86	3.7-4.43		2.60-2.90		**2.60-4.86**
HHCB	2.58-5.14	3.45-4.3				**2.58-5.14**
FLX	2.44-3.44					**2.44-3.44**
E2	2.22-3.34	2.30-2.83				**2.22-3.34**
E1	1.76-2.91	2.18-2.77				**1.76-2.91**
BPA					2.09-2.30	**2.09-2.30**
TMP	1.92-2.86					**1.92-2.86**
ERY			1.60-3.10			**1.60-3.10**
ROX	2.52-3.97	1.14-1.92	1.90-3.30			**1.14-3.97**
EE2	1.21-1.40	2.08-2.85				**1.21-2.85**
DZP	1.85-1.88					**1.85-1.88**
DCF		1.26-2.18	1.90-2.20			**1.26-2.20**
CBZ	1.60-2.27	1.31-1.83	1.60-2.00			**1.31-2.27**
SMX		0.77-1.79				**0.77-1.79**
IBP	1.03-1.76	1.00-1.78				**1.00-1.78**
NPX		1.03-1.71				**1.03-1.71**

S6. Biotransformation kinetics

The concentrations measured in the liquid and solid phase of the methanogenic effluent during the three kinetic experiments are displayed in Figures S2. An average methanogenic kinetics obtained from the three experiments is shown as well. Except for NP, EE2, SMX, TMP, and NPX, the resulting average values presented a low deviation, meaning that the concentrations of OMPs measured in three kinetic experiments were quite similar. Moreover, continuous lines depict the concentration patterns of OMPs in both phases if solely washout was the responsible for the removal. Therefore, the difference between the washout line and the experimental point corresponds to biotransformation.

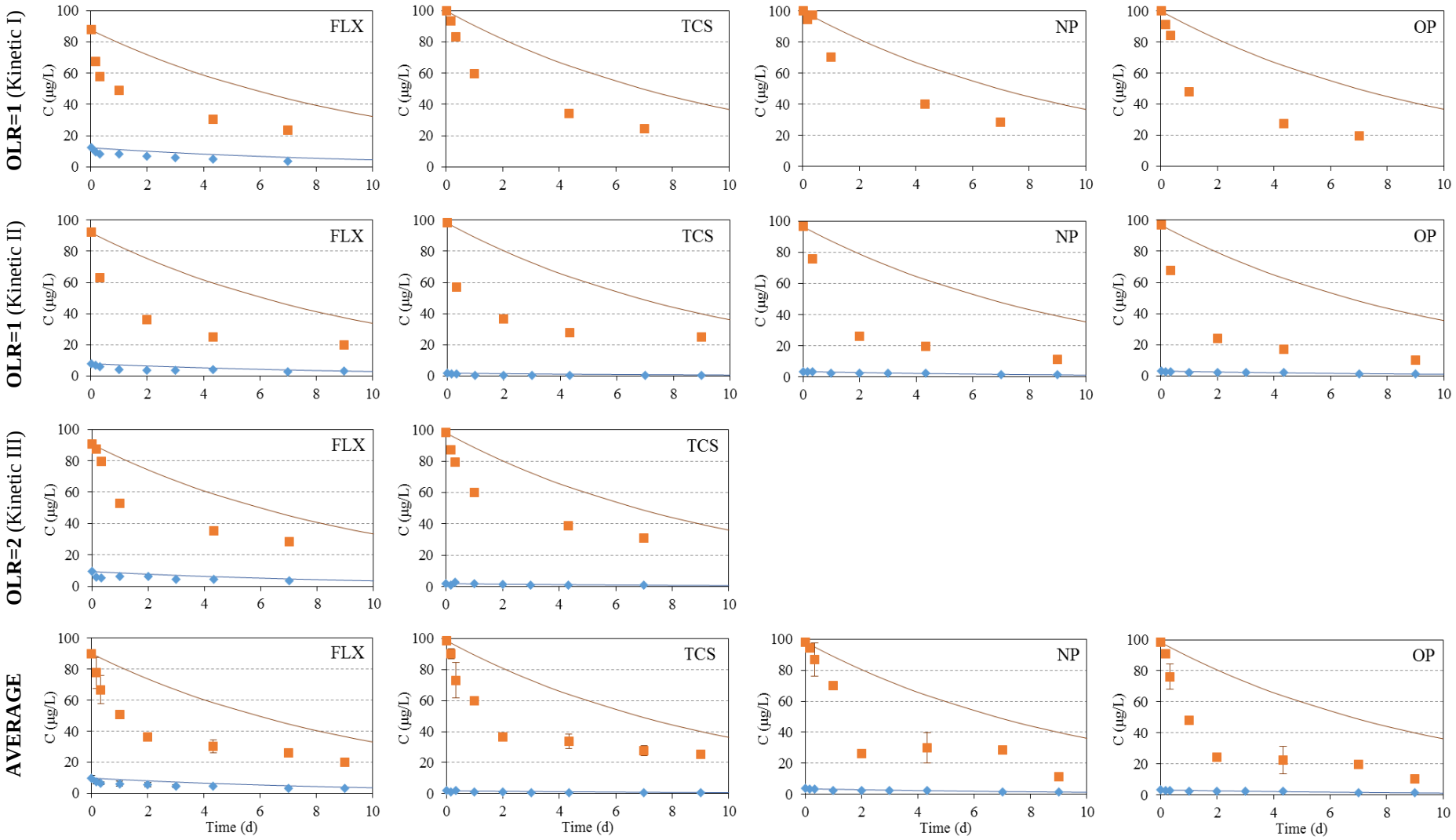

Fig. S2a. Measured concentrations in the liquid (blue diamonds) and solid (orange squares) phases of hydrophobic compounds (Group 3). Continuous lines show the removal of the OMP by washout in the liquid (blue) and solid phase (orange).

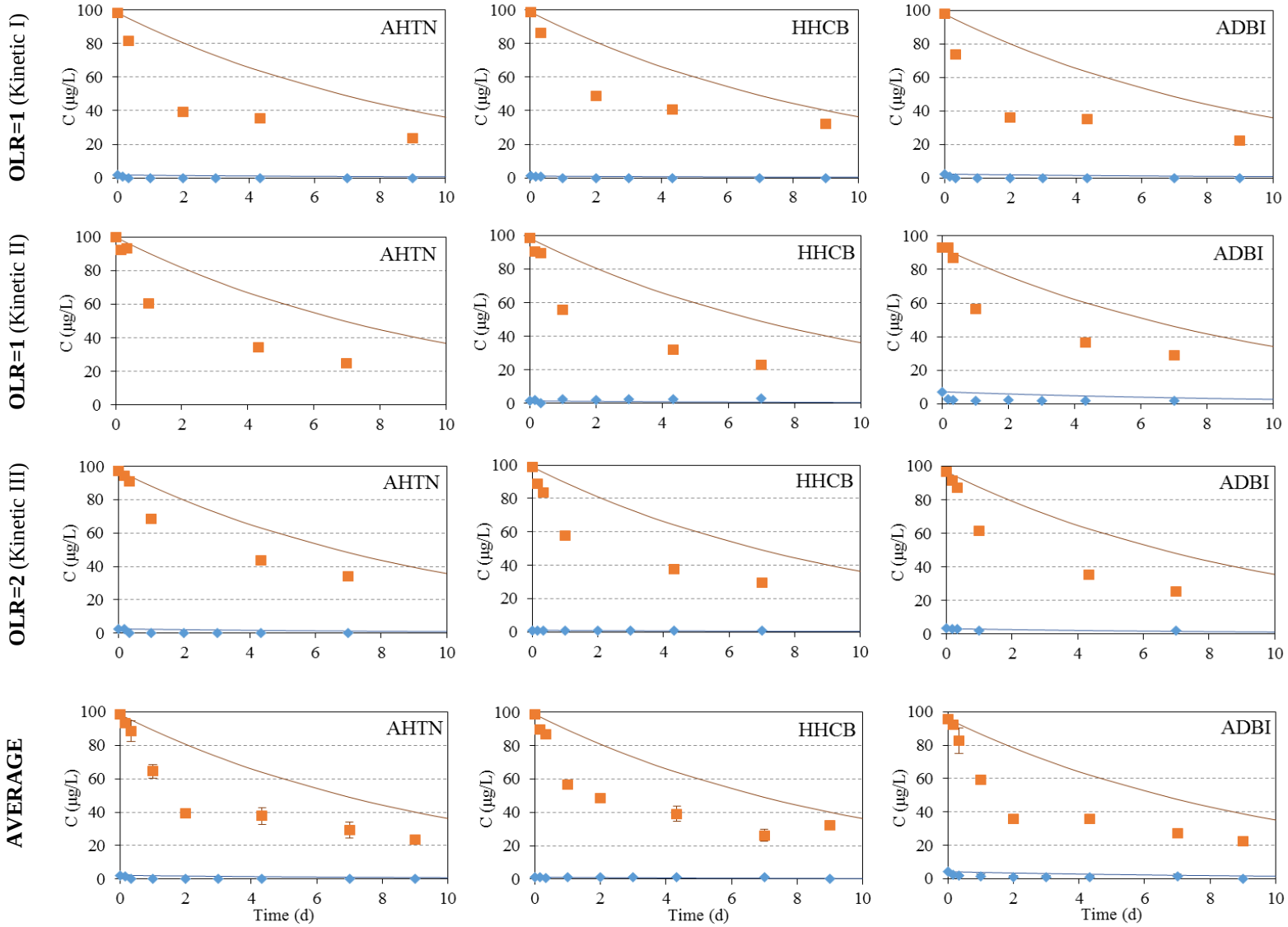

Fig. S2b. Measured concentrations in the liquid (blue diamonds) and solid (orange squares) phases of hydrophobic compounds (Group 3). Continuous lines show the removal of the OMP by washout in the liquid (blue) and solid phase (orange).

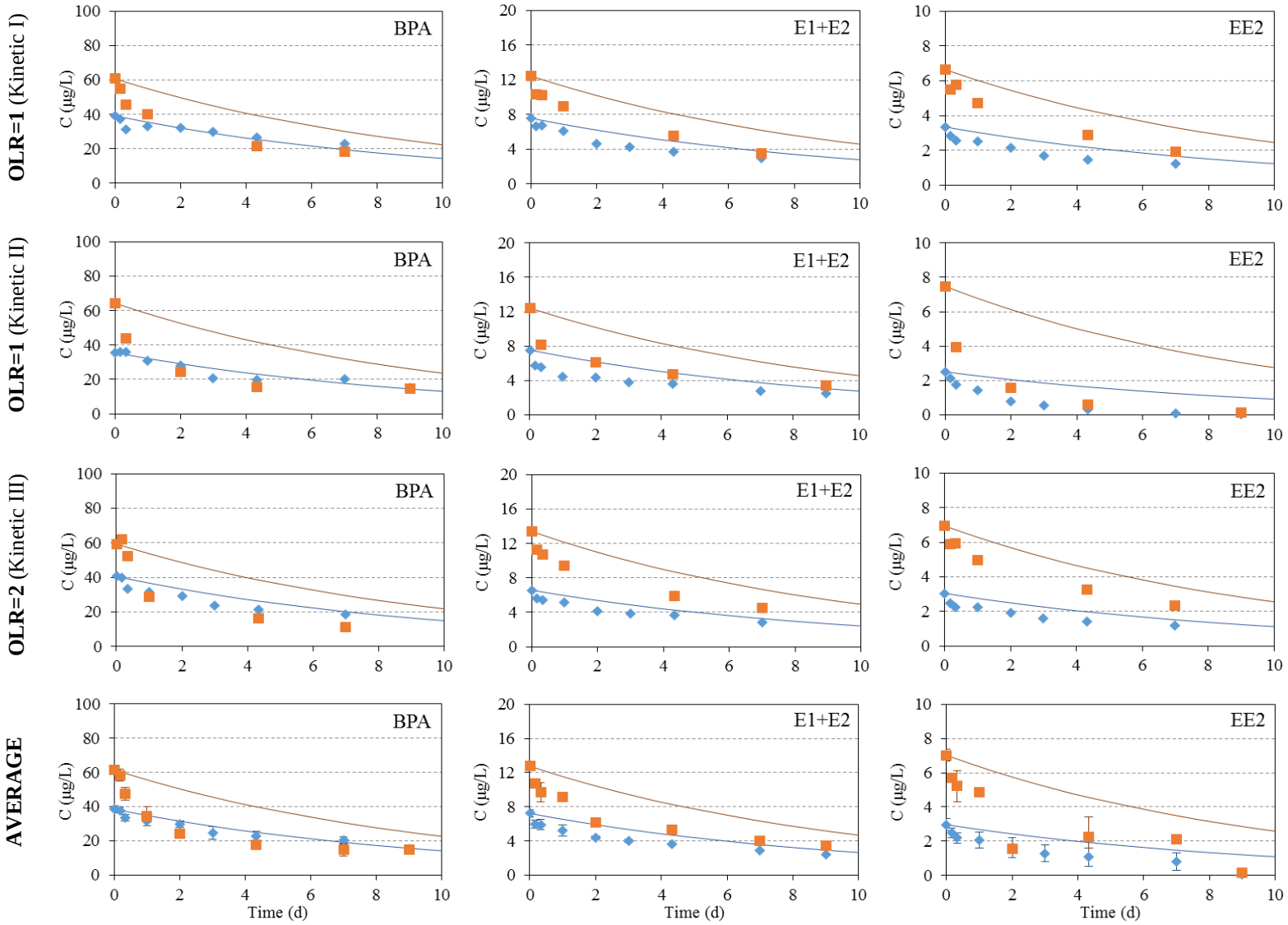

Fig. S2c. Measured concentrations in the liquid (blue diamonds) and solid (orange squares) phases of compounds with a medium hydrophobicity (Group 2). Continuous lines show the removal of the OMP by washout in the liquid (blue) and solid phase (orange).

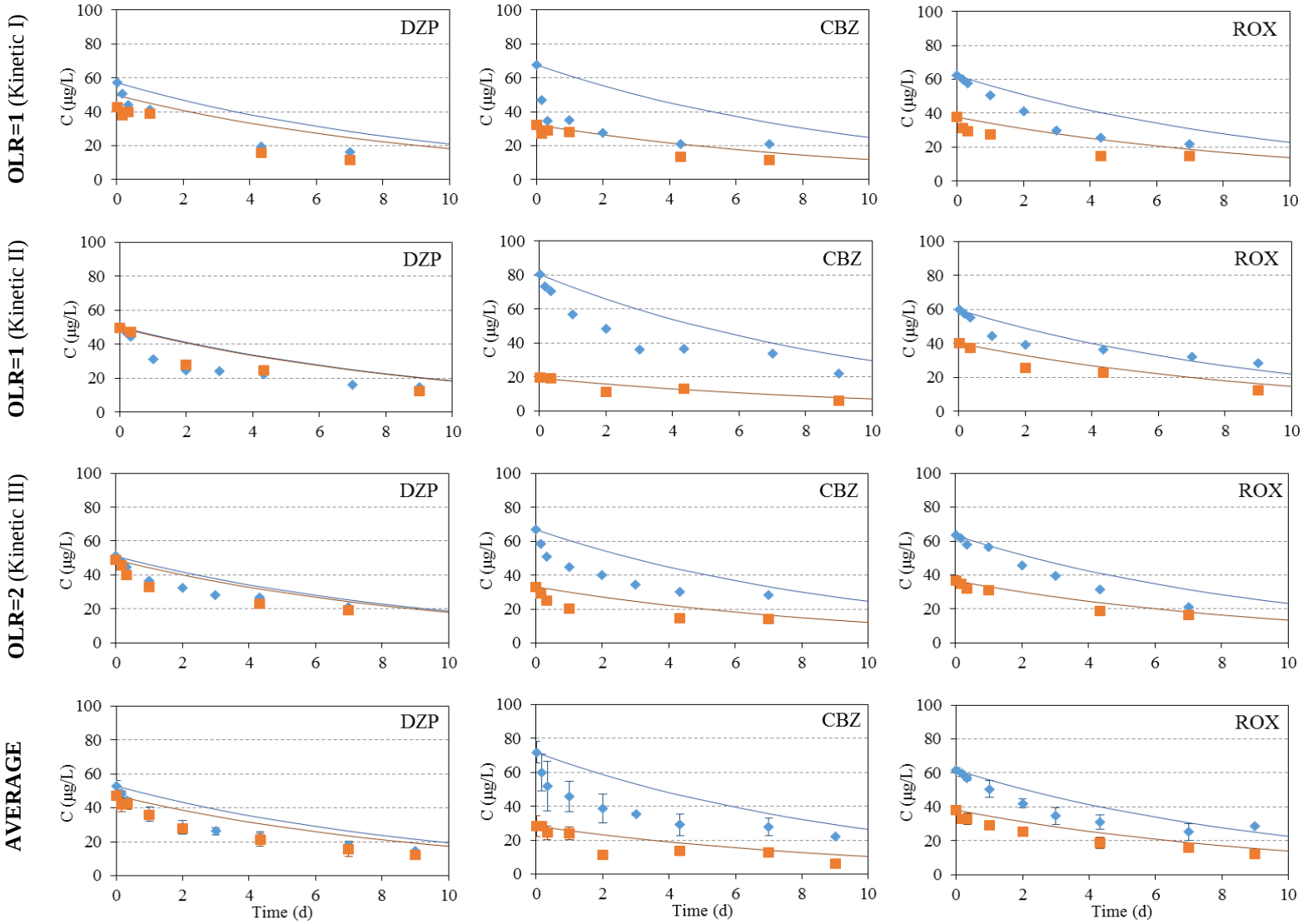

Fig. S2d. Measured concentrations in the liquid (blue diamonds) and solid (orange squares) phases of compounds with a medium (DZP, Group 2) or low hydrophobicity (CBZ and ROX, Group 1). Continuous lines show the removal of the OMP by washout in the liquid (blue) and solid phase (orange).

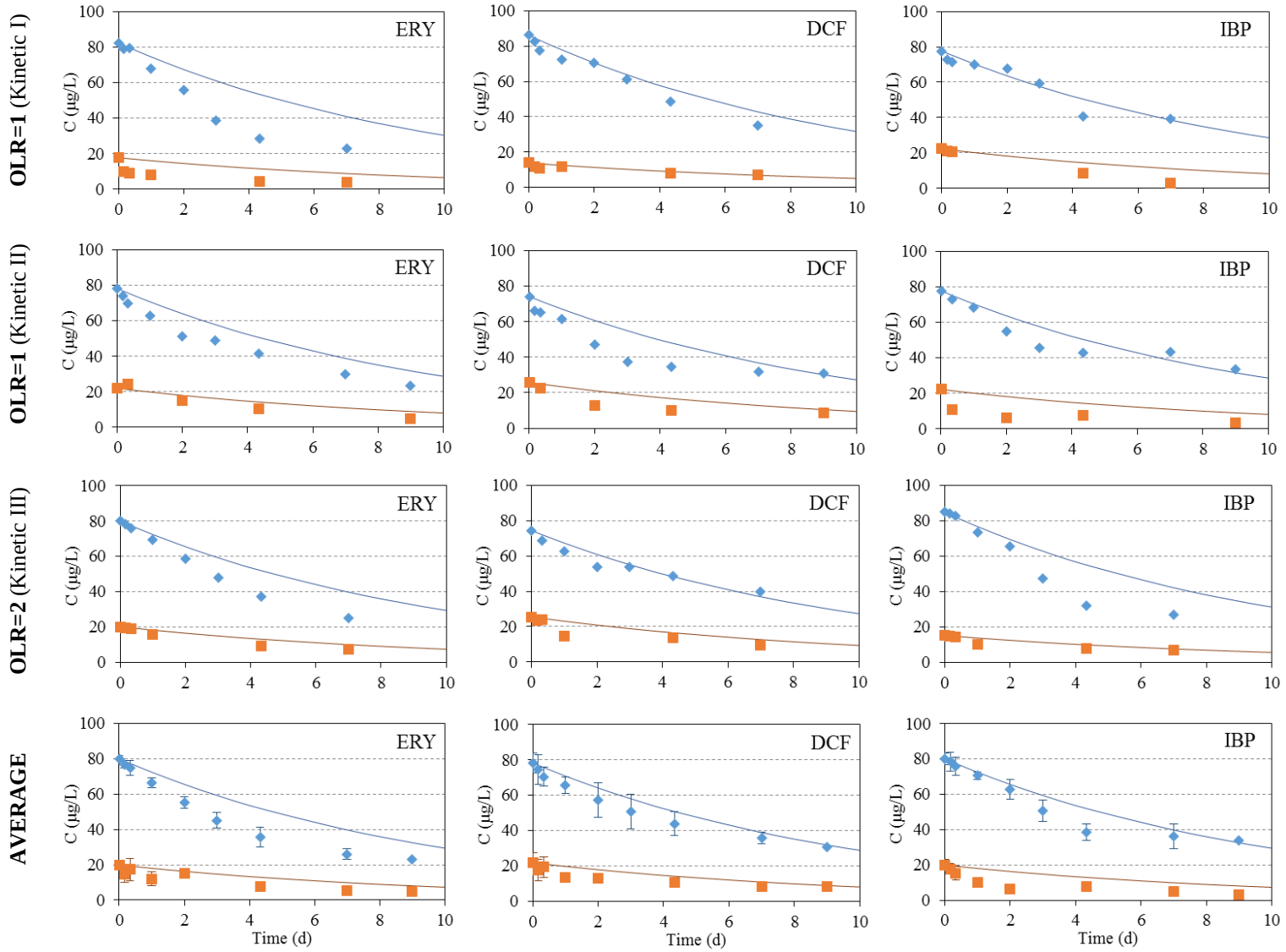

Fig. S2e. Measured concentrations in the liquid (blue diamonds) and solid (orange squares) phases of hydrophilic compounds (Group 1). Continuous lines show the removal of the OMP by washout in the liquid (blue) and solid phase (orange).

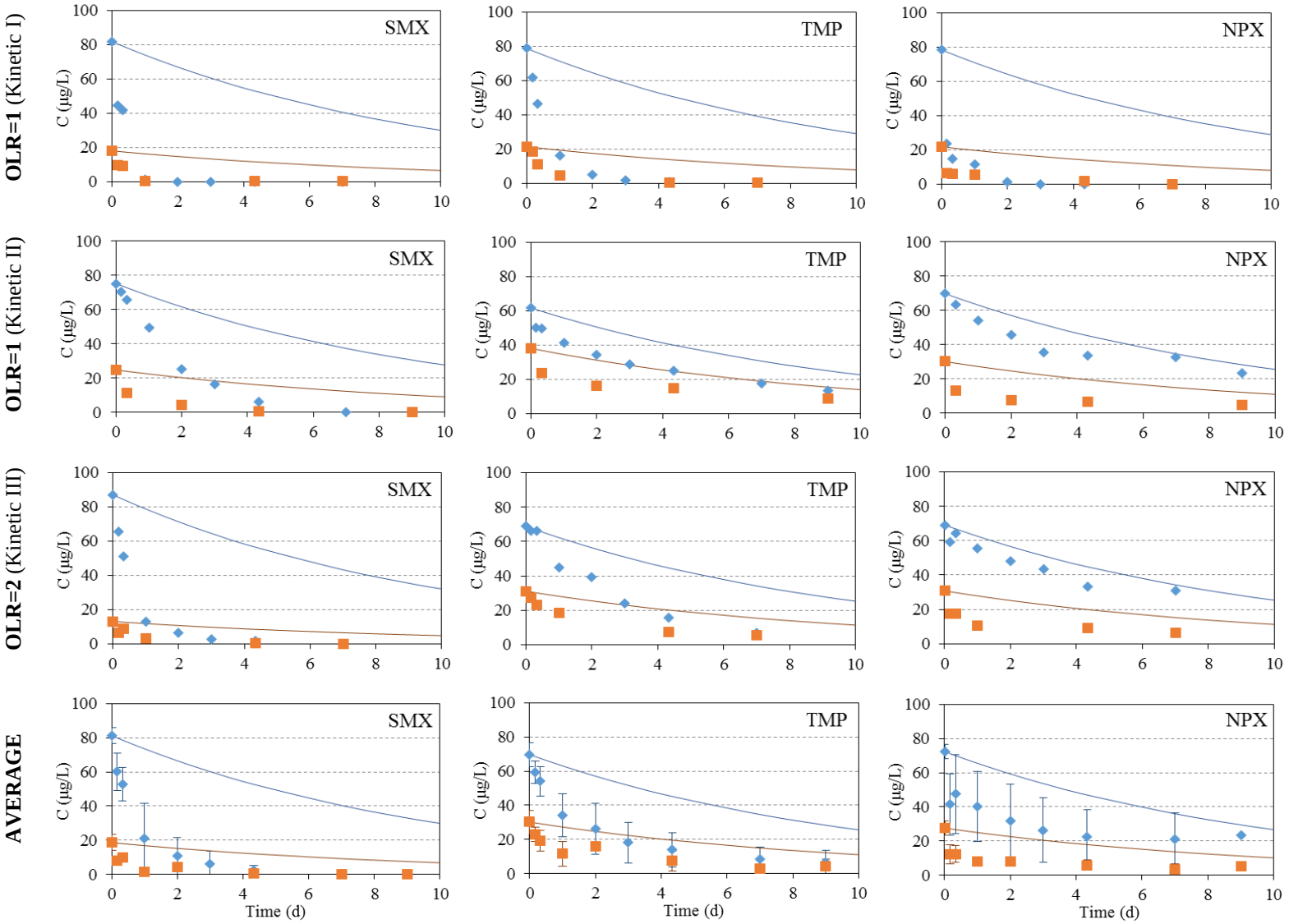

Fig. S2f. Measured concentrations in the liquid (blue diamonds) and solid (orange squares) phases of hydrophilic compounds (Group 1). Continuous lines show the removal of the OMP by washout in the liquid (blue) and solid phase (orange).

S7. Biotransformation rate constants in the methanogenic step

Table S5. Biotransformation kinetic constants (k_{biol}) calculated with the pseudo-first order model under methanogenic conditions. The estimated values are reported with the corresponding confidence interval (CI) [2.5, 97.5] % and the resulting root mean square errors (RMSE).

		Experiment I			Experiment II			Experiment III			Average
	OMPs	k_{biol} (L/gVSS d)	CI (95%)	RMSE (µg/L)	k_{biol} (L/gVSS d)	CI (95%)	RMSE (µg/L)	k_{biol} (L/gVSS d)	CI (95%)	RMSE (µg/L)	k_{biol} (L/gVSS d)
Group 1	NPX	0.043	[0.012, 0.107]	12	**2.055***	**[1.467, 3.299]**	9.5	0.020	[0.001, 0.056]	15	0.706
	SMX	**0.244***	**[0.212, 0.264]**	2.3	0.749	[0.617, 0.957]	6.3	0.631	[0.575, 0.692]	2.4	0.541
	TMP	0.056	[0.017, 0.117]	13	**0.457***	**[0.420, 0.488]**	2.1	0.100	[0.080, 0.120]	3.3	0.204
	CBZ	0.029	[0.012, 0.047]	7.2	0.026	[0.001, 0.200]	20	0.015	[0.000, 0.046]	15	0.023
	ERY	0.025	[0.015, 0.032]	4.1	0.035	[0.027, 0.052]	5.8	0.027	[0.025, 0.029]	1.2	0.029
	IBP	0.015	[0.000, 0.045]	11	0.011	[0.008, 0.018]	4.2	0.032	[0.022, 0.041]	3.9	0.019
	ROX	0.007	[0.000, 0.022]	7.9	0.019	[0.014, 0.037]	7.2	0.016	[0.011, 0.021]	3.0	0.014
	DCF	0.018	[0.002, 0.062]	11	0.007	[0.002, 0.011]	4.6	0.000	[0.000, 0.016]	8.0	0.008
Group 2	BPA	0.043	[0.018, 0.110]	11	0.008	[0.001, 0.022]	11	0.043	[0.012, 0.071]	10	0.031
	EE2	**0.472***	**[0.255, 0.827]**	0.8	0.025	[0.010, 0.050]	1.2	0.012	[0.000, 0.036]	1.4	0.170
	E1+E2	0.039	[0.002, 0.110]	3.0	0.021	[0.011, 0.034]	1.6	0.017	[0.002, 0.034]	2.1	0.026
	DZP	0.042	[0.011, 0.074]	10	0.031	[0.020, 0.047]	6.8	0.016	[0.000, 0.033]	9.3	0.030
Group 3	OP	0.284	[0.136, 0.608]	11	0.103	[0.031, 0.278]	12	n.a.			0.194
	NP	**0.217***	**[0.129, 0.393]**	10	0.032	[0.020, 0.044]	6.0	n.a.			0.125
	TCS	0.148	[0.020, 1.447]	20	0.046	[0.022, 0.088]	9.5	0.035	[0.012, 0.071]	12	0.076
	FLX	0.121	[0.039, 0.781]	15	0.036	[0.008, 0.280]	18	0.039	[0.008, 0.081]	12	0.065
	ADBI	0.106	[0.031, 0.423]	15	0.037	[0.012, 0.070]	11	0.051	[0.024, 0.080]	7.8	0.065
	AHTN	0.093	[0.033, 0.240]	13	0.047	[0.023, 0.070]	8.8	0.031	[0.010, 0.049]	7.2	0.057
	HHCB	0.044	[0.012, 0.121]	12	0.047	[0.017, 0.086]	9.8	0.039	[0.013, 0.093]	12	0.043

* Significantly different ($\alpha = 0.05$) k_{biol} values respect to the other two experiments. n.a. not available data

S8. Biotransformation phase

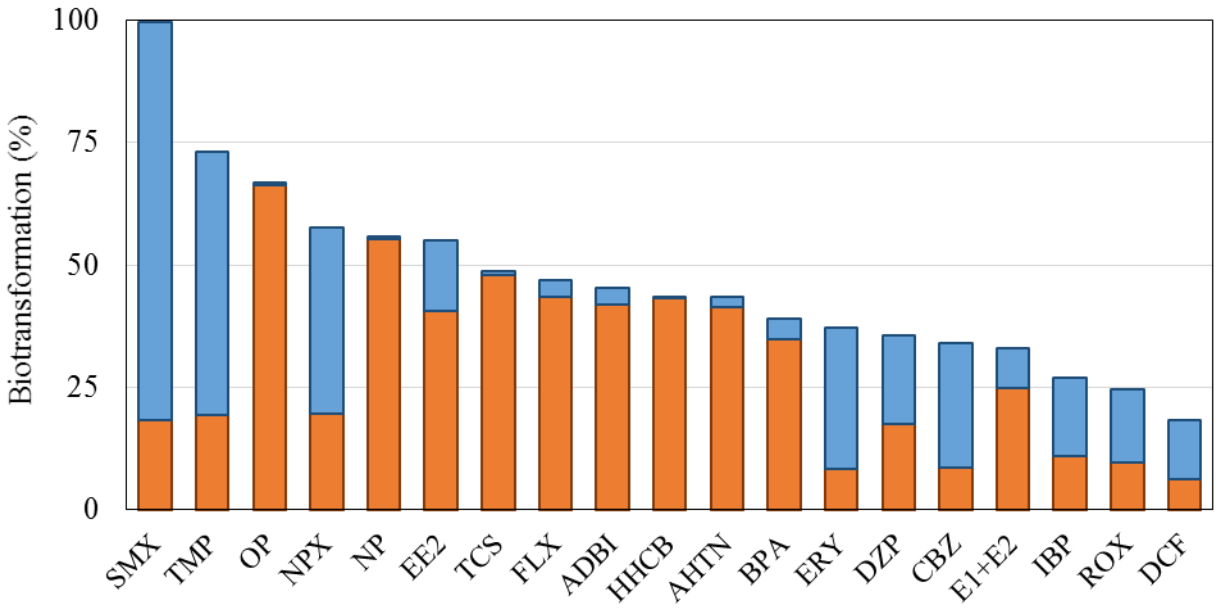

Fig. S3. Contribution of each phase to the total methanogenic biotransformation of OMPs. The apparent biotransformations measured in the solid (B_S) and liquid phases (B_L) are represented by the orange, lower bars and by the blue, upper bars, respectively.

S9. Removal of OMPs during sewage sludge AD

Table S6. Summary of reported OMPs removal during AD of sewage sludge under similar operational conditions. The average removal was calculated with the mean value of each reference and for those cases where two completely different values were reported both were considered.

OMP	AD removal (%)									
	Gonzalez-Gil et al. (2016)	Carballa et al. (2007)	Narumiya et al. (2013)	Samaras et al. (2014)	Malmborg & Magnér (2015)	Clara et al. (2011)	Paterakis et al. (2012)	Bergersen et al. (2012)	Yang et al. (2016)	Average
SMX	80	100	100	–	–	–	–	–	–	93 ± 12
NPX	100	85	–	85	85	–	–	–	90	89 ± 7
TMP	75	–	100	–	100	–	–	–	90	91 ± 12
NP	–	–	–	35	–	–	0/100	–	–	45 ± 51
FLX	70	–	–	–	0	–	–	30	30	33 ± 29
EE2	65	40/95	–	–	0	–	20	–	–	44 ± 37
TCS	15	–	30	65	–	–	–	–	50	40 ± 22
Musks	10	60	–	–	–	0/45	–	–	–	29 ± 28
BPA	–	–	–	80	–	–	–	–	0	40 ± 40
E1+E2	0	80	–	–	0	–	50	–	–	33 ± 39
CBZ	40	5	0	–	15	–	–	–	0	11 ± 15
DZP	50	30	–	–	–	–	–	–	–	40 ± 10
ERY	–	–	45	–	–	–	–	–	–	45
ROX	85	95	65	–	–	–	–	–	–	82 ± 15
DCF	–	0/80	25	95	25	–	–	–	0	38 ± 41
IBP	30	45	–	95	30	–	–	–	10	42 ± 32
OP	–	–	–	–	–	–	–	–	–	–
Operational conditions										
Continuous digester	Lab-scale	Lab-scale	Full-scale	Lab-scale	Lab-scale	Full-scale	Lab-scale	Lab-scale	Full-scale	
OMPs spike	No	Yes	No	Yes	Yes	No	No	Yes	No	
Substrate*	MixS	MixS	MixS	MixS	MixS	SS	MixS & PS	SS	PS	
Temperature (°C)	37	37	30-55	37	37	37	35	37	35	
HRT (d)	20-30	10-30	20-30	20	20	15-20	30	20	15-30	

*MixS: mixed primary and secondary sewage sludge; PS: primary sludge; SS: sewage sludge (type not specified).

References

Bergersen, O., Østnes, K., Vasskog, T., 2012. Anaerobic treatment of sewage sludge containing selective serotonin reuptake inhibitors. Bioresour. Technol. 117, 325–332.

Carballa, M., Omil, F., Ternes, T., Lema, J.M., 2007. Fate of pharmaceutical and personal care products (PPCPs) during anaerobic digestion of sewage sludge. Water Res. 41, 2139–50.

Clara, M., Gans, O., Windhofer, G., Krenn, U., Hartl, W., Braun, K., Scharf, S., Scheffknecht, C., 2011. Occurrence of polycyclic musks in wastewater and receiving water bodies and fate during wastewater treatment. Chemosphere 82, 1116–1123.

DrugBank database; http://www.drugbank.ca.

Gonzalez-Gil, L., Papa, M., Feretti, D., Ceretti, E., Mazzoleni, G., Steimberg, N., Pedrazzani, R., Bertanza, G., Lema, J.M., Carballa, M., 2016. Is anaerobic digestion effective for the removal of organic micropollutants and biological activities from sewage sludge? Water Res. 102, 211–220.

Ivashechkin, P., Corvini, P.-X., Dohmann, M., 2004. Behaviour of endocrine disrupting chemicals during the treatment of municipal sewage sludge. Water Sci. Technol. 50, 133–140.

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

Narumiya, M., Nakada, N., Yamashita, N., Tanaka, H., 2013. Phase distribution and removal of pharmaceuticals and personal care products during anaerobic sludge digestion. J. Hazard. Mater. 260, 305–12.

Paterakis, N., Chiu, T.Y., Koh, Y.K.K., Lester, J.N., McAdam, E.J., Scrimshaw, M.D., Soares, A., Cartmell, E., 2012. The effectiveness of anaerobic digestion in removing estrogens and nonylphenol ethoxylates. J. Hazard. Mater. 199–200, 88–95.

Samaras, V.G., Stasinakis, A.S., Thomaidis, N.S., Mamais, D., Lekkas, T.D., 2014. Fate of selected emerging micropollutants during mesophilic, thermophilic and temperature co-phased anaerobic digestion of sewage sludge. Bioresour. Technol. 162, 365–72.

Syracuse Research Corporation (SRC) PhysProp database; http://www.srcinc.com.

Varhanickova, D., Shiu, W.-Y., Mackay, D., 1995. Aqueous solubilities of alkylphenols and methoxyphenols at 25 ^{o}C. J. Chem. Eng. Data 40, 448–451.

Yang, S., Hai, F.I., Price, W.E., McDonald, J., Khan, S.J., Nghiem, L.D., 2016. Occurrence of trace organic contaminants in wastewater sludge and their removals by anaerobic digestion. Bioresour. Technol. 210, 153–159.