Supporting Information

Cometabolic enzymatic transformation of organic micropollutants under methanogenic conditions

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S1 Enzymatic pathways during acetate methanization

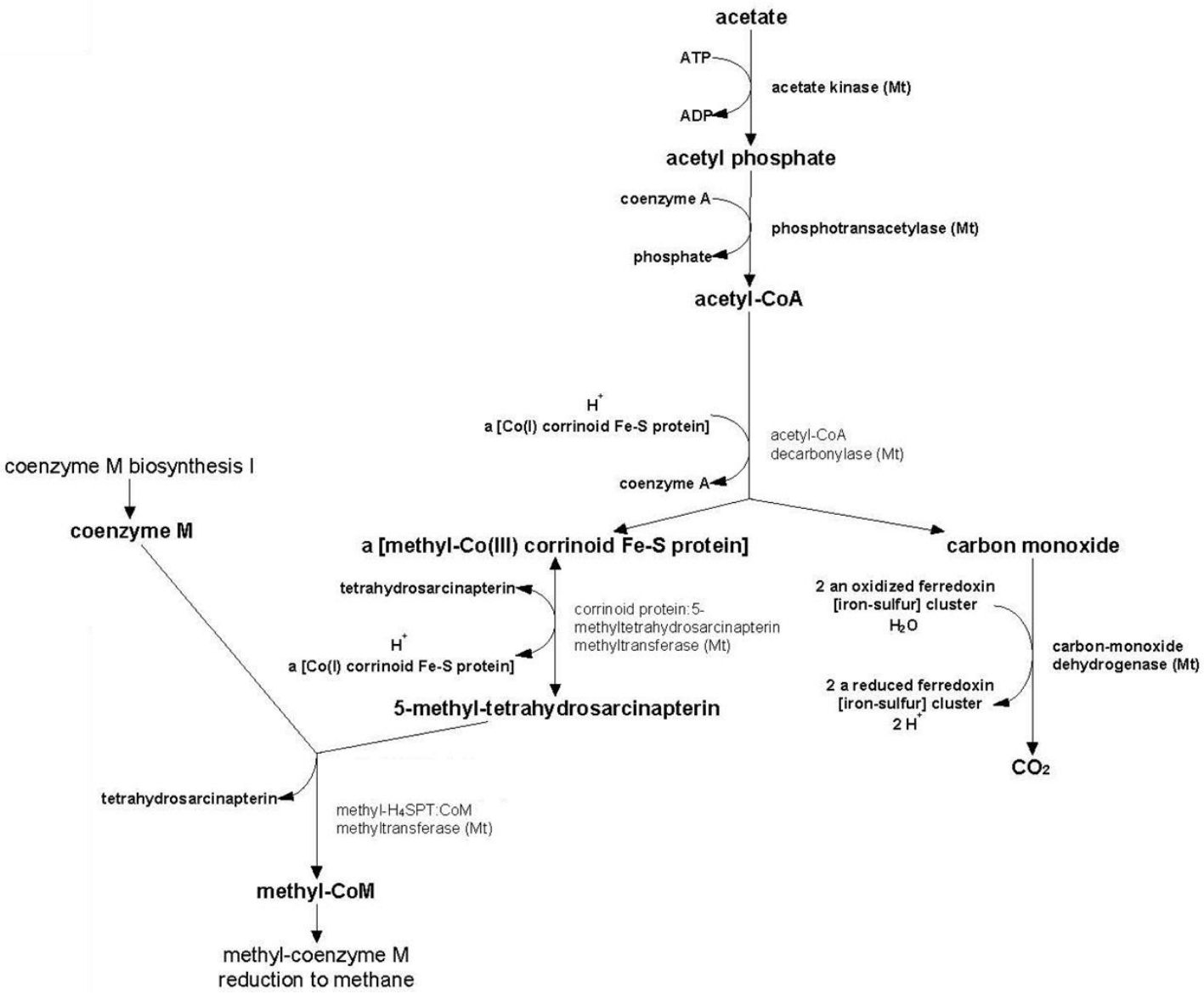


Figure S1. Enzymatic pathways involved in the aceticlastic methanogenesis of *Methanosarcina thermofila* (Mt).[1]

S2 Physicochemical characteristics of selected OMPs

Table S1. Chemical structures of the studied OMPs.

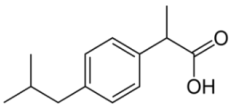
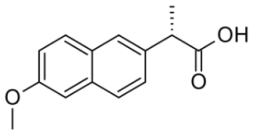
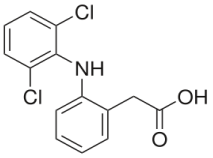
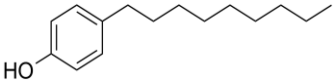
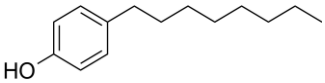
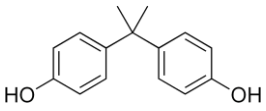
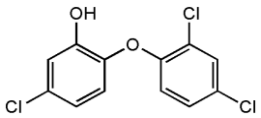
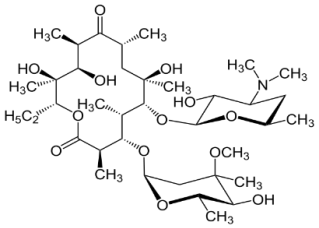
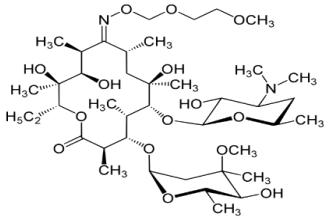
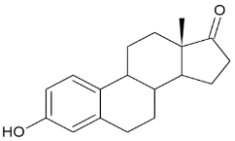
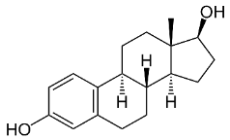
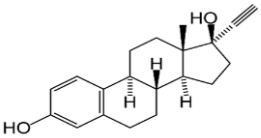
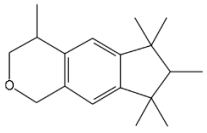
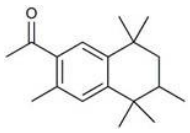
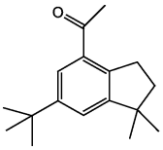
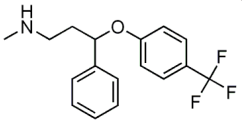
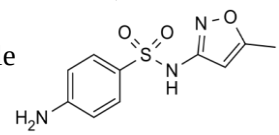
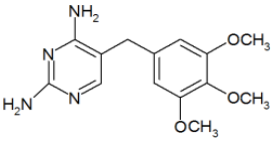
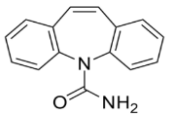
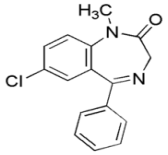
	Compound	Chemical structure	Compound	Chemical structure
GROUP 1 (carboxyl group)	Ibuprofen (IBP)		Naproxen (NPX)	
	Diclofenac (DCF)			
GROUP 2 (hydroxyl group)	Nonylphenol (NP)		Octylphenol (OP)	
	Bisphenol A (BPA)		Triclosan (TCS)	
	Erythromycin (ERY)		Roxithromycin (ROX)	
	Estrone (E1)		17β-estradiol (E2)	
	17α-ethinylestradiol (EE2)			
GROUP 3 (other functional groups)	Galaxolide (HHCB)		Tonalide (AHTN)	
	Celestolide (ADBI)		Fluoxetine (FLX)	
	Sulfamethoxazole (SMX)		Trimethoprim (TMP)	
	Carbamazepine (CBZ)		Diazepam (DZP)	

Table S2. Application and representative physicochemical properties of selected OMPs.

OMP	Application	MW [2] ($g\ mol^{-1}$)	s [2] ($mg\ L^{-1}$)	H [2] ($atm\ m^3\ mol^{-1}$)	pKa	log K_{ow} [2]
ERY	Antibiotic	733.9	1.4	$5.4 \cdot 10^{-29}$	8.9 [2]	3.1
ROX	Antibiotic	837.1	0.02	$5.0 \cdot 10^{-31}$	9.1 [3]	2.8
SMX	Antibiotic	253.3	610	$6.4 \cdot 10^{-13}$	6.2 [3]	0.9
TMP	Antibiotic	290.3	400	$2.4 \cdot 10^{-14}$	7.1 [2]	0.9
FLX	Antidepressant	309.3	60	$8.9 \cdot 10^{-8}$	9.8 [3]	4.1
CBZ	Anticonvulsant	236.3	112	$1.1 \cdot 10^{-10}$	15.9 [3]	2.5
DZP	Anxiolytic	284.7	50	$3.6 \cdot 10^{-9}$	3.4 [2]	2.8
E1	Estrogen	270.4	30	$3.8 \cdot 10^{-10}$	10.3 [3]	3.1
E2	Estrogen	272.4	3.6	$3.6 \cdot 10^{-11}$	10.3 [3]	4.0
EE2	Contraceptive/ estrogen	296.4	11.3	$7.9 \cdot 10^{-12}$	10.3 [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 [2]	3.97
NPX	Anti-inflammatory	230.3	15.9	$3.4 \cdot 10^{-10}$	4.2 [2]	3.2
DCF	Anti-inflammatory	296.2	2.4	$4.7 \cdot 10^{-12}$	4.2 [2]	4.2
OP	Surfactant	206.3	3.1	$8.5 \cdot 10^{-6}$	≈10 [4]	5.5
NP	Surfactant	220.4	7.0	$3.4 \cdot 10^{-5}$	≈10 [4]	5.8
TCS	Antiseptic	289.5	10	$5.0 \cdot 10^{-9}$	7.7 [3]	4.7
BPA	Fungicide/plasticizer	228.3	120	$1.0 \cdot 10^{-11}$	10.1 [2]	3.3

Molecular weight (MW), Henry's law constant (H), solubility at 25 °C (s), acid dissociation constant (pKa), octanol-water coefficient (K_{ow}).

S3 Analysis of organic micropollutants

Table S3. Limits of quantification (LOQ) and recovery ranges for the samples of the methanogenic reactors (MRs) and the acetate kinase (AK) assays.

	LOQ (ng L^{-1})		Recovery range (%)	
OMP	MR	AK assays	MR	AK assays
ERY	3.0	6.0	90-110	85-90
ROX	3.0	6.0	70-90	55-60
SMX	15	30	80-90	95-100
TMP	15	30	60-70	90-95
FLX	3.0	6.0	40-50	70-75
CBZ	15	30	70-80	95-100
DZP	15	30	50-60	95-100
E1	30	60	50-60	90-95
E2	30	60	60-70	95-100
EE2	30	60	60-80	95-100
ADBI	150	300	55-75	50-55
HHCB	150	300	65-95	90-95
AHTN	150	300	45-65	40-45
IBP	60	120	130-150	115-120
NPX	75	150	100-120	115-120
DCF	300	600	120-140	115-120
OP	60	120	130-150	65-70
NP	60	120	130-150	50-55
TCS	150	300	110-130	100-105
BPA	75	150	80-90	110-115

S4 Methanogenic reactors performance

The synthetic feeding was composed by a mixture of volatile fatty acids (VFA): acetic acid (HAc) (4.7 g L^{-1}), propionic acid (1.7 g L^{-1}) and butyric acid (1.4 g L^{-1}), in a COD proportion of 50:25:25, respectively. The feeding was supplemented with macro- (nitrogen and phosphorous) and micro-nutrients (Fe, Ca, Mg, Cr, Co, Cu, Mn, Mo, Ni, Se, Zn, Se, B),[5] as well as NaH_2CO_3 (5-10 g L^{-1}) as buffer and NaOH to adjust the pH to 6-7.

OMPs were spiked into the MRs at concentrations detected in sewage sludge, which can reach values above 100 µg L^{-1} for some OMPs (i.e. musk fragrances).[6,7] To avoid differences in their biotransformation kinetics, this concentration was used for all OMPs, except for hormones, whose values in sewage sludge[6–8] are usually below 10 µg L^{-1} and higher concentrations could strongly modify the estrogenicity of the reactors.

Table S4. Average operational, feeding and performance parameters of both methanogenic reactors (MRs) during steady-state period (1-2 months).

Operational parameters	
Temperature (°C)	37.0 ± 0.5
HRT (d)	10
OLR (g COD L^{-1} d^{-1})	1.0 ± 0.1
Feeding characteristics	
VFA (g HAc_{eq} L^{-1})	7.9± 0.7
COD (g L^{-1})	9.5 ± 1.0
Reactor performance	
pH	7.7 ± 0.3
VFA (g HAc_{eq} L^{-1})	1.4± 0.5
Intermediate/total alkalinity	0.29 ± 0.07
TSS (g L^{-1})	9.3 ± 1.9
VSS (g L^{-1})	2.9 ± 0.6
COD removal (%)	78 ± 9
Biogas production (L L^{-1} d^{-1})	0.50 ± 0.10
Biogas composition (% CH_4)	62 ± 5
CH_4 production (g COD L^{-1} d^{-1})	0.77 ± 0.12

S5 Determination of acetate kinase activity

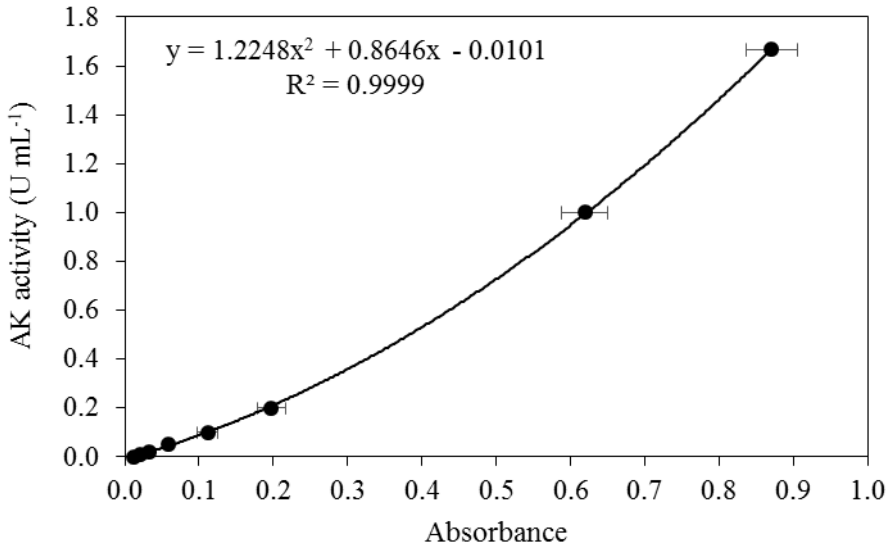


Figure S2. Standard curve to correlate the absorbance and AK activity.

Since 1 U of enzyme dephosphorylates 1 μmol of ATP per minute (incubation time 12 min), the consumption of ATP is correlated to the absorbance through Equation S1.

$$y = 14.698 \cdot x^2 + 10.375 \cdot x - 0.1212 \qquad \text{(Equation S1)}$$

where *y* is the ATP consumption (μmol mL^{-1}), and *x* is the absorbance. The correlation coefficient is R^2=0.9999.

S6 Preliminary assay with commercial acetate kinase

Table S5. Influence of temperature, pH and AK/OMPs ratio on the AK activity. The effect of temperature was tested at a pH of 7.4, the pH effect was evaluated at 25 °C and the influence of the ratio OMPs/AK was assessed at 25 °C and pH=7.4.

Theoretical AK (U mL^{-1})	AK activity (U mL^{-1})				
	37 °C	25 °C	pH=7.0	pH=7.6	[OMPs/AK, µg mg^{-1}]
0.010	0.010	0.009			0.009 [69]
0.020			0.019	0.022	
0.050	0.047	0.044			
0.200	0.230	0.185			0.197 [3.5]
1.000					0.954 [0.35]

The difference between measured and theoretical AK activity was never more than a 15%. Consequently, the tested parameters have no clear effect on AK activity.

S7 Removal of OMPs during sewage sludge AD

Table S6. Summary of reported OMPs removal during AD of sewage sludge and the main operational conditions employed. 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 el al.[7]	Carballa et al.[6]	Narumiya et al.[9]	Samaras et al.[10]	Malmborg and Magnér[11]	Clara et al.[12]	Paterakis et al.[8]	Bergersen et al.[13]	Yang et al.[14]	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									
Digester (cont.)	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 and 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).

S8 Enzymatic transformation in the presence and absence of acetate

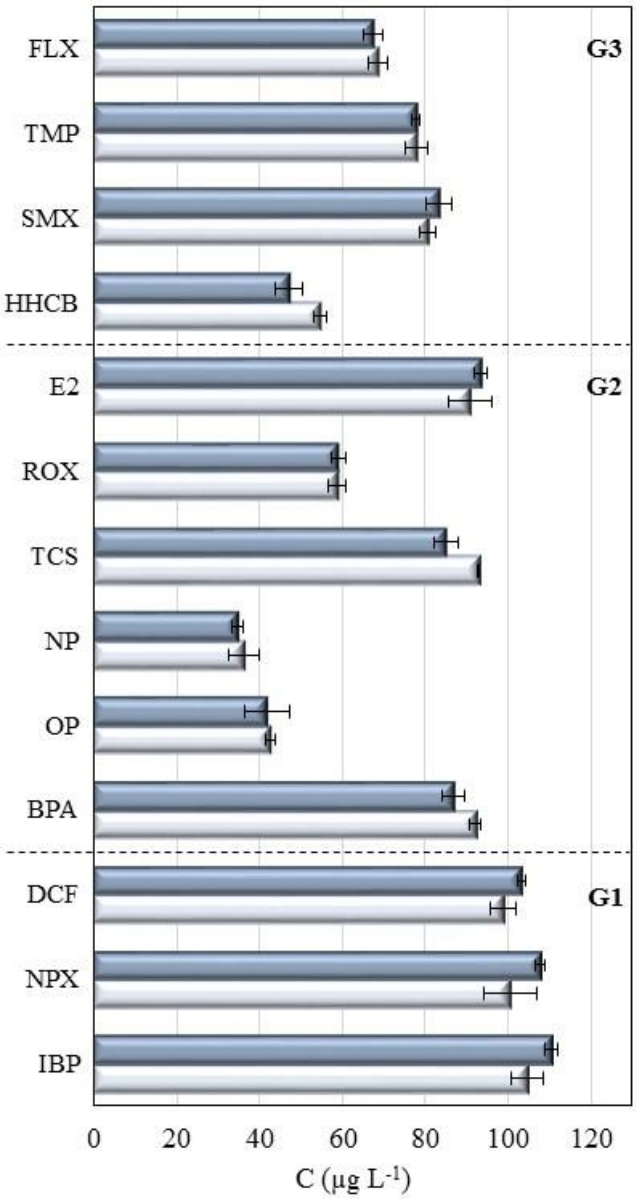


Figure S3. Concentration of OMPs in the AK assay with acetate (dark bars; n=2) and in the AK assay without acetate (white bars; n=2). No statistical differences were found between both assays (Student's t test; $p>0.05$). The compounds are sorted according to their chemical structures in three groups: (G1) OMPs with a carboxyl group, (G2) OMPs with a hydroxyl group and (G3) OMPs with other functional groups.

Acknowledgements

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References

(1) MetaCyc Database; http://biocyc.org/.

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

(3) DrugBank database; http://www.drugbank.ca.

(4) Varhanickova, D.; Shiu, W.-Y.; Mackay, D. Aqueous solubilities of alkylphenols and methoxyphenols at 25 ºC. *J. Chem. Eng. Data* **1995**, *40* (2), 448–451.

(5) Angelidaki, I.; Sanders, W. Assessment of the anaerobic biodegradability of macropollutants. *Rev. Environ. Sci. Biotechnol.* **2004**, *3* (2), 117–129.

(6) Carballa, M.; Omil, F.; Ternes, T.; Lema, J. M. Fate of pharmaceutical and personal care products (PPCPs) during anaerobic digestion of sewage sludge. *Water Res.* **2007**, *41* (10), 2139–2150.

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

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

(9) Narumiya, M.; Nakada, N.; Yamashita, N.; Tanaka, H. Phase distribution and removal of pharmaceuticals and personal care products during anaerobic sludge digestion. *J. Hazard. Mater.* **2013**, *260* (2013), 305–312.

(10) Samaras, V. G.; Stasinakis, A. S.; Thomaidis, N. S.; Mamais, D.; Lekkas, T. D. Fate of selected emerging micropollutants during mesophilic, thermophilic and temperature co-phased anaerobic digestion of sewage sludge. *Bioresour. Technol.* **2014**, *162*, 365–372.

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

(12) Clara, M.; Gans, O.; Windhofer, G.; Krenn, U.; Hartl, W.; Braun, K.; Scharf, S.; Scheffknecht, C. Occurrence of polycyclic musks in wastewater and receiving water bodies and fate during wastewater treatment. *Chemosphere* **2011**, *82* (8), 1116–1123.

(13) Bergersen, O.; Østnes, K.; Vasskog, T. Bioresource Technology Anaerobic treatment of sewage sludge containing selective serotonin reuptake inhibitors. *Bioresour. Technol.* **2012**, *117*, 325–332.

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