Fate of trace organic contaminants in oxic-settling-anoxic (OSA) process applied for biosolids reduction during wastewater treatment

Supplementary data

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Table S1. The basic properties of domestic sewage where *n*=number of samples

Property	Average	*n*
tCOD	474±292 mg/L	72
sCOD	101±54 mg/L	72
TOC	47.2±23.5 mg/L	70
TN	45.0±11.1 mg/L	70
NH_4^+-N	78.4±32.1 mg/L	69
PO_4^{3-}-P	30.3±14.7 mg/L	69
pH	7.2±0.5	61

Table S2. Sample preparation, solid phase extraction, and TrOC analysis

Table S2-a. Method description

Sample preparation. All samples (influent, effluent, and sludge) were initially centrifuged at 3720x*g* and 4 ºC for 10 min (Beckman Coulter, USA). To obtain TrOC concentration in the aqueous phase, the supernatant (from wastewater and sludge samples) was diluted to 500 mL in MilliQ water, and then sequentially filtered using 1 µm and 0.7 µm glass fibre filter papers. The filtered liquid later underwent solid phase extraction (SPE). To obtain TrOC concentration in the solid phase of sludge, the pellet (from sludge samples only) was freeze-dried (Christ GmbH, Germany) for 12 h. The dried sample was ground to powder using mortar and pestle, and then 0.5 g of powder was placed in a capped glass vial. In the first round of extraction, the powder was re-suspended in 10 mL of methanol, vortexed (Ratek, Australia), and then ultrasonicated for 10 min at 40 ºC. The mixture was centrifuged at 3720x*g* for 10 min, and then the supernatant was decanted and set aside. In the second round of extraction, the pellet from the previous extraction was re-suspended in 10 mL of dichloromethane and ethanol mixture (1:1 v/v), vortexed (Ratek, Australia), and then ultrasonicated for 10 min at 40 ºC. The mixture was centrifuged at 3720x*g* for 10 min, and then the supernatant was decanted and added to the previous extract. The combined extract was diluted to 500 mL with Milli-Q water, and then sequentially filtered using 1 µm and 0.7 µm glass fibre filter papers. These samples later underwent solid phase extraction (SPE).

Surrogate TrOC standards. All analytes had isotopically labelled standards except for metoprolol, benzotriazole, benzophenone, saccharin, oxybenzone, fenbofibrate, allopurinol, acesulfame, cyclamate, sucralose, aspartame, clofibric acid, dichloroprop, propylparaben, phenylphenol, and butylparaben. Those with standards were quantified using the isotope dilution method. Those without standards were determined using external calibration.

Solid phase extraction. Prior to SPE, the samples were spiked with 50 µL of a 1 mg/L solution of isotopically labelled surrogate TrOC standards (Supplementary Table S3) and mixed thoroughly. Then, the samples were loaded onto hydrophilic/lipophilic Oasis HLB cartridges (Waters, USA) that ~~have~~ had been sequentially conditioned with 5 mL of methyl-tert-butyl ether, 5 mL of methanol, and twice with 5 mL of Milli-Q water. The loading rate (15 mL/min) was controlled by adjusting the vacuum pressure in the SPE manifold. After loading, the cartridges were rinsed with 5 mL of MilliQ, gently dried using N_2 gas, and then stored in a sealed bag at 4ºC until elution and analysis.

Liquid Chromatography. Analytes were separated using an Agilent (Palo Alto, CA, USA) 1200 series high performance liquid chromatography (HPLC) system equipped with a 150 x 4.6 mm, 5 µm particle size, Luna C18 (*2*) column (Phenomenex, Torrence CA, USA) . A binary gradient consisting of 5 mM ammonium acetate in water (A) and 100% methanol (B) at a flow rate of 800 µL min^{-1} was used. For electrospray ionization (ESI) positive analyses, the gradient was as follows: 10% B held for 0.50 min, stepped to 50% B at 0.51 min and increased linearly to 100% B at 8 min, then held at 100% B for 2 min. For ESI negative analyses, the

gradient was as follows: 10% B held for 0.50 min, stepped to 60% B at 0.51 min and increased linearly to 100% B at 8 min, then held at 100% B for 3 min. A 5 min equilibration step at 10% B was used at the beginning of each run. For atmospheric pressure chemical ionization (APCI) analysis the eluants consisted of milli-Q grade water (A) and 0.1% v/v formic acid in methanol with the following ramp at a flow rate of 700 µL min^{-1}. 60% B held for 5 min, increased linearly to 100% B at 20 min, then held at 100% B for 3 min. A 3 min equilibrium step preceded injection. An injection volume of 10 µL was used for all methods. Analytical methods using ESI and APCI are based on that of Vanderford et al. (2006) and Vanderford et al. (2003), respectively.

Mass Spectrometry. Mass spectrometry was performed using an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a turbo-V ion source employed in both positive and negative electro-spray modes. Steroids were analysed the source configured for (APCI) in positive mode. Using multiple reaction monitoring (MRM) two mass transitions for all but three of the analytes were monitored for unequivocal confirmation. One mass transition for the labeled internal standard was monitored. Only the first transition was used for quantitiation. Relative retention times of the analyte and isotopically labeled internal standard were also monitored to ensure correct identification.

Calibration and limits of Detection. Standard solutions of all analytes were prepared at 1, 5, 10, 50, 100, 500 and 1000 ng/mL. A relative response ratio of analyte/internal standard over a 1 – 1000 ng concentration range was generated enabling quantitation with correction for losses due to ion suppression and incomplete SPE recovery. All calibration curves had a correlation coefficient of 0.99 or better. Detection limits were defined as the concentration of an analyte giving a signal to noise (s/n) ratio greater than 3. The Limits of Reporting were determined using a s/n ratio of greater than 10.

References:

[1] Vanderford, B.J., Snyder, S.A. 2006. Analysis of Pharmaceuticals in Water by Isotope Dilution Liquid Chromatography/Tandem Mass Spectrometry. Environ Sci Technol, **40**(23), 7312-7320.

[2] Vanderford, B.J., Pearson, R.A., Rexing, D.J., Snyder, S.A. 2003. Analysis of Endocrine Disruptors, Pharmaceuticals, and Personal Care Products in Water Using Liquid Chromatography/Tandem Mass Spectrometry. Anal Chem, **75**(22), 6265-6274.

Table S2-b. Transitions for compounds using ESI positive mode

Compound	Precursor Ion ($m\ z^{-1}$)	Product Ion ($m\ z^{-1}$)
paracetamol	152.1	110.1
paracetamol-15N13C	155.0	111.0
sulfamethoxazole-1	254.0	156.1
sulfamethoxazole-2	254.0	92.0
sulfamethoxazole-D4	258.1	160.1

caffeine-1	195.0	138.1
caffeine-2	195.0	110.1
caffeine-D9	204.1	144.2
trimethoprim-1	291.1	230.2
trimethoprim-2	291.1	261.1
trimethoprim-D9	300.3	234.2
benzotriazole	120.0	63.5
benzotriazole D4-1	124.0	67.9
aspartame-294-1	295.1	119.8
aspartame-294-2	295.1	179.8
triamterene-1	254.2	237.0
triamterene-2	254.2	104.0
triamterene-D5-1	259.2	242.2
primidone-1	219.2	162.2
primidone-2	219.2	119.0
primidone-D5-1	224.2	167.0
carazolol-1	299.1	115.6
carazolol-2	299.1	221.6
carazolol-D7-1	306.2	122.8
carazolol-D7-2	306.2	221.6
meprobamate-1	218.9	158.2
meprobamate-2	218.9	115.1
meprobamate-D3-1	221.9	161.2
bisoprolol-1	326.2	115.8
bisoprolol-2	326.2	73.5
bisoprolol-D-5-1	331.2	120.8
enalapril-1	377.1	234.1
enalapril-2	377.1	91.1
enalapril-D5	382.2	239.2
TCEP-1	284.9	62.9
TCEP-2	284.9	223.0
TCEP-D12-1	297.0	66.7
TCEP-D12-2	297.0	231.6
dilantin-1	253.1	182.1
dilantin-2	253.1	104.1
dilantin-D10	263.1	192.2
simazine-1	202.1	132.1
simazine-2	202.1	124.1
simazineD10-1	212.2	137.1
carbamazepine-1	237.0	194.2
carbamazpine-2	237.0	192.1
carbamazpine-D10	247.1	204.3
omeprazole-1	346.2	198.2
omeprazole-2	346.2	136.1
omeprazole-D3	349.2	198.0
DEET-1	192.2	119.0

DEET-2	192.2	108.9
DEET-D7-1	199.2	126.1
atrazine-1	216.0	174.2
atrazine-2	216.0	96.1
atrazine-D5	221.3	179.1
verapamil-1	455.4	165.1
verapamil-2	455.4	150.0
verapamil-D6-1	461.4	165.2
linuron-1	249.0	182.2
linuron-2	249.0	160.1
linuron-D6	255.0	160.1
diazepam-1	285.1	193.1
diazepam-2	285.1	154.2
diazepam-D5-1	290.1	198.1
benzophenone-1	183.0	104.2
benzophenone-2	183.0	76.5
benzophenone D10 1	193.1	109.0
saccharin	183.0	76.7
saccharin d4	183.1	104.0
clozapine-1	327.1	270.2
clozapine-2	327.1	192.1
clozapine-D4-1	331.2	272.0
amtriptyline-1	278.2	233.0
amtriptyline-2	278.2	117.1
amtriptyline-D6-1	284.4	233.1
TCPP d18	344.1	326.4
hydroxyzine-1	375.3	201.1
hydroxyzine-2	375.3	165.1
hydroxyzine-D8-1	383.3	201.1
oxybenzone-1	229.1	151.0
oxybenzone-2	229.1	104.7
Diazinon-1	305.1	169.1
Diazinon-2	305.1	115.0
DiazinonD10-1	315.2	170.1
fenofibrate-1	361.0	232.8
fenofibrate-2	361.0	138.8
Chlorpyrifos-1	351.9	96.2
Chlorpyrifos-2	349.9	197.9

Table S2-c. Transitions for compounds using ESI negative mode

Compound	Precursor Ion ($m\ z^{-1}$)	Product Ion ($m\ z^{-1}$)
saccharin 1	182.1	41.8
saccharin 2	182.1	105.3
saccharin D4 1	185.9	42.0

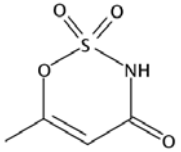
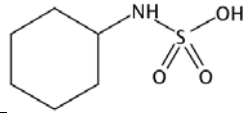
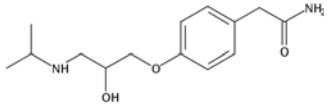
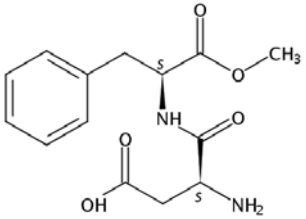
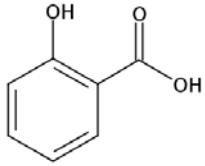
saccharin D4 2	185.9	105.9
acesulfame-1	161.8	81.8
acesulfame-2	161.8	77.9
salicyclic acid -1	136.8	93.0
salicyclic acid -2	136.8	64.7
salicilicAcidD6	140.9	96.9
cyclamate-1	177.9	79.4
cyclamate-2	177.9	80.3
cyclamate- D11	188.9	79.2
sucralose-1	398.9	36.7
sucralose-2	398.9	360.4
aspartame-2	293.0	199.0
aspartame-1	293.0	260.8
ketoprofen-1	252.8	208.8
ketoprofen-2	252.8	196.7
ketoprofen-D3	255.6	211.7
naproxen-1	228.9	184.6
naproxen-2	228.9	169.8
naproxen-D3	231.9	187.8
bisphenol A-1	226.9	211.8
bisphenol A-2	226.9	132.9
bisphenol A-D6	232.9	214.9
diclofenac-1	293.9	249.7
diclofenac-2	293.9	213.7
diclofenac-D4-1	297.9	253.8
propylparaben-1	178.9	92.0
propylparaben-2	178.9	136.2
diuron-1	230.8	185.4
diuron-2	230.8	149.7
diuronD6-1	236.9	185.8
Ibuprofen-1	204.9	160.8
Ibuprofen-2	204.9	158.8
ibuprofen-D3	208.0	163.9
phenylphenol-1	168.9	114.8
phenylphenol-2	168.9	140.8
gemfibrozil-1	248.9	120.5
gemfibrozil-2	248.9	126.8
gemfibrozil-D6	254.9	120.9
triclocarban-1	312.8	159.8
triclocarban-2	312.8	125.8
triclocarban-D4-1	317.0	159.8
Triclosan-1	286.6	35.0
Triclosan-2	286.7	141.6
Triclosan-D3	289.7	34.9
t-octylphenol-1	204.9	133.0
t-octylphenol-2	205.2	132.9

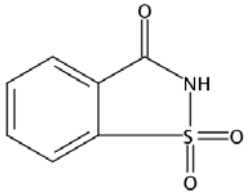
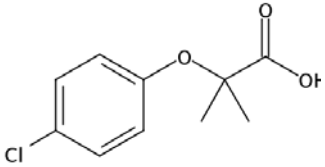
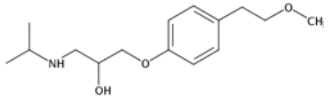
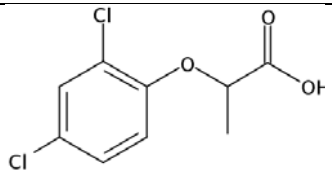
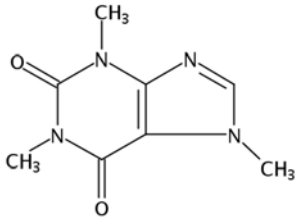
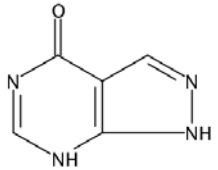
n-octylphenol-D17	222.1	108.0
nonylphenol-1	219.0	106.0
nonylphenol-D4-1	223.1	110.0
PFOA 1	413.0	368.5
PFOA 2	413.0	168.7
PFOA 13C8	421.0	375.8
PFOS 1	499.0	79.8
PFOS 2	499.0	98.7
PFOS 13C8	506.9	79.9
butylparaben-1	192.9	91.8
butylparaben 2	192.9	135.9

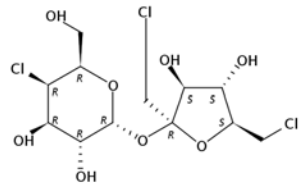
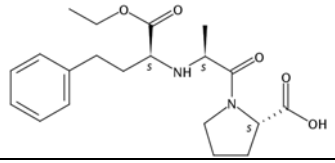
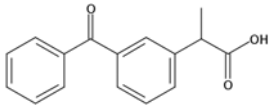
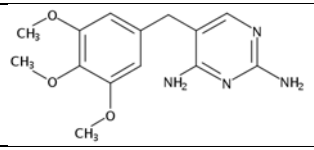
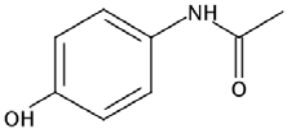
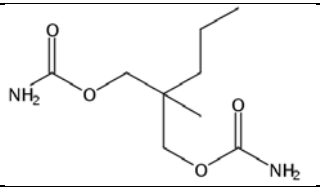
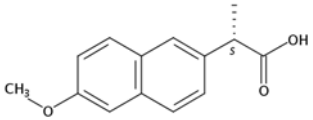
Table S2-d. Transitions for compounds using APCI positive mode

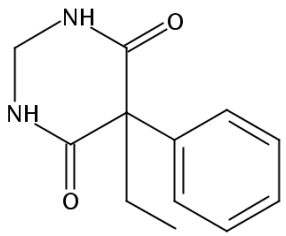
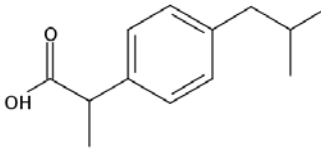
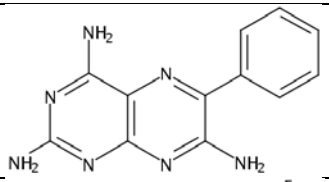
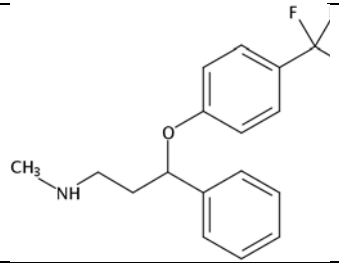
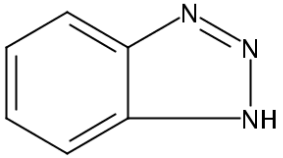
Compound	Precursor Ion ($m\ z^{-1}$)	Product Ion ($m\ z^{-1}$)
Estriol 1	271.1	253.1
Estriol 2	271.1	133.0
Estriol-D2	273.2	255.2
Androstendione 1	287.2	97.1
Androstendione 2	287.2	109.2
Androstendione-D3	290.2	100.1
Etiocholanolone 1	273.2	255.3
Etiocholanolone 2	273.2	91.1
Etiocholanolone-D2	275.2	257.1
Androsterone 1	273.2	255.2
Androsterone 2	273.2	91.0
Estrone 1	271.2	159.2
Estrone 2	271.2	133.0
Estrone-D4	275.1	161.0
17β-Estradiol 1	255.2	159.3
17β-Estradiol 2	255.2	133.2
17β-Estradiol-D4	259.1	161.1
17α-Estradiol 1	255.2	159.3
17α-Estradiol 2	255.2	133.2
17α-Ethynylestradiol 1	279.2	133.1
17α-Ethynylestradiol 2	279.2	159.2
17α-Ethynylestradiol-D4	283.1	135.1
Testosterone 1	289.2	97.2
Testosterone 2	289.2	109.1
Testosterone-D2	291.2	99.1

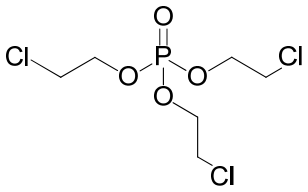
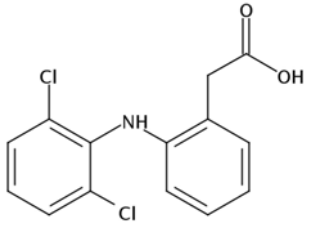
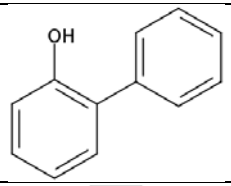
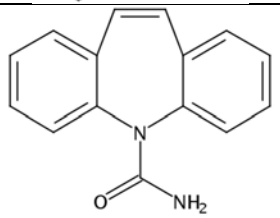
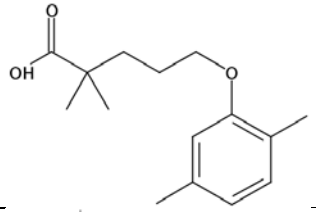
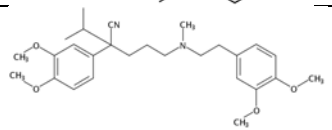
Table S3. Summary of TrOCs that were analysed and detected in the influent at different sampling periods. All analytes have isotopically-labelled surrogate standards except for those listed in Table S2. TrOC sampling and analysis were performed when SRT_{SBRs} was maintained at 10 d and SRT_{ext} was varied (10-40 d). The sampling campaigns occurred at different seasons.

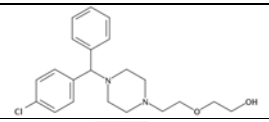
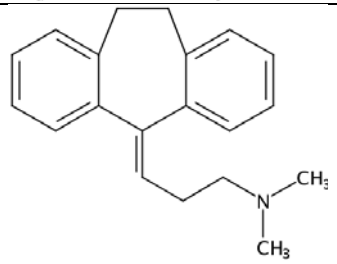
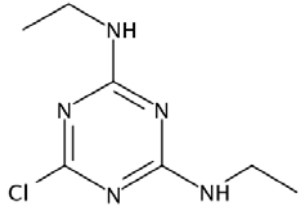
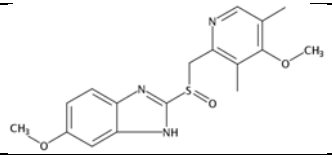
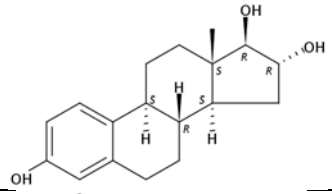
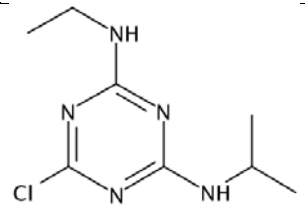
TrOC	Chemical Structure	Log D (pH 7; 25ºC)	Type or application	Detection limit (ng/L)	SRT_{ext} (d) / season					
					40 / winter		20 / spring		10 / summer	
					Analysed	Detected in influent	Analysed	Detected in influent	Analysed	Detected in influent
Acesulfame		-2.88	Artificial sweetener	5	No	-	Yes	No	Yes	No
Cyclamate		-2.46	Artificial sweetener	5	No	-	Yes	No	Yes	No
Atenolol		-2.09	Pharmaceutical (beta-blocker)	5	Yes	Yes	Yes	Yes	Yes	Yes
Aspartame		-1.99	Artificial sweetener	5	No	-	Yes	Yes	Yes	Yes
Salicylic acid		-1.13	Pharmaceutical	5	No	-	Yes	No	Yes	Yes

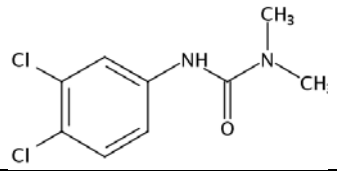
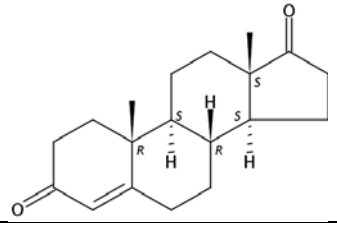
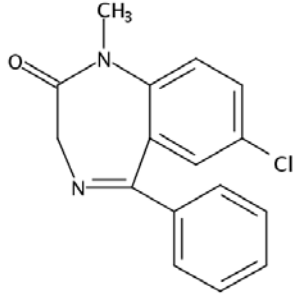
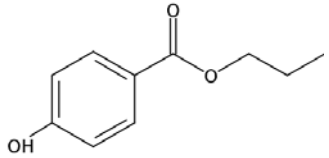
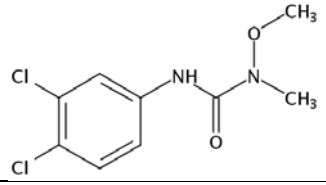
Saccharin		-1.09	Artificial sweetener	5	No	-	Yes	Yes	Yes	Yes
Clofibric acid		-1.06	Pesticide (herbicide)	5	No	-	Yes	No	Yes	No
Metoprolol		-0.81	Pharmaceutical (beta-blocker)	20	No	-	Yes	Yes	Yes	No
Dichloroprop		-0.77	Pesticide (herbicide)	20	No	-	Yes	Yes	No	No
Caffeine		-0.63	Food product (Stimulant)	5	Yes	Yes	Yes	Yes	Yes	Yes
Allopurinol		-0.55	Pharmaceutical (antidiuretic)	5	No	-	Yes	No	Yes	Yes

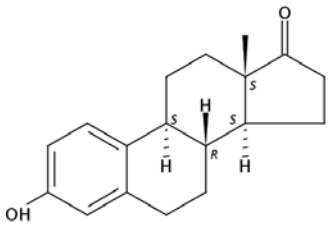
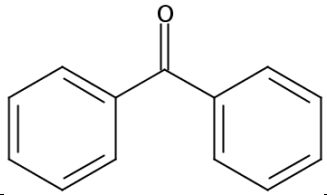
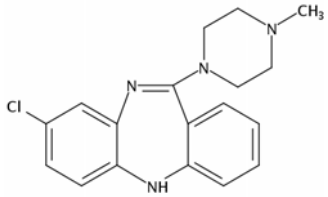
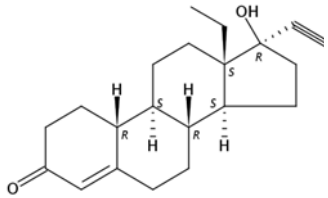
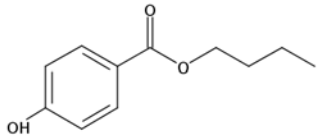
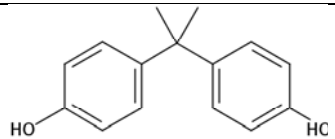
Sucralose		-0.23	Artificial sweetener	5	No	-	Yes	Yes	Yes	Yes
Enalapril		-0.14	Pharmaceutical (angiotensin converting enzyme inhibitor)	5	Yes	Yes	Yes	No	Yes	No
Ketoprofen		0.19	Pharmaceutical (nonsteroidal anti-inflammatory drug)	20	Yes	Yes	Yes	No	Yes	Yes
Trimethoprim		0.27	Antibiotic	20	Yes	Yes	Yes	Yes	Yes	Yes
Paracetamol		0.47	Pharmaceutical	20	Yes	Yes	Yes	Yes	Yes	Yes
Meprobamate		0.70	Pharmaceutical (tranquilizer)	5	Yes	No	No	-	No	-
Naproxen		0.73	Pharmaceutical (nonsteroidal anti-inflammatory drug)	5	Yes	Yes	Yes	Yes	Yes	Yes

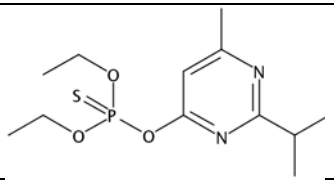
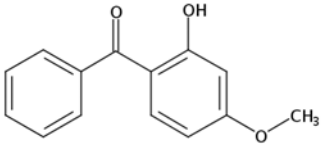
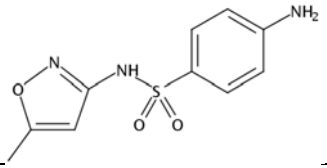
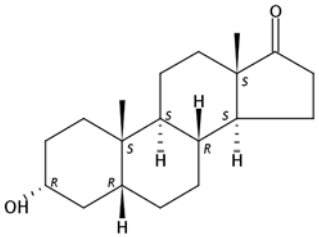
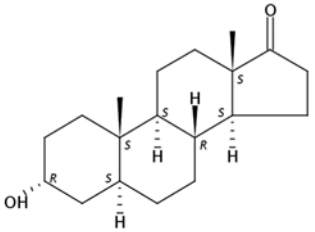
Primidone		0.83	Pharmaceutical (anticonvulsant)	20	Yes	Yes	Yes	Yes	Yes	Yes
Ibuprofen		0.94	Pharmaceutical (nonsteroidal anti-inflammatory drug)	20	Yes	Yes	Yes	Yes	Yes	Yes
Triamterene		1.03	Pharmaceutical (diuretic)	20	Yes	No	Yes	Yes	Yes	Yes
Fluoxetine		1.15	Pharmaceutical (antidepressant)	5	Yes	Yes	No	-	No	-
Benzotriazole		1.42	Industrial anticorrosive	20	No	-	Yes	Yes	Yes	Yes

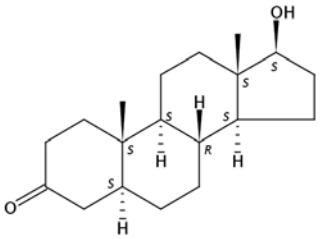
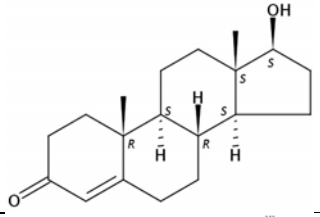
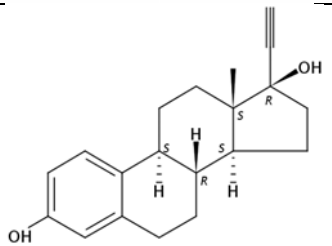
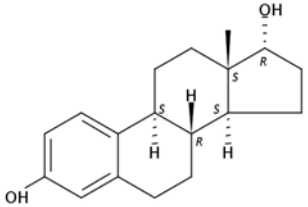
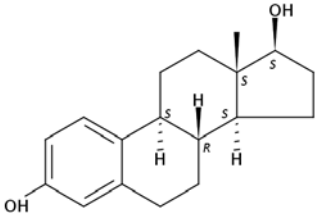
Tris(2-carboxyethyl) phosphene (TCEP)		1.47	Flame retardant	5	Yes	Yes	Yes	Yes	Yes	Yes
Diclofenac		1.77	Pharmaceutical (nonsteroidal anti-inflammatory drug)	20	Yes	Yes	Yes	Yes	Yes	Yes
Phenylphenol		1.88	Pesticide (fungicide)	5	Yes	Yes	Yes	No	Yes	Yes
Carbamazepine		1.89	Pharmaceutical (anticonvulsant and analgesic)	5	Yes	Yes	Yes	Yes	Yes	Yes
Gemfibrozil		2.07	Pharmaceutical (cholesterol and triglyceride reducer)	5	Yes	Yes	Yes	Yes	Yes	Yes
Verapamil		2.08	Pharmaceutical (calcium channel blocker)	5	Yes	Yes	Yes	Yes	Yes	Yes

Hydroxyzine		2.15	Pharmaceutical (antihistamine)	5	Yes	No	Yes	No	Yes	Yes
Amitriptyline		2.28	Pharmaceutical (antidepressant)	20	Yes	Yes	Yes	Yes	Yes	Yes
Simazine		2.28	Pesticide (herbicide)	5	Yes	Yes	Yes	No	Yes	No
Omeprazole		2.35	Pharmaceutical (anti-gastroesophaegal reflux)	20	Yes	No	Yes	No	Yes	No
Estriol		2.53	Hormone	5	Yes	Yes	Yes	Yes	Yes	Yes
Atrazine		2.64	Pesticide (herbicide)	5	Yes	No	Yes	No	Yes	No

Diuron		2.68	Pesticide (herbicide)	5	Yes	Yes	Yes	Yes	Yes	Yes
Androstenedione		2.72	Hormone	5	Yes	Yes	Yes	Yes	Yes	Yes
Diazepam		2.80	Pharmaceutical (muscle relaxant)	5	Yes	No	Yes	No	Yes	Yes
Propylparaben		2.88	Personal care product formulation	20	Yes	Yes	Yes	Yes	Yes	Yes
Linuron		3.12	Pesticide (herbicide)	5	Yes	No	Yes	No	Yes	No

Estrone		3.13	Hormone	5	Yes	Yes	Yes	Yes	Yes	Yes
Benzophenone		3.21	UV filter	5	No	-	Yes	Yes	Yes	Yes
Clozapine		3.23	Pharmaceutical (antipsychosis)	5	Yes	Yes	Yes	Yes	Yes	Yes
Levonorgestrel		3.37	Hormone	5	Yes	Yes	Yes	No	Yes	No
Butylparaben		3.38	Personal care product formulation	20	No	-	Yes	Yes	Yes	Yes
Bisphenol A		3.64	Industrial chemical (plastic production)	5	Yes	Yes	Yes	Yes	Yes	Yes

Diazinon		3.77	Pesticide	5	Yes	No	Yes	Yes	Yes	Yes
Oxybenzone		3.89	UV filter	20	No	-	Yes	Yes	Yes	Yes
Sulfamethoxazole		3.90	Antibiotic	20	Yes	Yes	Yes	Yes	Yes	Yes
Etiocholanolone		3.93	Hormone	5	Yes	Yes	No	-	No	-
Androsterone		3.93	Hormone	5	Yes	Yes	Yes	Yes	Yes	Yes

Di-hydrotestosterone		3.93	Hormone	5	Yes	No	Yes	Yes	Yes	Yes
Testosterone		4.11	Hormone	5	Yes	No	Yes	Yes	Yes	Yes
Ethynylestradiol		4.11	Xenoestrogen	5	Yes	Yes	Yes	Yes	Yes	Yes
17α-estradiol		4.15	Hormone	5	Yes	No	Yes	Yes	Yes	No
17β-estradiol		4.15	Hormone	5	Yes	Yes	Yes	Yes	Yes	Yes

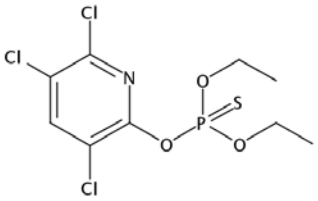
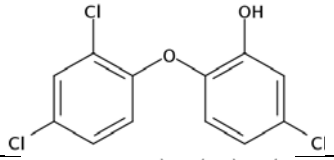
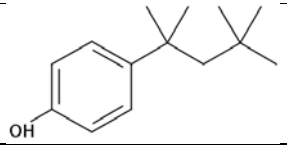
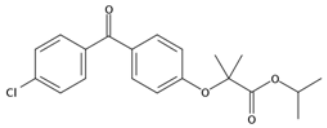
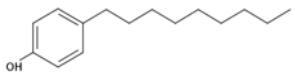
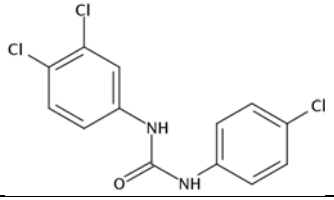
Chlorpyrifos		5.00	Pesticide	5	No	-	Yes	No	Yes	No
Triclosan		5.15	Antibiotic	20	Yes	Yes	Yes	Yes	Yes	Yes
4-tert-Octylphenol		5.18	Industrial surfactant	5	No	-	Yes	Yes	Yes	Yes
Fenofibrate		5.80	Pharmaceutical (cholesterol and triglyceride reducer)	20	No	-	Yes	Yes	Yes	Yes
Nonylphenol		7.63	Detergent breakdown product	10	Yes	Yes	Yes	Yes	Yes	No
Triclocarban		12.80	Industrial surfactant	20	Yes	Yes	Yes	Yes	Yes	Yes

Table S4. Estimation of TrOC concentration entering the external aerobic/anoxic and anoxic reactors and the control aerobic digester.

To analyse the biodegradation and sorption of TrOCs under aerobic/anoxic treatment, the TrOC concentration of sludge (in ng/L) going in to the reactor ($Y_{in\text{-}aerobic/anoxic}$) was estimated based on sludge flows from SBR_{OSA} ($Y_{SBR_{OSA}}$) and anoxic reactor (Y_{anoxic}):

$$Y_{in-aerobic/anoxic} = Y_{SBR_{OSA}} + Y_{anx} \quad \text{Equation S4.1}$$

$$Y_{in-aerobic/anoxic} = \frac{[(A_{SBR_{OSA}} + S_{SBR_{OSA}} \times MLSS_{SBR_{OSA}}) \times q_1 + (A_{anoxic} + S_{anoxic} \times MLSS_{anoxic}) \times q_4]}{(q_1 + q_4)} \quad \text{Equation S4.2}$$

Where A and S were the aqueous and solid phase TrOC concentration of sludge, MLSS was the sludge concentration, q_1 was the flow rate of sludge from SBR_{OSA} to aerobic/anoxic reactor (Figure 1a), and q_4 was the flow rate of sludge from anoxic to aerobic/anoxic reactor (Figure 1a).
Likewise, the TrOC concentration of sludge going in to the anoxic reactor was estimated based on sludge flow from the aerobic/anoxic reactor ($Y_{aerobic/anoxic}$):

$$Y_{in-anoxic} = Y_{aerobic/anoxic} \quad \text{Equation S4.3}$$

$$Y_{in-anoxic} = A_{aerobic/anoxic} + S_{aerobic/anoxic} \times MLSS_{aerobic/anoxic} \quad \text{Equation S4.4}$$

Notably, the flow rate of sludge from the aerobic/anoxic to the anoxic reactor (q_3) was equal to the rate at which sludge was withdrawn from the anoxic reactor (q_4+q_5) (Figure 1a).

The TrOC concentration of sludge going in to the aerobic digester was also estimated based on sludge flow from $SBR_{control}$:

$$Y_{in-aerobic} = Y_{SBR_{control}} \quad \text{Equation S4.5}$$

$$Y_{in-aerobic} = A_{SBR_{control}} + S_{SBR_{control}} \times MLSS_{SBR_{control}} \quad \text{Equation S4.6}$$

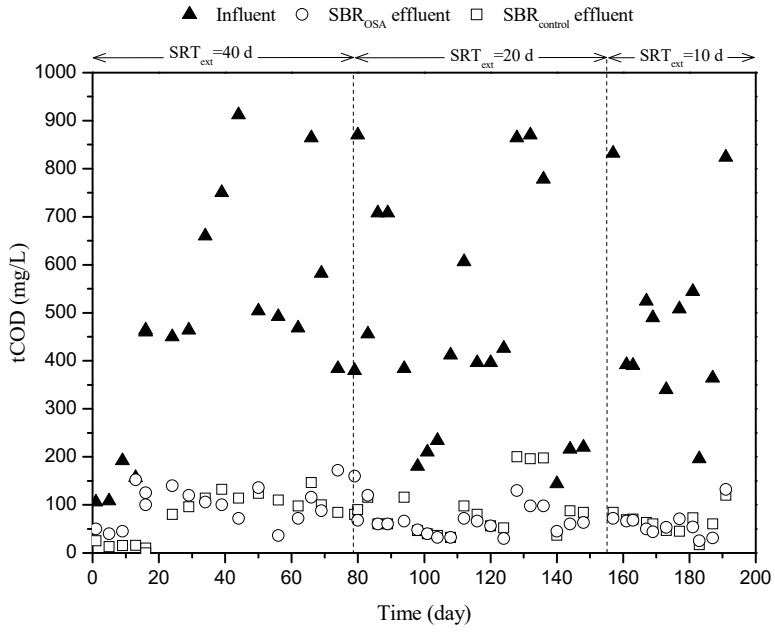


Figure S5. tCOD of SBR_{OSA} and $SBR_{control}$ when SRT_{SBR} was 10 days and SRT_{ext} was varied (10-40 d). The dashed line indicates different SRT_{ext}.

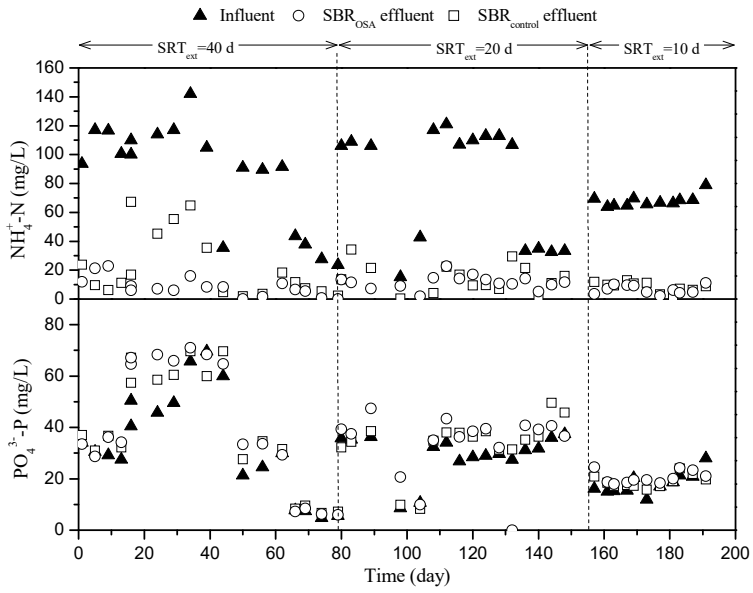
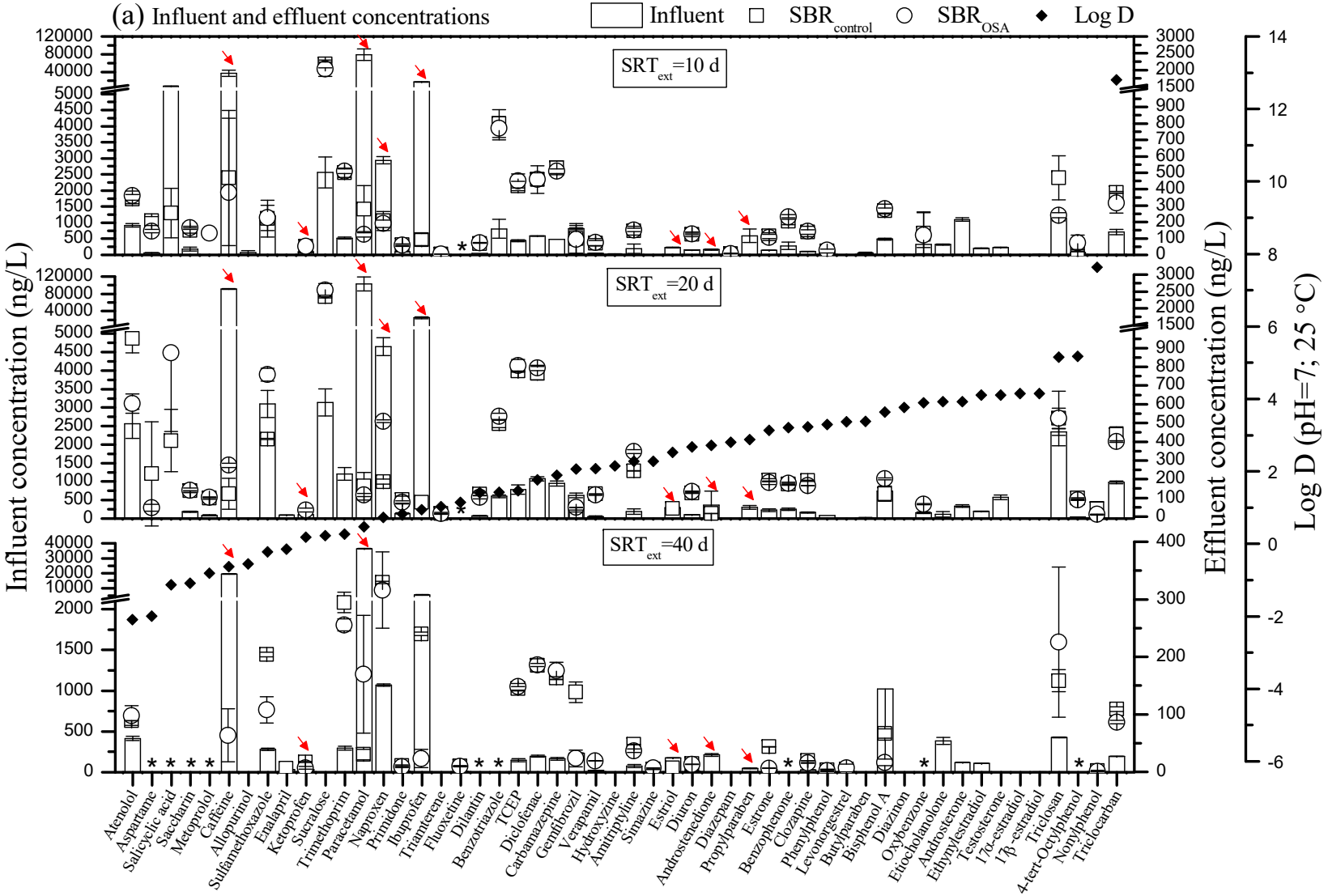


Figure S6**.** Ammonia and orthophosphate concentration of $SBR_{control}$ and SBR_{OSA} when SRT_{SBR} was 10 days and $SRT_{ext.reactors}$ was varied (10-40 d). The dashed line indicates different SRT_{ext}.


(a) Influent and effluent concentrations
Influent
SBR control
SBR OSA
Log D
SRT ext =10 d
SRT ext =20 d
SRT ext =40 d
Influent concentration (ng/L)
Effluent concentration (ng/L)
Log D (pH=7; 25 °C)
Atenolol
Aspartame
Salicylic acid
Saccharin
Metoprolol
Caffeine
Allopurinol
Sulfamethoxazole
Enalapril
Ketoprofen
Sucralose
Trimethoprim
Paracetamol
Naproxen
Primidone
Ibuprofen
Triamterene
Fluoxetine
Dilantin
Benzotriazole
TCEP
Diclofenac
Carbamazepine
Gemfibrozil
Verapamil
Hydroxyzine
Amitriptyline
Simazine
Estriol
Diuron
Androstenedione
Diazepam
Propylparaben
Estrone
Benzophenone
Clozapine
Phenylphenol
Levonorgestrel
Butylparaben
Bisphenol A
Diazinon
Oxybenzone
Etiocholanolone
Androsterone
Ethynylestradiol
Testosterone
17α-estradiol
17β -estradiol
Triclosan
4-tert-Octylphenol
Nonylphenol
Triclocarban

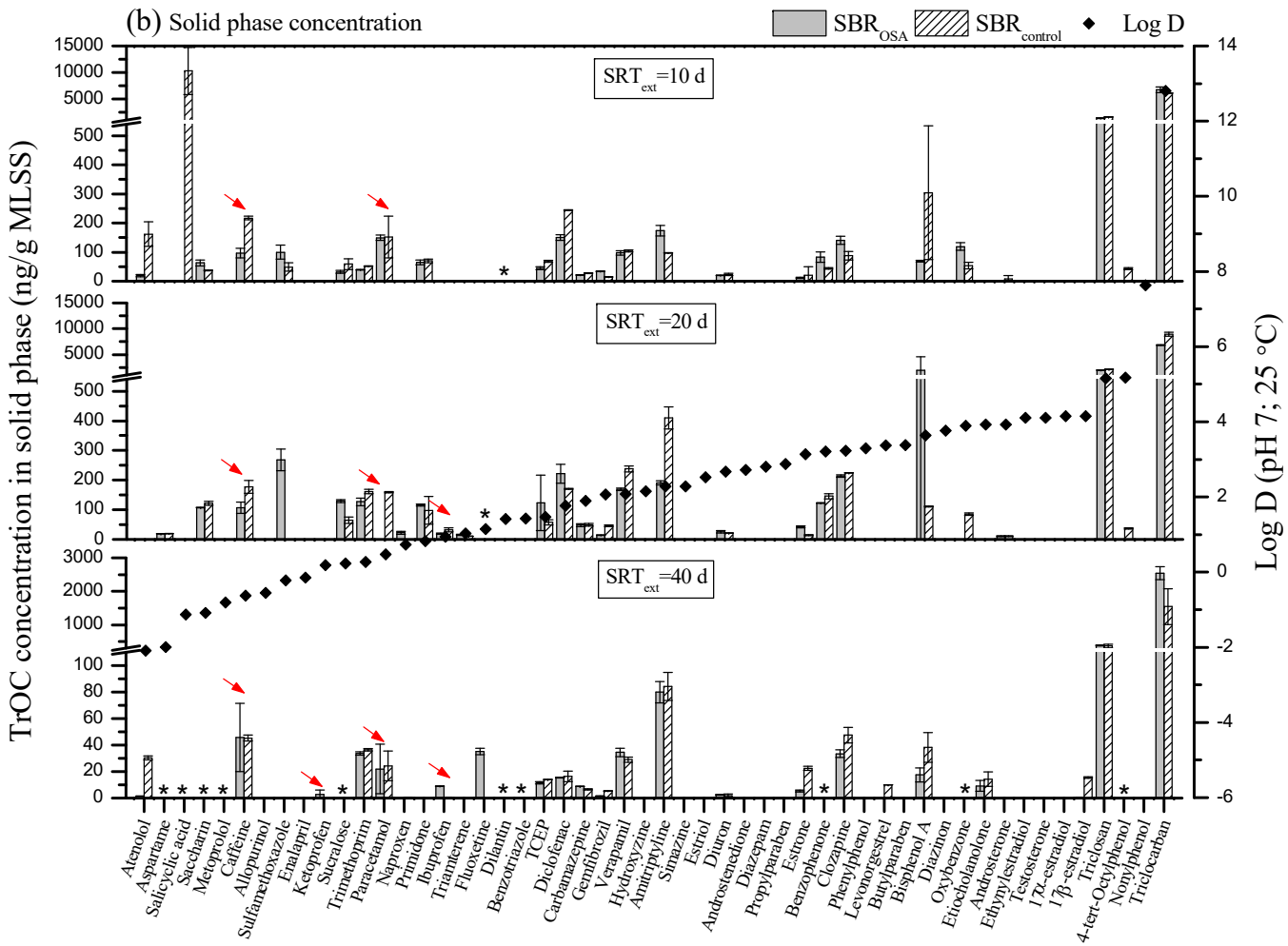


Figure S7. TrOC concentrations in the (a) influent, effluent, and (b) solid phase of sludge of SBR_{OSA} and $SBR_{control}$ when SRT_{SBR} was maintained at 10 d and SRT_{ext} was varied (10-40 d). The values are the average of two measurements (n=2). The asterisks (*) represent TrOCs that were not analysed in a particular sampling campaign. The arrows (→) denote contaminants that were highly biodegraded.

Table S8. TrOCs with notable variation (>30% difference) in SBR_{OSA} and $SBR_{control}$ effluents when SRT_{SBR} was maintained at 10 d and SRT_{ext} was varied (10-40 d); The values are the average of two measurements (*n*=2).

SRT_{ext} (d)	TrOC	Influent (ng/L)	Effluent (ng/L)	
			SBR_{OSA}	$SBR_{control}$
10	Aspartame	63±6	145±11	207±27
	Paracetamol	79,000±12,728	125±16	278±144
	Triclosan	1,126±71	241±16	469±134
	4-tert-octylphenol	73±35	73±48	26±7
20	Atenolol	2,560±396	604±51	952±76
	Aspartame	20±0	48±19	229±287
	Salicylic acid	Below detection limit	874±419	406±167
	Caffeine	91,500±707	274±11	122±82
	Ketoprofen	107±4	35±11	Below detection limit
	Paracetamol	103,100±16,263	116±8	163±73
	Naproxen	4,650±240	509±7	187±13
	Ibuprofen	24,700±2,121	Below detection limit	77±0
	Estriol	291±25	Below detection limit	43±0
	Androstenedione	368±374	Below detection limit	17±0
	Bisphenol A	745±1	202±6	123±40
	Oxybenzone	161±20	66±3	Below detection limit
	Sulfamethoxazole	3,100±368	758±34	414±3
	Nonylphenol	20±1	13±0	44±36
40	Caffeine	19,500±283	63±46	563±26
	Ketoprofen	32±4	7±2	17±1
	Paracetamol	36,450±70	170±102	30±9
	Ibuprofen	5,525±247	23±16	240±3
	Gemfibrozil	232±4	23±15	138±17
	Estriol	175±0	Below detection limit	7±0
	Estrone	32±0	6±1	43±1
	Bisphenol A	1,020±1203	16±6	67±8
	Sulfamethoxazole	280±13	107±23	204±4
	Triclosan	428±5	226±131	158±19

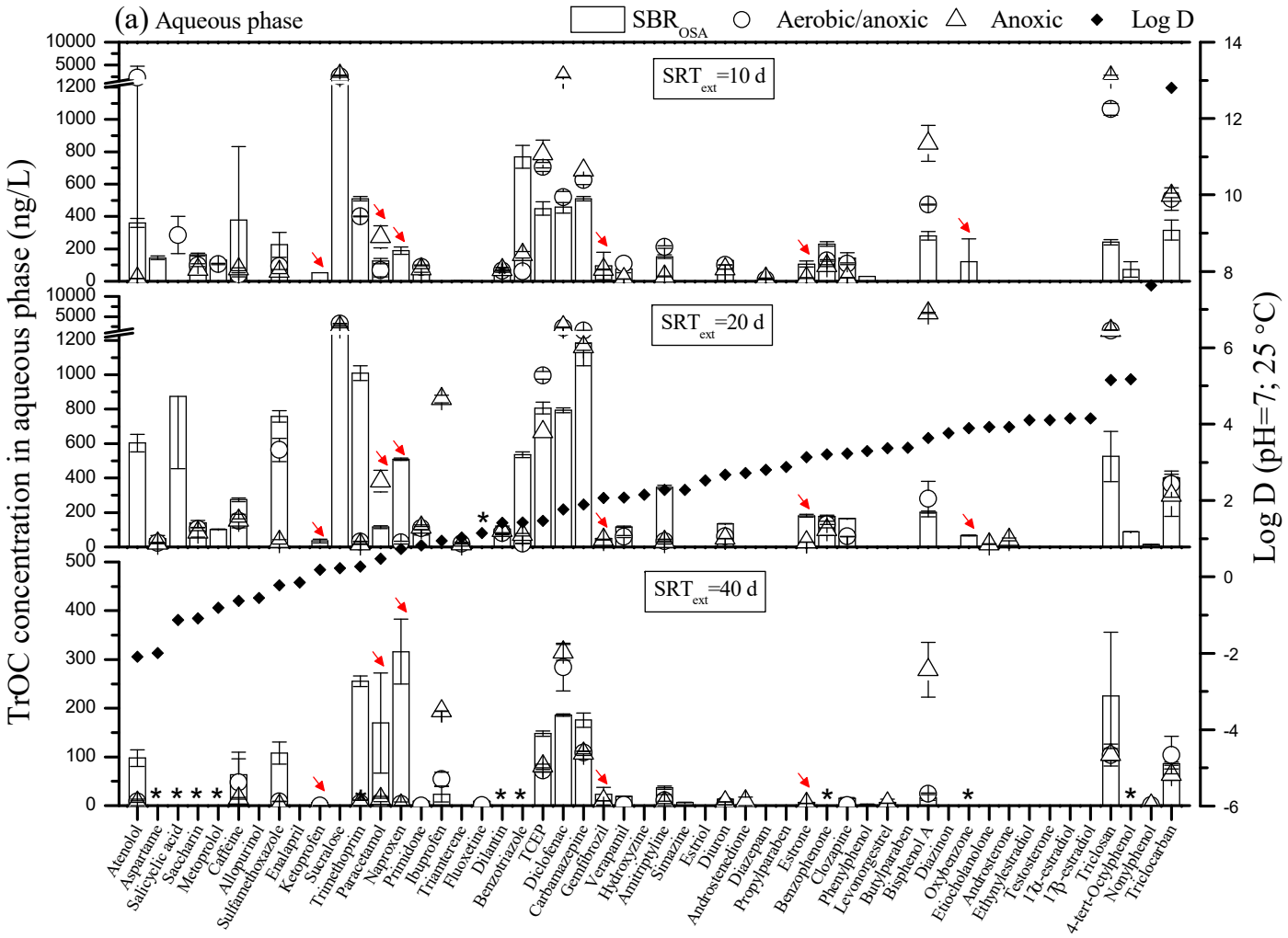
Table S9. Flow rate and change in receiving media during sludge interchange in the OSA system at different external reactor SRT_{ext}

SRT_{ext} (d)	**q_1 (mL/d) [a] / Δ receiving media (%) [b]**	**q_2 (mL/d) [c] / Δ receiving media (%) [b]**	**q_3 [d] (mL/d)**	**q_4 [e] (mL/d) / Δ receiving media (%) [b]**	**q_5 (mL/d) [f] / Δ receiving media (%) [b]**
10	468 / 23.4	200/10	400	132/6.6	68/1.4%
20	234 / 11.7	100/5	200	66/3.3	34/0.7%
40	117 / 5.9%	50/2.5	100	33/1.7	17/0.4%

[a] q_1= SBR_{OSA} to aerobic/anoxic
[b] Δ receiving media (%) = volume transferred to the reactor/total volume of the reactor x 100
[c] q_2= aerobic/anoxic to anoxic
[d] q_3= wasted from aerobic/anoxic
[e] q_4= anoxic to aerobic/anoxic
[f] q_5= anoxic to SBR_{OSA}

Table S10. TrOCs with notable variation (>30 difference) in the solid phase of SBR_{OSA} and $SBR_{control}$ when SRT_{SBR} was maintained at 10 d and SRT_{ext} was varied (10-40 d); The values are the average of two measurements (n=2).

SRT_{ext} (d)	**TrOC**	**SBR_{OSA} (ng/g dry solids)**	**$SBR_{control}$ (ng/g dry solids)**
10	TCEP	45±5	68±4
	Atenolol	19±42	162±42
	Salicylic acid	Below detection limit	10,283±4,434
	Caffeine	97±16	217±7
	Diclofenac	150±9	244±2
	Amtriptylene	173±18	97±1
	Estrone	11±2	21±29
	Benzophenone	83±18	44±3
	Clozapine	141±14	88±15
	Bisphenol A	69±3	304±230
	Oxybenzone	119±13	53±11
	Sulfamethoxazole	100±24	49±14
20	TCEP	123±93	58±9
	Caffeine	107±19	178±22
	Sucralose	129±5	65±11
	Paracetamol	Below detection limit	159±2
	Ibuprofen	19±2	33±17
	Diclofenac	222±32	171±1
	Gemfibrozil	15±0	46±3
	Verapamil	169±4	238±10
	Amtriptylene	191±7	410±37
	Estrone	43±3	14±2
	Bisphenol A	1,922±2,620	111±1
	Oxybenzone	Below detection limit	85±4
	Sulfamethoxazole	268±37	Below detection limit
	Triclocarban	6,810±91	8,931±412
40	Atenolol	Below detection limit	30±1
	Estrone	5±1	22±1
	Clozapine	33±3	47±33
	Bisphenol A	17±5	38±11
	Triclocarban	2543±195	1,541±533


(a) Aqueous phase
SBR$_{OSA}$
Aerobic/anoxic
Anoxic
Log D
SRT$_{ext}$=10 d
SRT$_{ext}$=20 d
SRT$_{ext}$=40 d
TrOC concentration in aqueous phase (ng/L)
Log D (pH=7; 25 °C)
Atenolol
Aspartame
Salicylic acid
Saccharin
Metoprolol
Caffeine
Allopurinol
Sulfamethoxazole
Enalapril
Ketoprofen
Sucralose
Trimethoprim
Paracetamol
Naproxen
Primidone
Ibuprofen
Triamterene
Fluoxetine
Dilantin
Benzotriazole
TCEP
Diclofenac
Carbamazepine
Gemfibrozil
Verapamil
Hydroxyzine
Amitriptyline
Simazine
Estriol
Diuron
Androstenedione
Diazepam
Propylparaben
Estrone
Benzophenone
Clozapine
Phenylphenol
Levonorgestrel
Butylparaben
Bisphenol A
Diuzinon
Oxybenzone
Etiocholanolone
Androsterone
Ethynylestradiol
Testosterone
17α-estradiol
17β-estradiol
Triclosan
4-tert-Octylphenol
Nonylphenol
Triclocarban

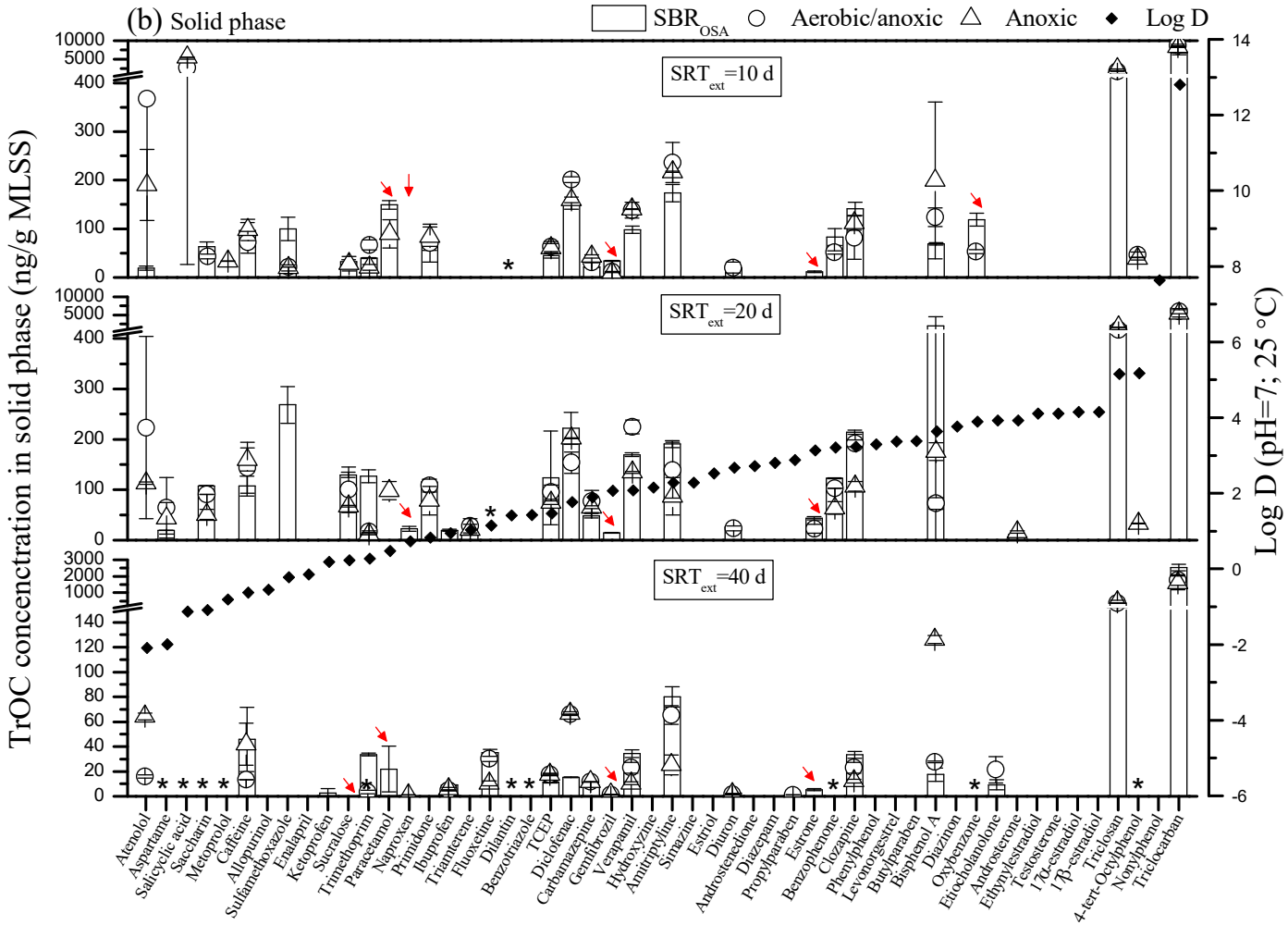


Figure S11. TrOC concentration in the (a) aqueous and (b) solid phase of sludge in the external aerobic/anoxic and anoxic reactor of OSA when SRT_{SBR} was maintained at 10 d and SRT_{ext} was varied (10-40 d). The values are the average of two measurements (n=2). The asterisks (*) represent contaminants that were not analysed in a particular sampling campaign. The arrows (→) denote denote contaminants that were highly biodegraded in the aerobic/anoxic reactor. No contaminant was highly biodegraded in the anoxic reactor.

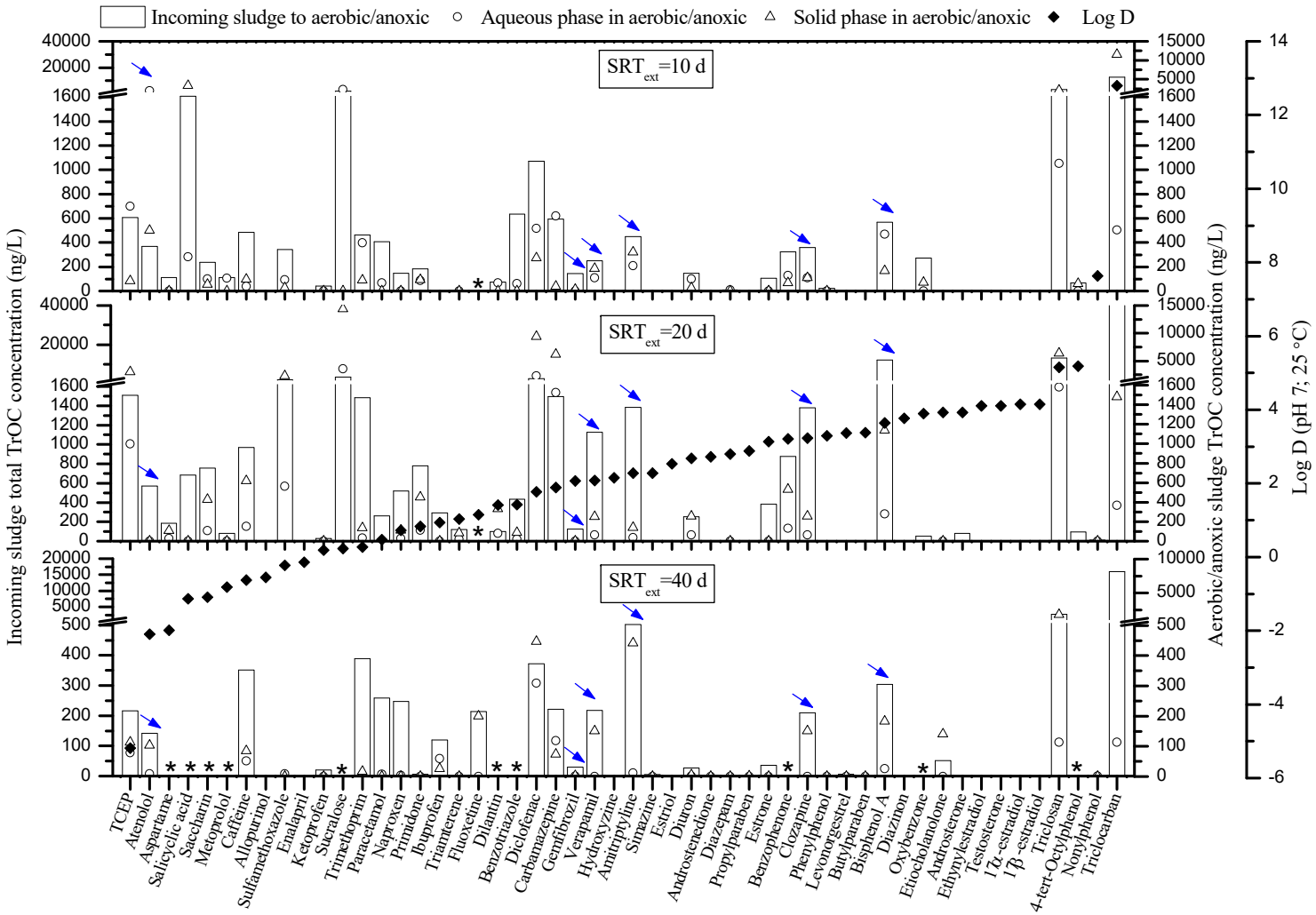


Table S12. The concentration TrOCs entering the aerobic/anoxic reactor ($Y_{in\text{-}aerobic/anoxic}$, labelled as "incoming sludge") *vs.* the concentration of TrOCs in aqueous and solid phase of sludge in aerobic/anoxic reactor when SRT_{SBR} was maintained at 10 d and SRT_{ext} was varied (10-40 d). The values are the average of two measurements. The asterisks (*) represent contaminants that were not analysed in a particular sampling campaign. The biodegradation of some TrOCs (denoted by arrows →) increased when SRT_{ext} was increased from 10 to 20 d, but decreased when SRT_{ext} was further increased to 40 d.

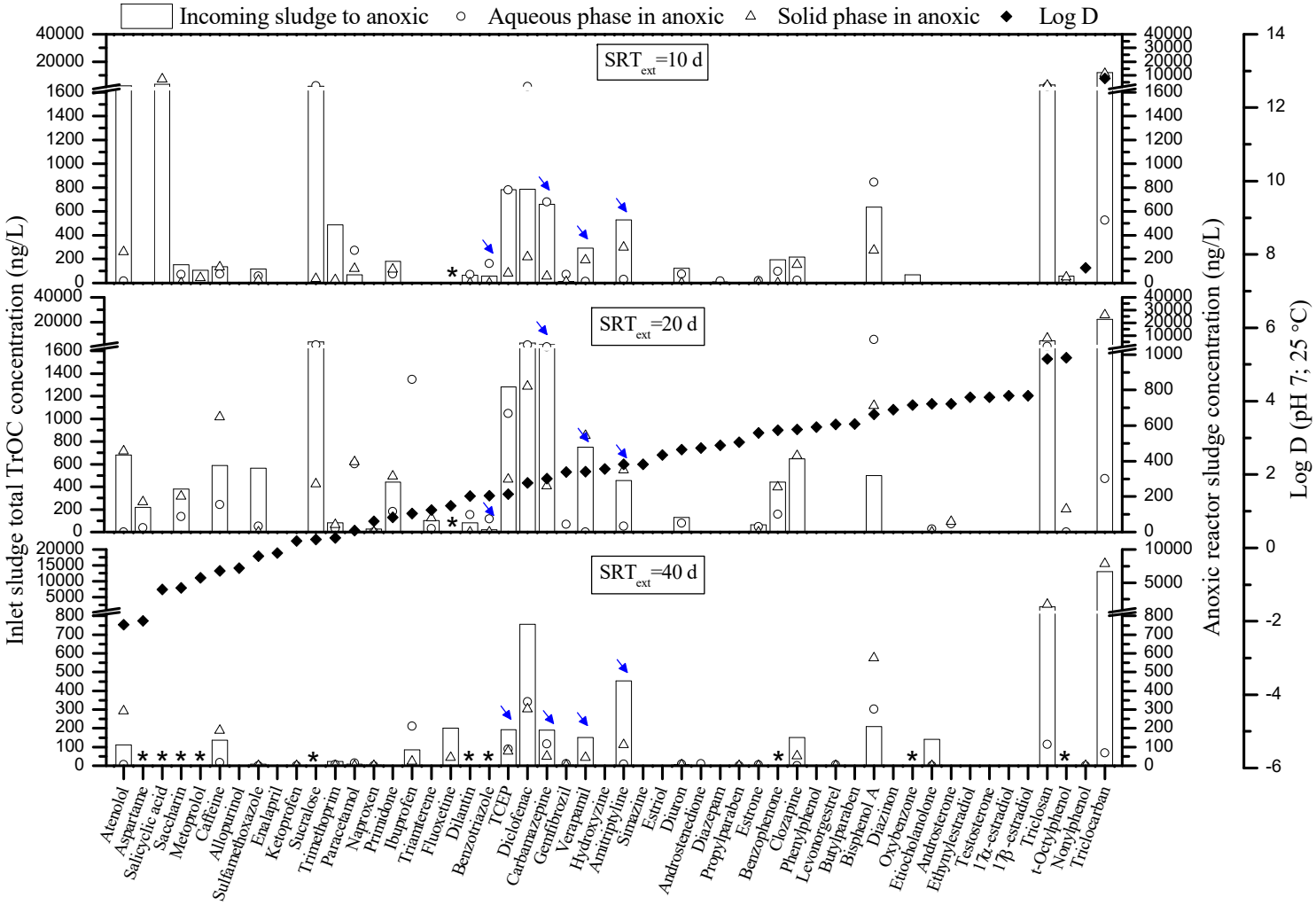
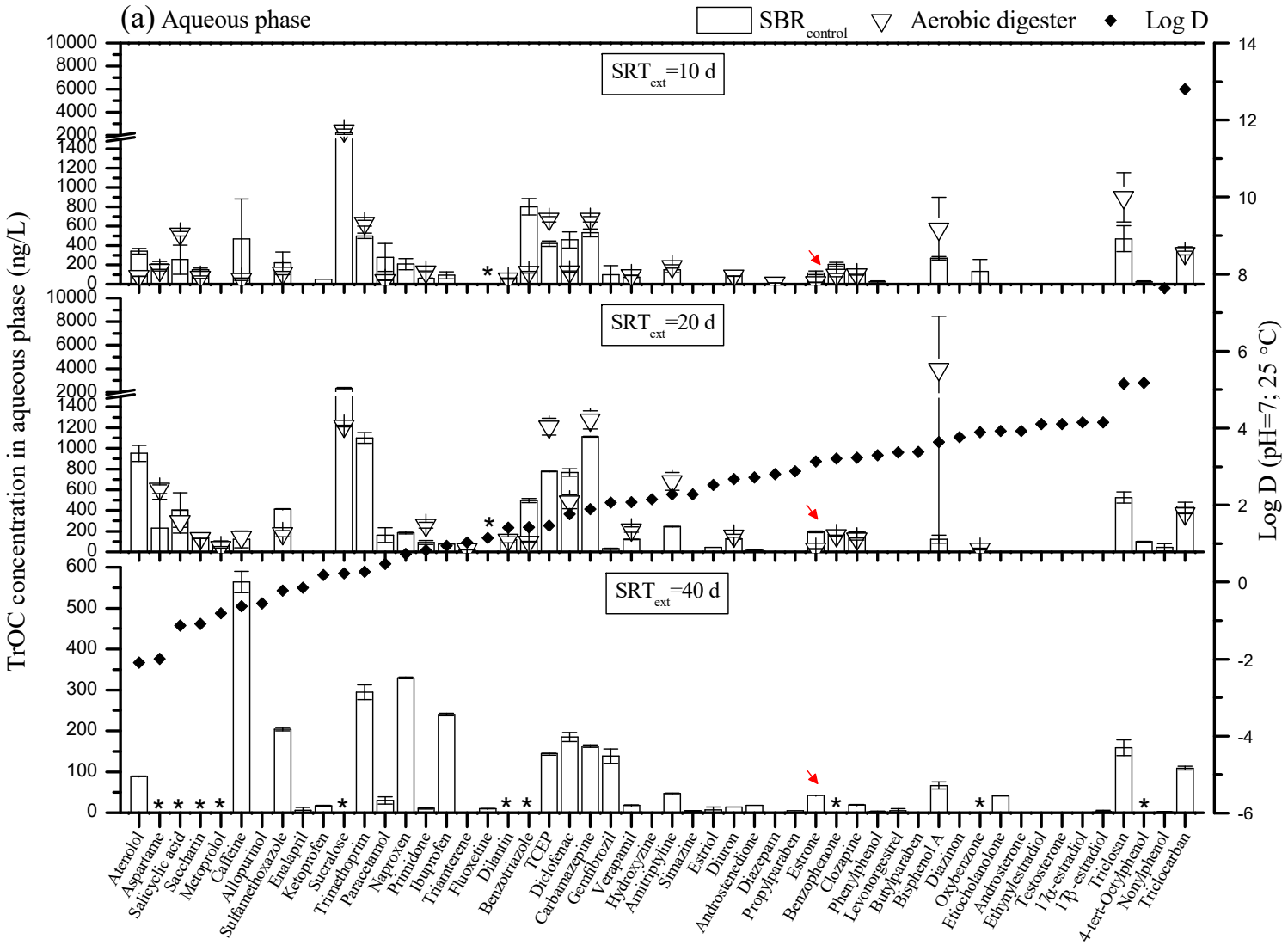


Figure S13. The concentration TrOCs entering anoxic reactor ($Y_{in\text{-}anoxic}$, labelled as "incoming sludge") *vs.* the concentration of TrOCs in aqueous and solid phase of sludge in anoxic reactor when SRT_{SBR} was maintained at 10 d and SRT_{ext} was varied (10-40 d). The values are the average of two measurements. The asterisks (*) represent contaminants that were not analysed in a particular sampling campaign. The biodegradation of some contaminants (denoted by arrows →) increased when SRT_{ext} was increased from 10 to 40 d.


(a) Aqueous phase
SBR control
Aerobic digester
Log D
SRT ext =10 d
SRT ext =20 d
SRT ext =40 d
TrOC concentration in aqueous phase (ng/L)
Log D (pH=7; 25 °C)
Atenolol
Aspartame
Salicyclic acid
Saccharin
Metoprolol
Caffeine
Allopurinol
Sulfamethoxazole
Enalapril
Ketoprofen
Sucralose
Trimethoprim
Paracetamol
Naproxen
Primidone
Ibuprofen
Trianterene
Fluoxetine
Dilantin
Benzotriazole
TCEP
Diclofenac
Carbamazepine
Gemfibrozil
Verapamil
Hydroxyzine
Amitriptyline
Simazine
Estriol
Diuron
Androstenedione
Diazepam
Propylparaben
Estrone
Benzophenone
Clozapine
Phenylphenol
Levonorgestrel
Butylparaben
Bisphenol A
Diazinon
Oxybenzone
Etiocholanolone
Androsterone
Ethynylestradiol
Testosterone
17α-estradiol
17β-estradiol
Triclosan
4-tert-Octylphenol
Nonylphenol
Triclocarban

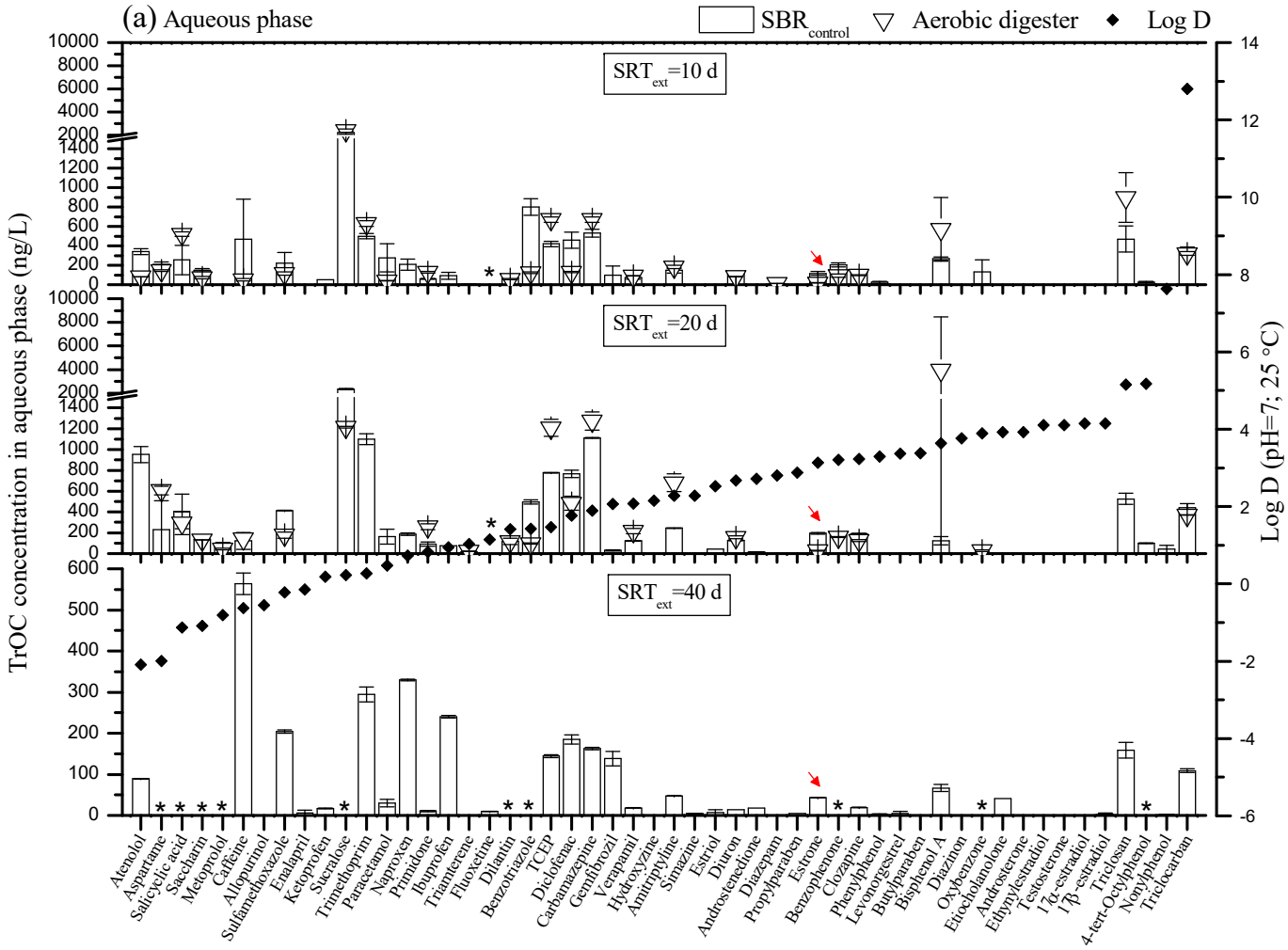


Figure S14. TrOC concentration in the (a) aqueous and (b) solid phase of sludge in the aerobic digester when SRT_{SBR} was maintained at 10 d and SRT_{ext} was varied (10-40 d). The values are the average of two measurements (n=2). The asterisks (*) represent contaminants that were not analysed in a particular sampling campaign. The arrows (→) denote contaminants that were highly biodegraded.

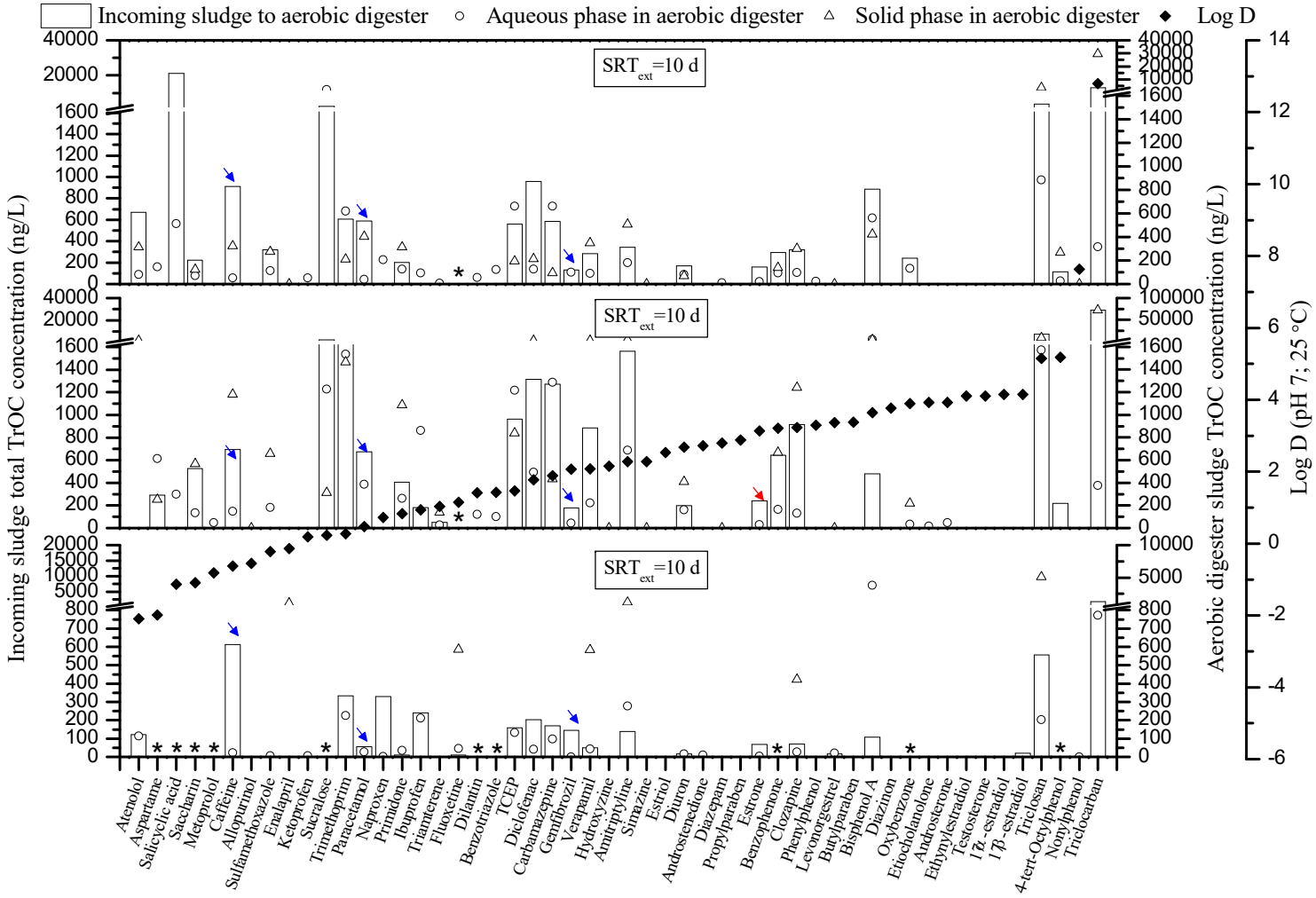


Figure S15. The concentration TrOCs entering control aerobic digester ($Y_{in\text{- aerobic}}$, labelled as "incoming sludge") *vs.* the concentration of TrOCs in aqueous and solid phase of sludge in the aerobic digester when SRT_{SBR} was maintained at 10 d and SRT_{ext} was varied (10-40 d). The values are the average of two measurements. The asterisks (*) represent contaminants that were not analysed in a particular sampling campaign.The biodegradation of some TrOCs (denoted by arrows →) increased when SRT_{ext} was increased from 10 to 40.