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SUPPLEMENTARY MATERIAL TO

Biodegradation of a mixture of benzophenone, benzophenone-3, caffeine and carbamazepine in a laboratory test filter

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ADDITIONAL EXPERIMENTAL DETAILS

TABLE S-I. Overview of the experiment

Phase	Initial level of PPCPs μg L^{-1}	Duration time	Description	Performed measurements	Sampling frequency
BFD[1]	-	4 week	The filter is conditioned with fresh nonfiltered river water, which is percolated through the test filter. Water in the system is changed every seven days (in total 4 cycles)	pH; O_2; conductivity; t, $KMnO_4$ consumption microscopic analysis of sand sample	1st and 7th day of each cycle at the end of fourth cycle
A1[2]	20	8 days	Adaptation of microorganisms	pH; O_2; conductivity; t; $KMnO_4$ consumption PPCPs toxicity tests	1st and 8th day for each parameter
B1[3]	20	8 days	The spiked filtered river water is percolated through the test filter. Biodegradation is determined by taking samples after define time intervals	pH; dissolved O_2; conductivity; t; $KMnO_4$ consumption PPCPs toxicity tests	1st and 8th day every day 1st and 8th day

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TABLE S-I. Continued

Phase	Initial level of PPCPs μg L^{-1}	Duration time	Description	Performed measurements	Sampling frequency
A2[4]	60	7 days	Adaptation of microorganisms at a higher concentration	pH; dissolved O_2; conductivity; t; $KMnO_4$ consumption toxicity tests	1st and 7th day for each parameter
B2[5]	60	7 days	Determination of biodegradation at a higher concentration of PPCPs after defined time intervals	pH; dissolved O_2; conductivity; t; $KMnO_4$ consumption	1st and 7th day
				PPCPs	every day
				toxicity tests	1st and 7th day

[a]The biofilm development phase; [b]adaptation phase 1; [c]biodegradation phase 1; [d]adaptation phase 2; [e]Biodegradation phase 2

PPCPs analysis

A 2 μL sample was injected into GC (splitless mode 0.1 min, purge flow 60 mL min^{-1}, at 250 °C). The GC oven was programmed as follows: an initial temperature of 60 °C, held for 3 min, and then was ramped at 5 °C/min to 300 °C, held for 10 min. Samples were analyzed using an GC/MS (Agilent Technologies 7890B, with 5977A MSD). Separation was achieved using a HP-5 MS 30 m×0.25 mm×0.25 μm (Agilent J&W, CA, USA) capillary column with helium as the carrier gas.

Toxicity tests

Daphnia magna *acute toxicity test.* Less than 24 h-old daphnias (neonates) were used in tests. Neonates were transferred into 50-mL glass vessels containing 25 mL of test solution and controls – standard M4 medium,[30] and the filtered river Danube sample, in four replicates (5 animals per test vessel) each. The immobilisation of the neonates was recorded after 24 and 48 h, and the results were compared to the controls. Test acceptability criterion for *D. magna* acute toxicity tests is ≥ 90 % survival rate in the control. The dissolved oxygen concentration (mg L^{-1}) was measured prior to *D. magna* toxicity tests, in control / test solutions. The measured values were in the range 4.45-6.19 mg L^{-1}, and did not change considerably over *D. magna* toxicity test.

Vibrio fischeri *luminescence inhibition test. V. fischeri* culture (strain NRRL B-11177), supplied by Macherey-Nagel GmbH&Co. KG, Duren, Germany as freeze-dried bacteria (BioFix®Lumi), was reconstituted, prior to testing, using the commercial reactivation solution from the same manufacturer. The initial luminescence (I_0) was measured in reactivated bacterial suspension after 15 min

long adjustment period at 15 °C. To achieve a minimum required quantity of bacteria for accurate luminescence measurement,[31] 0.2 mL of the bacterial test suspension was added into 0.8 mL of test solution / control. The final luminescence (I_{30}) was measured after 30 min exposure of the bacterial suspension to the test solutions / controls. The tests were carried out in triplicates. The results were calculated as the percentage of luminescence inhibition (H_{30}) in test solutions relative to the corresponding controls (standard 2 % NaCl solution / filtered river Danube sample (FD) with addition of 2 % NaCl for salinity adjustment).

The correction factor (fk_{30}) was calculated as follows:

$$fk_{30} = \frac{Ik_{30}}{I_0} \tag{1}$$

where Ik_{30} stands for the bioluminescence in control after 30 min; I_0 stands for the initial bioluminescence.

The corrected values of I_0 were calculated as follows:

$$Ic_t = I_0 fk_{30} \tag{2}$$

The inhibition in each test solution was calculated as follows:

$$H_{30} = \frac{Ic_t - I_{30}}{Ic_t} 100 \tag{3}$$

where H_{30} stands for the inhibition of bioluminescence after 30 min, %; I_3 stands for the bioluminescence after 30 min.

TABLE S-II. List of PPCPs studied and their properties

Name	Abbreviation	Molecular weight, g mol^{-1}	Octanol / water partition coefficient
Benzophenone[1]	BP	182.222	3.18
Benzophenone-3[2]	BP-3	228.247	3.79
Carbamazepine[3]	CBZ	236.274	2.45
Caffeine[4]	CF	194.194	-0.07

TABLE S-III. List of target ions (*m*/*z*) of compounds used for analysis

Analyte	Target ion, *m*/*z*
BP	105
BP-3	151
CBZ	193
CF	194

TABLE S-IV. Parameters for river water during phase of biofilm development

Parameter	I cycle		II cycle		III cycle		IV cycle	
	Day							
	I	VII	I	VII	I	VII	I	VII
pH	8.1	8.2	8.3	7.5	8.02	8.1	8.1	8.02
Conductivity, μS cm^{-1}	390	396	394	392	377	393	351	342
Temperature, °C	17	23	26.3	24.2	23.5	26	19	26
$KMnO_4$ consumption, mg L^{-1}	5.8	2.5	9	6.3	14	12.6	11.4	10

TABLE S-V. Results of weathering tests in glass beakers; removal, %

Phase of experiment	BP		BP-3		CF		CBZ	
	Night-day circle	Dark	Night-day circle	Dark	Night-day circle	Dark	Night-day circle	Dark
B1	83	25	15	54	54	47	Not calculated	Not calculated
B2	90	29	7	53	54	51	0	0

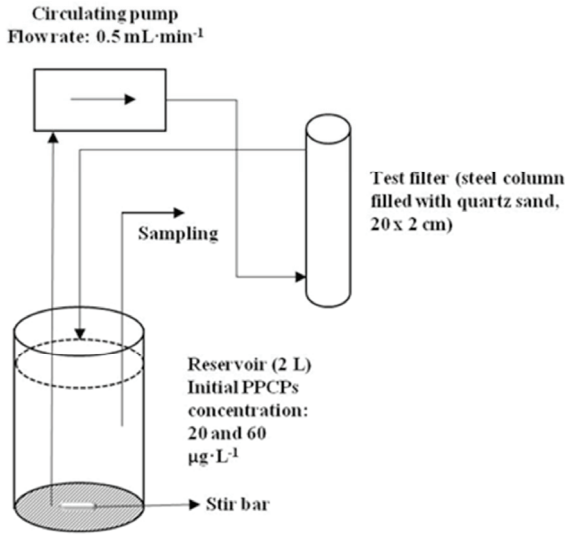


Fig. S-1. Biologically test filter set-up.

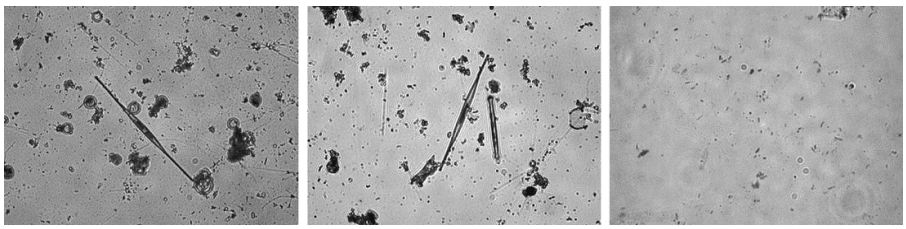
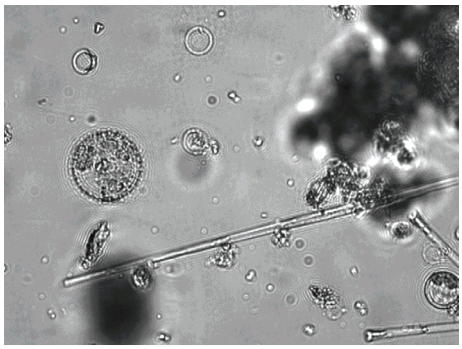
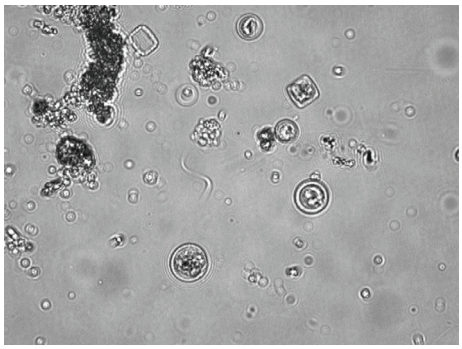


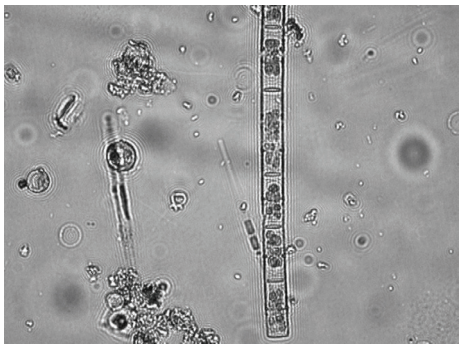
Fig. S-2. Images of stained samples showing bacteria (coccal and rod forms) and diatoms.



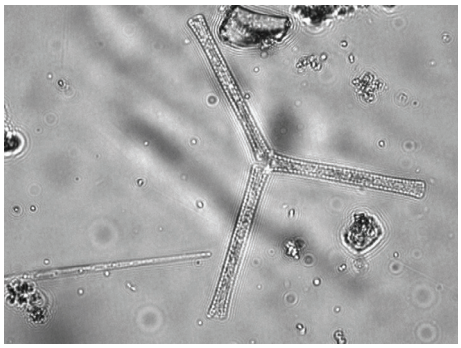
a) *Synedra sp.*



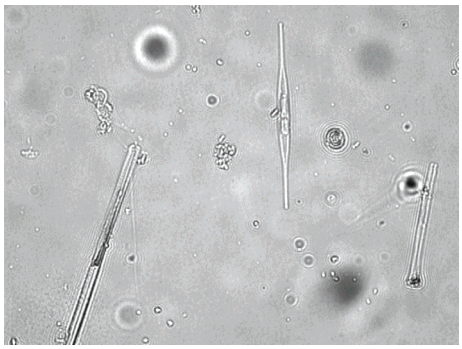
b) *Cyclotella sp.*



c) *Melosira sp.*



d) *Asterionella sp.*



e) *Nitzschia sp.*

Fig. S-3. Images of native samples showing diatoms present in the biofilm.

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