Biodegradation of the artificial sweetener acesulfame in biological wastewater treatment and sandfilters

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Supplementary Information

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Formation of SA by transformation of ANSA (c₀ =400 µg/L) in batch experiments (n=3) conducted with activated sludge from SBR which was repeatedly washed and resuspended in 50 mM phosphate buffer.

Formation of SA by transformation of ACE (c₀ =400 µg/L) in batch experiments (n=3) conducted with activated sludge from SBR which was repeatedly washed and resuspended in 50 mM phosphate buffer.

List of retention times (RTs) and precursors and product ions from MS and MS² spectra obtained from ACE and ACE TPs using IC-LTQ-Orbitrap-MS with electrospray ionization in negative ionization mode.

Identification of TPs

Possible chemical structures of TP180a and TP 180b.

References
Tab. S1: Overview about the biological treatment steps, selected operational parameters as well as the average ACE concentrations (± standard deviation) detected in the influents and effluents of 13 municipal WWTPs which were sampled over a period of two weeks (24 h composite samples, n=6). The operational parameters were provided by the WWTPs. Values of the chemical oxygen demand (COD) and NH4-N represent either the annual average or (in parentheses) the average of the sampling period. Influent samples were taken from raw wastewater before biological treatment and effluent samples were taken from the final effluent before discharge into the receiving water (i.e. after secondary clarification or sand filtration).

| WWTP | Capacity [PE] | Discharge [m³/d] | Biological treatment steps | SRT [d] | TSS [g/L] | COD Influent [mg/L] | COD Effluent [mg/L] | COD Removal [%] | NH4-N Influent [mg/L] | NH4-N Effluent [mg/L] | NH4-N Removal [%] | ACE Influent [µg/L] | ACE Effluent [µg/L] | ACE Removal [%] |
|------|--------------|------------------|---------------------------|--------|---------|---------------------|---------------------|----------------|---------------------|---------------------|----------------|----------------|----------------|----------------|----------------|
| A    | 50,000       | 13,000           | AS (pD, N)                | 11     | ND      | 376                 | 18                  | 95.2           | 24                  | 1.0                 | 95.8           | 32 ± 9         | 1.0 ± 0.1       | 96 ± 3         |
| B    | 44,500       | 12,000           | AS (pD, N), SF            | 20     | ND      | (650)              | (13)               | (98)           | (37)               | (<0.1)             | (>99.7)        | 46 ± 6         | 1.4 ± 0.3       | 97 ± 1         |
| C    | 50,000       | 8100             | AS (pD, N)                | 20     | 3.5     | 319                 | 20                  | 93.7           | 28                  | 0.35                | 99.2           | 28 ± 6         | 0.97 ± 0.21     | 96 ± 1         |
| D    | 47,500       | 7500             | AS (pD, N)                | 12     | 4       | 639                 | 24                  | 96.2           | 34                  | 0.54                | 98.4           | 35 ± 7         | 2.2 ± 0.2       | 93 ± 2         |
| E    | 30,000       | 6130             | AS-s (iD, N)              | 29     | 6       | 437                 | 18                  | 95.9           | 27                  | 0.2                 | 99.3           | 39 ± 10        | 2.7 ± 0.3       | 92 ± 4         |
| F    | 80,000       | 62,400           | AS (pD, N)                | 17     | 3.8     | 496                 | (321)              | 96.4           | (95.0)             | 26                  | 0.7            | 96.3           | 26 ± 7         | 1.9 ± 0.2       | 92 ± 3         |
| G    | 78,000       | 8500             | AS (pD, N), SF            | 11     | ND      | 879                 | 26                  | 97.0           | 19                  | 0.51                | 97.3           | 20 ± 5         | 1.7 ± 0.7       | 90 ± 6         |
| H    | 54,000       | 25,000           | AS (pD, N), FB (pD, N), SF| 10     | ND      | 650                 | 23                  | 96.5           | 20                  | 0.29                | 98.6           | 25 ± 9         | 2.8 ± 1.1       | 86 ± 11        |
| I    | 29,000       | 4420             | AS (pD, N)                | ND     | 3.5     | (370)               | (22)               | (94.1)         | (54)               | (<0.1)             | (>99.8)        | 36 ± 5         | 4.8 ± 0.5       | 87 ± 1         |
| J    | 440,000      | 88,400           | AS (pD, N), SF            | 14     | 4.5     | (527)               | (15)               | (97.4)         | ND                  | ND                  | ND             | 26 ± 3         | 3.1 ± 0.2       | 88 ± 1         |
| K    | 350,000      | 60,300           | AS (iD, N)                | 12     | 3.5     | 440                 | (359)              | 92.5           | 50                  | 4.9                 | 90.3           | 81 ± 28        | 18 ± 14         | 77 ± 13        |
| L    | 220,000      | 27,400           | AS (pD, N)                | 13.5   | 4       | 703                 | 37                  | 94.7           | 40                  | 0.56                | 98.6           | 48 ± 2         | 13 ± 1         | 72 ± 2         |
| M    | 25,500       | 3200             | H-BF-AS (pD, N)           | 5      | ND      | (121)               | (19)               | (84.2)         | (34)               | (0.13)              | (99.6)         | 43 ± 6         | 19 ± 3         | 57 ± 8         |

AS: activated sludge treatment, AS-s: activated sludge treatment with sludge stabilization, pD: pre-denitrification, iD: intermittent denitrification, N: nitrification, FB: fixed-bed biofilm reactor, H-BF-AS hybrid biofilm-activated sludge process, SF: sand filtration; ND: no data.
S1 Description of the analytical methods

S1.1 Quantitative analysis of ACE by LC-MS/MS

Quantitative analysis of ACE in samples from WWTPs and the waterworks was conducted by LC-MS/MS. After addition of the surrogate standard (4 µg/L ACE-d₄), 80 µL of the samples were injected into an Agilent 1260 Series liquid chromatography system (Agilent Technologies, Waldbronn, Germany) coupled to a SCIEX QTrap 5500 mass spectrometer (SCIEX, Darmstadt, Germany). Chromatographic separation was achieved using a Zorbax Eclipse Plus C18 column (2.1 x 150 mm, 3.5 µm, Agilent Technologies, Waldbronn, Germany). Ultrapure water was used as mobile phase A and methanol as mobile phase B, both acidified with 0.1% formic acid. The gradient elution applied was as follows: 100% A for 1 min, decrease to 80% A within 1 min, further decrease to 0% A within 14.5 min, hold isocratic at 0% A for 5.5 min, increase to initial conditions (100% A) within 0.1 min and hold isocratic for 6 min. The total run time was 28 min and the flow rate and the column temperature were set to 0.3 mL/min and to 30°C, respectively. The mass spectrometer was operated with negative ion electrospray ionization (ESI) using multiple reaction monitoring (MRM). The ion source conditions were as follows: collision gas, medium; curtain gas 40 psi; ion source gas 1, 40 psi; ion source gas 2, 45 psi; source temperature 500°C; ion spray voltage, -4.5 kV; entrance potential, -10 V. The monitored mass transitions and compound specific MS parameters are described in Tab. S2.

S1.2 Quantitative analysis of ACE and the TPs SA and ANSA by IC-MS/MS

Quantitative analysis of ACE and the TPs SA and ANSA in samples from batch experiments was realized with an ion chromatography system (Metrohm 881 Compact IC pro with chemical suppressor, Metrohm GmbH, Filderstadt, Germany), coupled to an API 4000 triple quadrupole mass spectrometer (SCIEX, Darmstadt, Germany). ACE-d₄ was used as surrogate standard. Chromatographic separation was achieved on a Metrosep A Supp 5 anion exchange column (150 x 4 mm) connected to a Metrosep A Supp 4/5 guard column (Metrohm GmbH, Filderstadt, Germany). Ultrapure water and a carbonate buffer (5 mM Na₂CO₃ and 3.1 mM NaHCO₃) both amended with 10% methanol were used as mobile phase A and B, respectively. The gradient elution applied was as follows 36% A for 8 min, decrease to 0% A within 2 min, hold isocratic at 0% A for 30 min, increase to initial conditions (36% A) within 1 min and hold isocratic at 36% A for 4 min. The total run time was 45 min and the flow rate and the column temperature were set to 0.7 mL/min and to 50°C, respectively. The injection volume was 10 µL. The mass spectrometer was operated with negative electrospray ionization (ESI) using multiple reaction monitoring (MRM). The ion source conditions were as follows: collision gas, medium; curtain
gas 30 psi; ion source gas 1, 50 psi; ion source gas 2, 50 psi; source temperature 600°C; ion spray voltage, -4.2 kV; entrance potential, -10 V. The monitored mass transitions and compound specific MS parameters are described in Tab. S2.

**Tab. S2**: Monitored mass transitions and compound specific MS parameters used for quantification of acesulfame (ACE), sulfamic acid (SA) and acetoacetamide-N-sulfonic acid (ANSA).

| Compound | MRM 1 [m/z] | MRM 2 [m/z] | Dwell time [ms] | DP [V] | CE [eV] | CXP [V] |
|----------|-------------|-------------|-----------------|--------|---------|--------|
| ACE      | 162.0/82.0  | 162.0/78.0  | 30              | -50/-50| -22/-38 | -5/-3  |
| ACE-d4   | 166.0/86.0  | -           | 30              | -50/-  | -22/-   | -5/-   |
| SA       | 96.0/80.0   | -           | 30              | -50/-  | -24/-   | -6/-   |
| ANSA     | 180.0/96.0  | 180.0/80.0  | 30              | -40/-40| -23/-55 | -5/-10 |

DP: declustering potential, CE: collision energy, CXP: cell exit potential

**S1.3 Calibration and determination of the limit of quantification (LOQ)**

For quantification of ACE, SA and ANSA an internal standard calibration with linear fitting and a weighing factor of 1/x was used. The limit of quantification was derived from the signal-to-noise (S/N) ratio in the native samples. At the LOQ, the S/N ratio of the mass transitions used for quantification (MRM 1) and confirmation (MRM 2, not available for SA) had to be at least 10 and 3, respectively.

**S1.4 TP identification by high-resolution mass spectrometry**

High-resolution mass spectra and MS² fragmentation experiments for the detection, identification and characterization of ACE TPs were obtained by Orbitrap-MS (LTQ Orbitrap Elite, Thermo Scientific, Bremen, Germany) operated in negative and positive ionization mode. The Orbitrap system was coupled to an ion chromatography system (Metrohm 881 Compact IC pro with chemical suppressor, Metrohm GmbH, Filderstadt, Germany). Chromatographic conditions were the same as described above (S.1.2) for quantification of the TPs SA and ANSA.

The source parameters for the IC-LTQ-Orbitrap-MS measurements were set as follows (values for the positive ionization mode are given in parenthesis): capillary temperature, 325°C (325°C); capillary voltage, 3.2 kV (2.8 kV); heater temperature, 450°C (350°C); sheath gas flow rate, 45 AU (40 AU); aux gas flow rate, 15 AU (15 AU); S-lens RF level, 60% (60%).

Data dependent acquisition was used to give rise to spectra as follows: A full scan (50–300 m/z) was performed followed by MS² scans for the two most intense ions with intensities of > 1000 and > 500, respectively. CID (collision-induced dissociation) and HCD (higher energy collisional dissociation) with normalized collision energies of 35% and 120%, respectively.
were used for fragmentation. In addition, dynamic exclusion was applied (exclusion of masses for which two MS\(^2\) experiments have been performed; exclusion duration, 15 s; repeat duration, 30 s). External calibration was performed before the analysis of each batch to assure accurate mass determinations with a resolution of 60,000.

To avoid the missing of TPs not amenable to chromatographic separation by IC, the Orbitrap system was also interfaced with a Thermo Scientific Accela U-HPLC system (Accela pump and autosampler) equipped with a reversed-phase (RP) column (Hydro-RP, 150 x 4.6 mm, 4 µm) from Phenomenex (Aschaffenburg, Germany). Ultrapure water and acetonitrile (both amended with 0.1% formic acid) were used as mobile phase A and B, respectively. The gradient elution applied was as follows 100% A for 5 min, decrease to 60% A within 10 min, further decrease to 5% A within 3 min, hold isocratic at 5% A for 5 min, increase to initial conditions (100% A) within 0.1 min and hold isocratic at 100% A for 6.9 min. The total run time was 30 min and the flow rate and the column temperature were set to 0.5 mL/min and to 50°C, respectively. The injection volume was 10 µL.

**S1.5 Determination of basic operational parameters**

Basic operational parameters in experiments with activated sludge were measured with a spectral photometer (DR 5000 UV-VIS, Hach Lange GmbH, Düsseldorf, Germany) using cuvette tests (NO\(_3\)-N: LCK 339, NH\(_4\)-N: LCK303 and LCK304, DOC: LCK385, all Hach Lange GmbH, Düsseldorf, Germany).

In batch-experiments with sand, analyses of dissolved organic carbon (DOC) was performed according to German standard DIN EN 1484 (DIN, 1997) by combustion catalytic oxidation using a TOC analyser (TOC-L series, Shimadzu, Kyoto, Japan).
Tab. S3: Basic operational parameters measured in laboratory batch experiments for the approaches A and B (all concentrations as mg/L).

| Setup | time [h] |  |  | replicate |  |  |  |  |  |  | TSS |
|-------|----------|---|---|-----------|---|---|---|---|---|---|-----|
|       |          | 1 |   |           | 2 |   | 3 |   |   |   |     |
|       |          | NH₄-N | NO₃-N | DOC | NH₄-N | NO₃-N | DOC | NH₄-N | NO₃-N | DOC |     |
| A1    | 0        | 15.3 | 1.4 | 14.7 | 15.3 | 1.5 | 15.4 | - | - | - | -   |
|       | 4        | < LOQ | 13.8 | - | 1.7 | 9.3 | - | - | - | - | -   |
|       | 6        | < LOQ | 15.6 | 11.8 | < LOQ | 13.9 | - | - | - | - | -   |
|       | 27       | < LOQ | 27.4 | - | < LOQ | 25.9 | - | - | - | - | -   |
| A2    | 0        | 15.0 | 1.2 | 17.3 | 14.8 | 1.2 | 16.2 | - | - | - | -   |
|       | 4        | - | - | - | - | - | - | - | - | - | -   |
|       | 6        | 15.8 | 1.0 | 14.1 | 15.8 | - | - | 15.7 | - | - | -   |
|       | 27       | 20.2 | 2.9 | - | 17.3 | 2.8 | - | - | - | - | -   |
| A3    | 0        | < LOQ | 8.0 | 11.9 | < LOQ | 7.8 | 12.5 | - | - | - | -   |
|       | 4        | - | - | - | - | - | - | - | - | - | -   |
|       | 6        | < LOQ | 9.9 | 13.4 | < LOQ | 9.9 | - | - | - | - | -   |
|       | 27       | < LOQ | 23.9 | - | < LOQ | 25.4 | - | - | - | - | -   |
| A4    | 0        | 19.3 | 7.6 | 12.6 | 19.5 | 7.6 | 11.7 | - | - | - | 1900 |
|       | 4        | < LOQ | 24.2 | - | 4.1 | 18.7 | - | - | - | - | -   |
|       | 6        | < LOQ | 26.2 | 10.9 | < LOQ | 23.6 | - | - | - | - | -   |
|       | 27       | < LOQ | 39.8 | - | < LOQ | 35.6 | - | - | - | - | -   |
| A5    | 0        | 3.1 | 158.0 | 16.8 | 3.3 | 155.0 | 17.0 | - | - | - | -   |
|       | 4        | 3.0 | 161.0 | 16.6 | 3.3 | 151.0 | 17.0 | - | - | - | -   |
|       | 6        | - | - | - | - | - | - | - | - | - | -   |
|       | 27       | 3.1 | - | - | 3.2 | - | - | - | - | - | -   |
| B1    | 0        | - | < LOQ | - | - | < LOQ | - | - | < LOQ | - | -   |
|       | 8        | - | - | - | - | - | - | - | - | - | -   |
|       | 24       | - | - | - | - | - | - | - | - | - | -   |
|       | 48       | - | 26.3 | - | - | 27.7 | - | - | 25.9 | - | -   |
|       | 96       | - | 37.8 | - | - | 42.3 | - | - | 37.0 | - | -   |
| B2    | 0        | - | 204 | - | - | 211 | - | - | 214 | - | 1640 |
|       | 8        | - | 188 | - | - | 192 | - | - | 197 | - | -   |
|       | 24       | - | 176 | - | - | 172 | - | - | 176 | - | -   |
|       | 48       | - | 150 | - | - | 147 | - | - | 153 | - | -   |
|       | 96       | - | 117 | - | - | 116 | - | - | 118 | - | -   |
| B3    | 0        | - | < LOQ | - | - | < LOQ | - | - | < LOQ | - | -   |
|       | 8        | - | < LOQ | - | - | < LOQ | - | - | < LOQ | - | -   |
|       | 24       | - | - | - | - | - | - | - | - | - | -   |
|       | 48       | - | < LOQ | - | - | < LOQ | - | - | < LOQ | - | -   |
|       | 96       | - | < LOQ | - | - | < LOQ | - | - | < LOQ | - | -   |
Tab. S4: Basic operational parameters measured in laboratory batch experiments for the approach C (all concentrations as mg/L).

| Setup | time | replicate |
|-------|------|-----------|
|       | [d]  | [h]       |           |
|       |      | NH₃-N     | NO₂⁻-N   | DOC     | NH₃-N | NO₂⁻-N | DOC     | NH₃-N | NO₂⁻-N | DOC     |
| C1 &  | 0    | 0         |           | 0.1     | 4.96  | 12.9    | 0.1     | 4.96  | 12.9    | 0.1     | 4.96  | 12.9    |
| C2    | 0    | 2         | -        | -       |       |         | 13.3    |       |         | 0.1     | 4.96  | 12.9    |
|       | 2    | 48        | < LOQ    | 24.7    | 12.9  | < LOQ   | 25.28   | 13.2  | < LOQ   | 0.1     | 56    | 17.4    |
|       | 6    | 144       | < LOQ    | 52.6    | 14.4  | 0.1     | 56      | 17.4  | 0.1     | 55.9    | 16.5  |
|       | 10   | 240       | -        | -       |       |         | 18.4    |       |         | -       | -     | 21.8    |
|       | 10   | 241       | -        | -       |       |         | 18.4    |       |         | -       | -     | 21.8    |
| C1    | 14   | 336       | 1.7      | 76.6    | 17.1  | 4.1     | 77.7    | 20.8  | < LOQ   | 2.4     | 77.4  | 19.7    |
|       | 14   | 337       | -        | -       |       |         | 17.1    |       |         | -       | -     | 19.7    |
|       | 16   | 384       | -        | -       |       |         | 18.8    |       |         | -       | -     | 19.7    |
|       | 20   | 481       | -        | -       |       |         | 19.2    |       |         | -       | -     | 17.6    |
|       | 21** | 505       | 0.2      | 94.6    | 19.8  | 0.2     | 86.1    | 19.7  | 0.4     | 90.4    | 21.8  |
|       | 23   | 552       | -        | -       |       |         | 19.8    |       |         | -       | -     | 19.8    |
|       | 28   | 672       | 0.1      | 106     | 16.3  | 0.1     | 95.3    | 17.6  | 0.1     | 98.9    | 17.1  |
|       | 29   | 696       | -        | -       |       |         | 22.1    |       |         | -       | -     | 27.2    |
| C2    | 0    | 2         | -        | -       |       |         | 25.4    |       |         | -       | -     | 24.9    |
|       | 2    | 48        | < LOQ    | 27.0    | 25.6  | < LOQ   | 25.8    | 25.7  | < LOQ   | 0.3     | 61    | 32.4    |
|       | 6    | 144       | < LOQ    | 53.4    | 25.8  | 0.2     | 59.3    | 33.0  | 0.3     | 61      | 32.4  |
|       | 10   | 240       | -        | -       |       |         | 24.2    |       |         | -       | -     | 40.4    |
|       | 10   | 241       | -        | -       |       |         | -       |       |         | -       | -     | -       |
|       | 14   | 336       | 0.4      | 75.2    | 17.4  | 6.2     | 78.4    | 35.8  | 5.4     | -       | -     | 35.4    |
|       | 14   | 337       | -        | -       |       |         | 37.6    |       |         | -       | -     | 44.4    |
|       | 16   | 384       | -        | -       |       |         | 34.9    |       |         | -       | -     | 42.4    |
|       | 20   | 481       | -        | -       |       |         | 21.8    |       |         | -       | -     | 28.4    |
|       | 21** | 505       | < LOQ    | 86.9    | 39.8  | 0.1     | 91.7    | 45.2  | 1.3     | -       | -     | 40.8    |
|       | 23   | 552       | -        | -       |       |         | 43.6    |       |         | -       | -     | 39.8    |
|       | 28   | 672       | < LOQ    | 96.1    | 18.2  | 0.6     | 99.4    | 21.7  | 0.0     | -       | -     | 26.6    |
|       | 29   | 696       | -        | -       |       |         | 23.7    |       |         | -       | -     | 29.8    |

*before aliquoting into separate test-vessels
** after respike
Tab. S5: Basic operational parameters measured in bench scale reactors in batch mode (all concentrations as mg/L).

| Setup | time [h] | NH$_4$-N | NO$_3$-N | DOC |
|-------|---------|---------|---------|-----|
| R1    | 0       | 11.8    | 1.7     | 19.5|
|       | 1       | < LOQ   | 11.9    | 13.6|
|       | 2       | < LOQ   | 12.3    | 12.9|
|       | 4       | < LOQ   | 12.7    | 13.3|
|       | 7       | < LOQ   | 14.0    | 13.1|
|       | 11      | < LOQ   | 16.1    | 13.3|
|       | 24      | < LOQ   | 24.6    | 13.9|
|       | 51      | < LOQ   | 44      | 14.9|
| R2    | 0       | 12.0    | 0.3     | 18.1|
|       | 1       | < LOQ   | 12.5    | 12.8|
|       | 2       | < LOQ   | 12.8    | 12.5|
|       | 4       | -       | -       | -   |
|       | 7       | -       | 18.7    | -   |
|       | 11      | -       | 21.6    | 11.9|
|       | 24      | < LOQ   | 33.3    | 13.1|
|       | 51      | < LOQ   | 62.1    | 15.5|
| R3    | 0       | 7.8     | 210     | 12.9|
|       | 1       | -       | 222     | 12.5|
|       | 2       | -       | 235     | 12.6|
|       | 4       | -       | 264     | 12.4|
|       | 7       | 6.7     | 298     | 12.6|
|       | 11      | -       | 348     | 11.1|
|       | 24      | -       | 504     | 11.3|
|       | 51      | 13.2    | 806     | 11.9|
| R4    | 0       | < LOQ   | 10.8    | 11.9|
|       | 1       | < LOQ   | 11.6    | 10.3|
|       | 2       | < LOQ   | 11.8    | 10.4|
|       | 4       | -       | -       | -   |
|       | 7       | -       | 15.3    | -   |
|       | 11      | -       | 15.4    | 9.7 |
|       | 24      | < LOQ   | 15.9    | 9.5 |
|       | 51      | < LOQ   | 20.6    | 8.7 |
Tab. S6: Basic operational parameters measured in influent and effluent of the bench scale reactors in sequencing batch mode (all concentrations as mg/L).

| Date       | Influent | R1 effluent | R2 effluent | R3 effluent | R4 effluent |
|------------|----------|-------------|-------------|-------------|-------------|
|            | NH$_4$-N | DOC         | NH$_4$-N | NO$_3$-N | DOC | NH$_4$-N | NO$_3$-N | DOC | NH$_4$-N | NO$_3$-N | DOC |
| 23.03.2015 | 43.8     | 89.6        | < LOQ     | 10.5      | 10.7 | 0.32      | 9.6      | 11.8 | 5.4       | 216      | 10.1 |
| 26.03.2015 | 49.0     | 85.2        | < LOQ     | 12.0      | 11.2 | 0.47      | 9.7      | 12.2 | 4.9       | 230      | 10.0 |
| 09.04.2015 | 47.4     | 91.2        | < LOQ     | 11.9      | 11.1 | 0.21      | 8.8      | 11.4 | 6.7       | 243      | 10.6 |
| 13.04.2015 | 46.2     | 72.8        | 0.09       | 11.4      | 11.2 | 0.26      | 9.2      | 12.3 | 13.8      | 218      | 11.8 |
| 24.04.2015 | 48.0     | 87.2        | 0.23       | 11.4      | 12.4 | < LOQ     | 10.9     | 12.0 | 17.9      | 221      | 10.8 |
| 27.04.2015 | 45.0     | 87.6        | 0.30       | 10.6      | 11.7 | < LOQ     | 10.0     | 12.1 | 12.6      | 231      | 12.4 |
| 25.05.2015 | 48.0     |             | 0.28       | 10.3      |     | 0.10      | 9.2      |     | 16.9      | 260      |     |
| 01.06.2015 | 36.6     | 70.2        | 0.17       | 7.3       | 10.1 | 0.19      | 6.5      | 11.8 | 17.1      | 245      | 10.4 |
| 05.06.2015 | -        |             | -          | 8.2       |     | -         | 7.0      |     | -         | 250      |     |
| 08.06.2015 | 59.8     | 73.4        | 0.24       | -         | 11.5 | 0.22      | -        | 12.4 | 17.8      | -        | 10.8 |
| 13.07.2015 | 46.0     | 77.2        | 0.20       | 8.0       | 10.5 | < LOQ     | 6.1      | 11.3 | 11.8      | 255      | 10.8 |
| 18.12.2015 | 35.8     | 71.6        | < LOQ     | 8.4       | 11.1 | < LOQ     | 7.6      | 12.7 | 20.4      | 188      | 10.3 |
| 21.12.2015 | 51.4     | 99.2        | < LOQ     | 9.6       | 11.9 | 0.14      | 8.3      | 15.7 | 19.6      | 198      | 10.3 |
**Fig. S1:** Concentration [µg/L] of ACE in surface water and in the respective collection well after slow sand filtration and short underground passage.

**Fig. S2:** Ratio [%] of the sum of molar concentrations of ACE and SA and the spiked ACE concentration (ACE+SA/spiked ACE) in the high spike batch experiment (c₀ (ACE) = 40 mg/L) with activated sludge. The error bars represent the standard deviation (n=3). *respike of ACE
Fig. S3: Formation of SA by transformation of ANSA (c₀ = 400 µg/L) in batch experiments (n=3) conducted with activated sludge from SBR which was repeatedly washed and resuspended in 50 mM phosphate buffer. The error bars represent the standard deviation.

Fig. S4: Formation of SA by transformation of ACE (c₀ = 400 µg/L) in batch experiments (n=3) conducted with activated sludge from SBR which was repeatedly washed and resuspended in 50 mM phosphate buffer. The error bars represent the standard deviation.
**Tab. S7:** List of retention times (RTs) and precursors and product ions from MS and MS² spectra obtained from ACE and ACE TPs using IC-LTQ-Orbitrap-MS with electrospray ionization in negative ionization mode.

| Compound | Precursor ion | Measured MW (Error in ppm) | Elemental composition | Fragment ions | Intensity [%] | Proposed fragmentation |
|----------|---------------|-----------------------------|-----------------------|--------------|--------------|----------------------|
| Acesulfame | 161.9867 (0.546) C₄H₄NO₃S | 101.9763 (1.5) 98.0249 (1.8) 82.0300 (2.4) | C₄H₄NO₃S 3 | CID (35%) | [M-C₄H₄O₃]⁻ |
| TP 180a | 179.9972 (-0.04) C₆H₆NO₄S | 121.9558 (2.6) 95.9765 (3.8) | CNO₂S 2 | CID (35%) | [M-C₅H₄O]⁻ |
| TP 180b | 179.9971 (-0.42) C₆H₆NO₄S | 135.9711 (0.36) 121.9555 (1.0) 105.9606 (1.2) 72.0093 (2.7) | C₆H₆NO₄S 100 | CID (35%) | [M-C₅H₄O]⁻ |
| TP 178 | 177.9814 (-0.65) C₆H₆NO₄S | 134.9758 (0.43) 98.0249 (1.4) | C₆H₆NO₂ 100 | CID (35%) | [M-CHNO]⁻ |
| TP 192 | 191.9613 (2.2) C₆H₆NO₄S | 112.0045 (4.4) 84.0096 (5.6) | C₆H₆NO₃ 100 | CID (35%) | [M-SO₃⁻]⁻ |

CID: collision-induced dissociation, HCD: higher energy collisional dissociation

**S2 Identification of TPs**

Based on the measured exact mass of m/z 95.9763 suggesting a sum formula of H₂NSO₃ TP96 was identified as sulfamic acid (SA). This was confirmed by comparison of retention times and MS-fragmentation-spectra with the acquired reference standard of SA.

The high resolution mass spectrometric analysis of TP180a (RT 23.8 min) and TP180b (RT 10.7 min) revealed an exact mass of 179.9972 which indicated the introduction of an oxygen (+O) and two hydrogen (+2H) atoms. Four plausible structures (see Fig. S5) were assumed for TP180a and TP180b: i) a hydrolysis of the amide bond leading to an open ring structure with a carboxylic group at the former C-4 position and a sulfamate moiety ((2Z)-3-(sulfamoyloxy)but-2-enoic acid, structure A), ii) a water addition in α-position (C-5) forming 5-hydroxy-6-methyl-4-oxo-1,2,3-oxathiazinan-3-ide 2,2-dioxide (structure B), iii) a water addition in β-position (C-6) of the keto group forming 6-hydroxy-6-methyl-4-oxo-1,2,3-oxathiazinan-3-ide 2,2-dioxide.
(structure C) and hydrolysis of the hemiacetal of structure C leading to acetoacetamide-N-sulfonic acid (ANSA, structure D). The latter compound could be obtained as reference standard and based on the comparison of the RT and MS spectrum TP180a could be identified as ANSA.

The MS$^2$ spectrum of TP180b was completely different from that of TP180a and to the best of our knowledge has not been described so far. The MS$^2$ spectrum exhibited the following fragments: m/z 135.9711, C$_2$H$_2$NO$_4$S, [M-C$_2$H$_4$O]; m/z 121.9555, CNO$_4$S, [M-C$_3$H$_6$O]; m/z 105.9606, CNO$_3$S, [M-C$_3$H$_6$O$_2$]; m/z 72.0093, C$_2$H$_2$NO$_2$, [M-C$_2$H$_4$O$_3$S]. The latter fragment indicated that the additional oxygen is located in α-position (C-5) of the keto group of ACE. Since this only applies to structure B, TP180b was tentatively identified as 5-hydroxy-6-methyl-4-oxo-1,2,3-oxathiazinan-3-ide 2,2-dioxide.

For **TP178** (RT 14.7 min) an exact mass of 177.9814 was determined and indicated an hydroxylation either at the C-5 position or at the C-7 position. The former would lead to 6-methyl-4,5-dioxo-1,2,3-oxathiazinan-3-ide 2,2-dioxide which differs from the assumed structure of TP180b only by a keto group instead of an alcohol group at the C-5 position. However, since the MS$^2$ spectrum of TP 178 only exhibited one dominant fragment (m/z 98.0249, C$_4$H$_4$NO$_2$, [M-SO$_3$]) which was comparable to that of acesulfame but not to any of those of TP180b, it was assumed that TP 178 still bears the double bond between C-5 and C-6 and is more likely hydroxylated at the C-7 position. It was therefore tentatively identified as 6-(hydroxymethyl)-4-oxo-4H-1,2,3-oxathiazinan-3-ide 2,2-dioxide.

The measured exact mass of **TP192** (m/z 191.9613, C$_4$H$_2$NO$_6$S) suggested the introduction of two additional oxygens and the cleavage of 2 hydrogens. TP192 was also detected doubly charged (m/z 95.4769) which indicated the presence of an acidic moiety. However, the MS$^2$ spectrum gave no indication of a carboxyl group (no cleavage of CO$_2$ or CH$_2$O$_2$). Instead the only plausible structure was that of 6-formyl-4,5-dioxo-1,2,3-oxathiazinan-3-ide 2,2-dioxide exhibiting a 1,3-dicarbonyl moiety. The deprotonation of TP 192 leading to the double charged species in the ESI source can be explained by the formation of enolate forms and their stabilization by delocalization of the charge over three atoms. Except for SA, TP 192 was the only TP for which no further degradation was observed.

However, the proposed chemical structures of the minor TPs TP180b, TP178 and TP192 could not be further confirmed, since reference standards were not available and the quantities were too low for structural elucidation by NMR.
Fig. S5: Possible chemical structures of TP180a and TP 180b. TP 180a was unambiguously identified as structure D by comparison with a reference standard. TP 180b was tentatively identified as structure B.

S3 References

DIN, 1997. Water analysis - Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC); German version EN 1484-1997.