Front page for deliverables

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1 Introduction

In addition to phase partitioning, degradation rates are major determinants of the environmental fate of compounds. However, currently available mathematical models were developed mainly for simple compounds and most of the underlying experimental data were generated in qualitative form (e.g. ready vs. not ready biodegradable). Furthermore, available data sets are typically related to water-only systems and not to sediments, where sorption, ageing, sequestration, and cross coupling may affect the bioavailability, transformation and degradation.

As a consequence, the prediction of outdoor system half-lives by mathematical models is hampered by the lack of quantitative or at least semi-quantitative data that apply to more realistic conditions. To overcome these shortcomings, a new water-sediment test system was developed following OECD Guideline 301 C (MITI I) to generate biodegradation data of more complex organic compounds.

The equipment to perform standardised OECD 301 water-only test is commercially available (e.g. the OxiTop®-system of WTW, D-82362 Weilheim, Germany). The OxiTop®-system determines manometric changes, which occur when oxygen is consumed to transform organic carbon into carbon dioxide. In the closed system the carbon dioxide is trapped by an absorbent (e.g. soda lime). A decrease in pressure is used by the OxiTop®-system for calculating the biochemical oxygen demand (BOD). The new water-sediment test system as well as materials and methods were described in detail in Deliverable D.2.3.1 of October 31, 2005.

In the following, the experimental work between October 2005 and April 2006 is reported, including the following main aspects:

- The observed difference in the fate of the model substance aniline in the standardised water-only and in the new water-sediment system was investigated in experiments, which run under identical conditions in parallel (3 approaches).
- Experimental biodegradation kinetics for a first set of four test compounds in water and sediment are presented.

2 Material and Methods

2.1 Difference in the fate of aniline in the standardised water-only and in the new water-sediment system

As reported in deliverable D.2.3.1, several differences in the fate of aniline in the standardised water-only and in the new water-sediment system could be observed, which had to be examined. Biodegradation rates are presented based on % ThOD (Theoretical Oxygen Demand), which is tantamount to 'ultimate biodegradation'.

- The exponential phase of the degradation curves of aniline in the water-only and in the water-sediment system ends at approximately 60 – 80% ThOD and 35% ThOD, respectively (Fig. 1). In addition, the biodegradation rate [% ThOD] at the plateau is clearly lower in the water-sediment system and the degradation curves of the water-sediment system were less steep. Experiments using water-only systems performed by Reuschenbach (2000) and Strotmann (2004) also resulted in this characteristic curve progression.

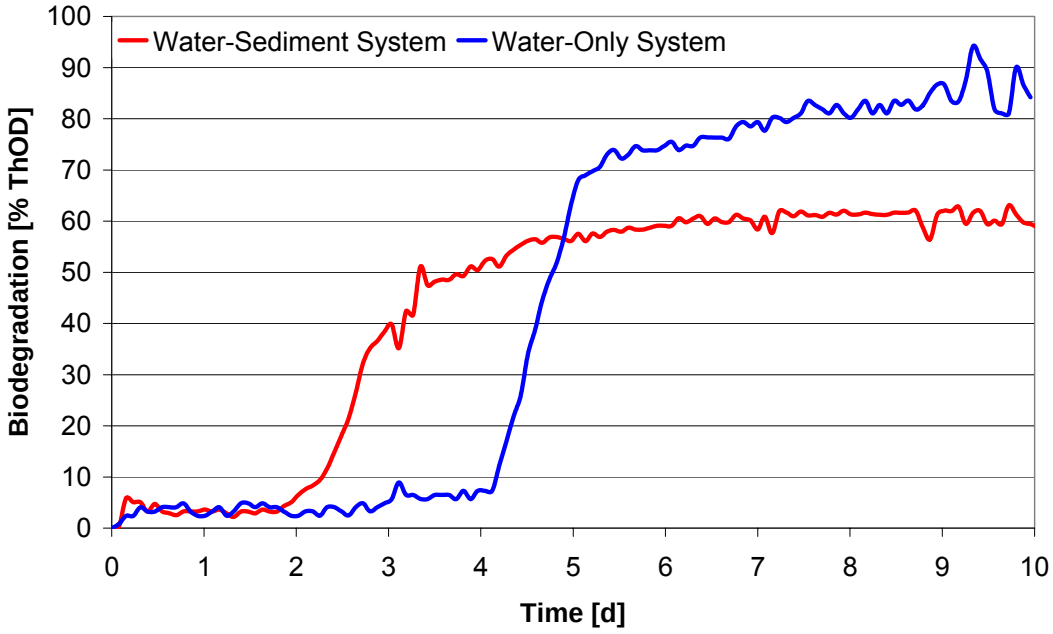


Fig. 1: Comparison of the biodegradation of 75 mg/L aniline in the water-only and in the water-sediment system.

- Furthermore, at the same time the exponential phase ends, an oxygen minimum was detected in the overlying water (Fig. 2). This corresponds to observations by Storhas et al. (2000). They assume that the end of the exponential degradation phase is caused by an oxygen deficiency in the water phase.

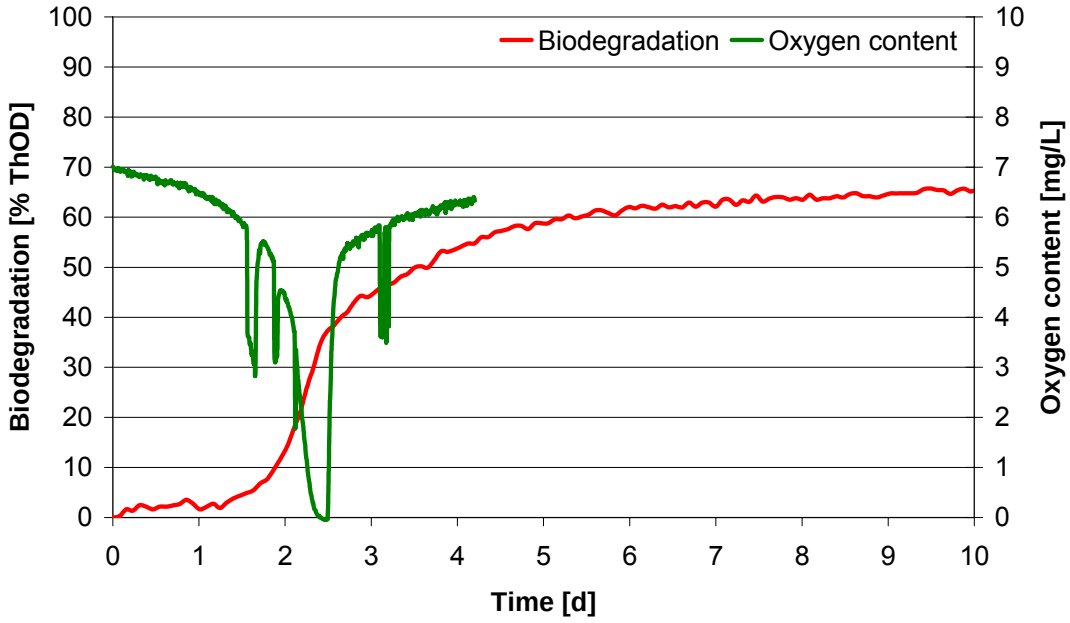


Fig. 2: Biodegradation of 100 mg/L aniline in the new water-sediment system and measurement of oxygen content in the overlying water

- In the water-only system, 60 – 80% of the aniline is used by the bacteria for energy production and is oxidised to CO_2, whereas 20 – 40% are used for building up microbial biomass (Strotmann 2000, Pagga 2000). The shortened exponential phase and the lower degradation rate at the plateau in the water-sediment system pose the question of the whereabout of the remaining test substance.

In order to submit the differences between the two test systems to a careful examination, the following aspects were investigated:

1. <u>Influence of the test substance concentration and the enhancement of oxygen flux into the water body</u>

   In order to check whether the end of the exponential phase was caused by an oxygen deficiency, the test design was modified. The test substance concentration of 100 mg/L was

reduced to 75 mg/L. Thereby, the ThOD could be reduced. In addition, the sediment surface was covered by a stainless steel mesh. This allowed for a higher stirring speed whereby the oxygen entry into the water body should be increased without re-suspension of sediment particles.

2. Influence of $CaCO_3$ in the sediment
   Since the modified artificial sediment according to OECD Guideline No. 218 is prepared using $CaCO_3$ in order to adjust pH, some of the produced CO_2 might react according to the equation $CaCO_3 + CO_2 + H_2O \leftrightarrow Ca(HCO_3)_2$. Consequently, the produced CO_2 is not stripped from the water body and therefore no decrease in pressure could be detected. In order to check this assumption, two tests were run in parallel using the artificial sediment containing $CaCO_3$ on the one hand and sediment without $CaCO_3$ but with a phosphate buffer (K_2HPO_4 combined with KH_2PO_4) on the other hand.

3. Interrelationship between biological oxygen demand and dissolved organic carbon
   In addition to the biological oxygen demand (BOD), the dissolved organic carbon (DOC) was measured in the water phase at several time points during the test.

2.2 Biodegradation tests for a first set of four test compounds in water and sediment

2.2.1 Test design

The mixed microbial inoculum (MITI I, OECD 1992) used in the tests was collected at ten sites (4 STPs, 3 rivers and 3 lakes) in the Rhein-Main region in Germany and was kept in the laboratory at test conditions since April 2005.

With the intention to enable the comparison of experimental data from water-only (OECD 301) and the new water-sediment system, two substances were tested in parallel. The tests with ivermectin and atenolol were performed between February 15th and March 15th, 2006. The Tests with 3,5-dichloroaniline and 2,4-dinitrophenol started on March 27th and were terminated on April 24th. Due to the rapid photodegradation of ivermectin and atenolol (DT_{50} < 1 d), all test vessels were covered with aluminium foil (Fig. 3). The medium/overlying water was stirred continuously using a magnetic stirrer. The test vessels were equipped with OxiTop®-C measuring heads (WTW, Germany). The test conditions are summarised in Tab. 1.

2.2.2 Test substances

The physico-chemical parameters of the test substances are shown in Tab. 2. For atenolol, 3,5-dichloroaniline and 2,4-dinitrophenole, stock solutions were prepared by dissolving the test item in test medium. Due to its very low water solubility, ivermectin was added directly to the test vessels. Aniline was used as reference substance. All test substances were tested at 100 mg/L.

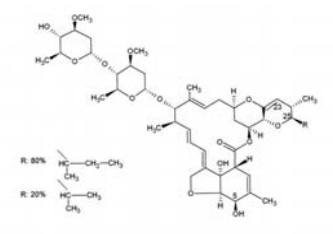
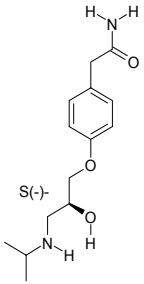
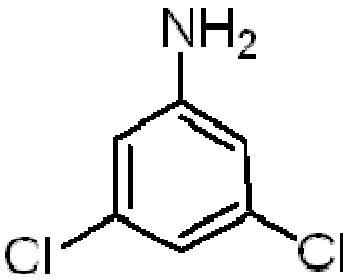
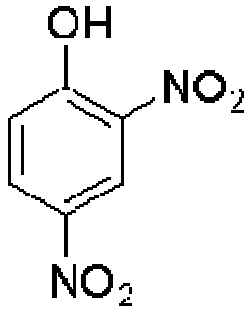
Fig. 3: Left: Experimental setup in the test with ivermectin and atenolol. Right: Newly developed water-sediment system and standard water-only system

Tab. 1: Summary of the test conditions in the tests using the water-only and the water-sediment system

Test Parameter	Water-Only System	Water-Sediment System
Type of sediment:	-	Artificial sediment based on OECD 218 (2004b) modified according to Egeler *et al.* 1997 and Meller *et al.* 1998
Type of medium / overlying water:	Mineral medium according to OECD 301 C	Mineral medium according to OECD 301 C
Inoculum:	MITI I inoculum according to OECD 301 C	MITI I inoculum according to OECD 301 C
Volume of test vessel:	500 mL	1647 mL
Volume of test medium / overlying water per test vessel:	225 mL (ivermectin, atenolol) 250 mL (3,5-dichloroaniline, 2,4 dinitrophenole)	450 mL
Amount of sediment per test vessel:	-	170 g (fresh weight)
Sediment-water volume ratio:	-	1 : 4
Test substance concentration:	100 mg/L	100 mg/L
Concentration of nitrification inhibitor	10 mg/L	10 mg/L

Test Parameter	Water-Only System	Water-Sediment System
allyl thiourea (ATU):		
Amount of inoculum as suspended solids:	30 mg/L (dry weight)	30 mg/L (dry weight)
Temperature:	25 ± 1°C	25 ± 1°C
Light intensity:	complete darkness	complete darkness
pH of test solution:	7 ± 1	7 ± 1
Test duration:	28 d	28 d
Measuring intervals:	112 min	112 min
No. of control replicates:	2	2
No. of abiotic control replicates:	2 (1 per test substance)	-
No. of replicates per test substance:	4	4
No. of replicates for the reference substance aniline:	2	-

Tab. 2: Physico-chemical parameters of the test substances used in the studies with the water-only and the water-sediment system

	Ivermectin B1a, B1b	(+/-)-Atenolol	3,5-Dichloroaniline	2,4-Dinitrophenole
CAS-No.:	70288-86-7 [1]	29122-68-7 [2]	626-43-7	51-28-5
Empirical formula:	$B_{1a} = C_{48}H_{74}O_{14}$ $B_{1b} = C_{47}H_{72}O_{14}$	$C_{14}H_{22}N_2O_3$	$Cl_2C_6H_3NH_2$	$(O_2N)_2C_6H_3OH$
Structural formula:				
Molecular weight [g/mol]:	B_{1a} = 875.10; B_{1b} = 861.07	266.34	162.02	184.11
Water solubility:	4 mg/L [9]	1.33 * 10^4 mg/L (meas.) [4]	784 (25°C; meas.) [12]	2.790 (20°C; meas.) [12]
Log P_{ow}:	3.22 [10]	0.16 (meas.) [5]	2,9 (meas.) [12]	1,67 (meas.) [12], [14]
K_{oc}:	12600 – 15700 [11]	148 [6]	309 [14]	200 (est.) [14]
Vapour pressure [Pa]:	Ivermectin: < 2.0*10^{-7} [10] B_{1a} = 1.60*10^{-28} [7] B_{1b} = 4.24*10^{-28} [7]	1.03*10^{-7} (calc.) [7]	2.8 [13]	5.2*10^{-2} [13]
Hydrolysis (DT_{50}):	n.d.	n.d.	Not expected [14]	6.16*10^{-13} (25°C; est.) [12]
Photolysis (DT_{50}):	0.13 d [11]	0.077 d [8]	n.d.	n.d.
ThOD $_{(NH4)}$ in mg/mg [3]:	2.106	1.982	1.185	0.521

[1] Ivermectin is a mixture of at least 80% ivermectin B1a (=22,23-dihydro-avermectin B1a; CAS-No. 71827-03-7) and not more than 20% ivermectin B1b (=22,23-dihydro-avermectin B1b; CAS-No. 70209-81-3). The mixture (CAS-No. 70288-86-7) used for the tests contains 94% ivermectin B1a and 2.8% ivermectin B1b. [2] Atenolol is a mixture of two enantiomers R(+)-atenolol (CAS-No. 56715-13-0) and S(-)-atenolol (CAS-No. 93379-54-5). [3] The theoretical oxygen demand (without nitrification) per mg test substance was calculated according to OECD 301 (OECD 1992a); [4] Experimental Data from PhysProp Database (EPI v3.12); McFarland, J.W. et al. (2001); [5] Experimental Data from PhysProp Database (EPI v3.12); Hansch, C. et al. (1995); [6] PCKOCWIN v1.66 (EPI v3.12), estimate; [7] MPBWIN v1.41 (EPI v3.12), Modified Grain method; [8] AopWIN v1.91 (EPI v3.12), 12-hr day; 1.5E+6 OH/cm³); [9] Dossier; [10] Halley, B.A.; Nessel, R.J.; Lu, A.Y.H. (1989): Environmental Aspects of Ivermectin Usage in Livestock: General Considerations. In W.C. Campbell (ed.): Ivermectin and Abamectin. Springer, New York; Chapter 11, pp. 162-172; [11] Halley, B.A.; Jacob, T.A.; Lu, A.Y.H. (1989): The environmental impact of the use of ivermectin: Environmental effects and fate. Chemosphere 18 (7-8): 1543-1563; [12] SRC PhysProp data base (http://www.syrres.com); [13] eigene Berechnung basierend auf [1]; [14] Hazardous Substances Data Bank (HSDB), National Library of Medicine (NLM): http://www.nlm.nih.gov/

3 Results and Discussion

3.1 Investigation of the differences between the water-only and the new water-sediment system

3.1.1 Influence of the test substance concentration and the enhancement of oxygen flux into the water body

In order to optimise the test performance and the degradation curves, the oxygen depletion in the overlying water during the exponential degradation phase was minimised. Two approaches were tested to meet this goal: 1) enhancement of the oxygen flux into the water body; 2) reduction of the test substance concentration to reduce the bacterial oxygen demand.

Compared to the test with 100 mg aniline/L the course of the oxygen content in the water phase testing 75 mg aniline/L was similar, but due to the reduced bacterial oxygen demand and the higher oxygen flux into the water body, the oxygen was not consumed completely during exponential growth. However, the exponential phase still ends at approximately 35% ThOD (Fig. 4). Thus, it can be assumed that the oxygen depletion/deficiency observed in earlier experiments is not the reason for the shortened exponential phase in the water-sediment system.

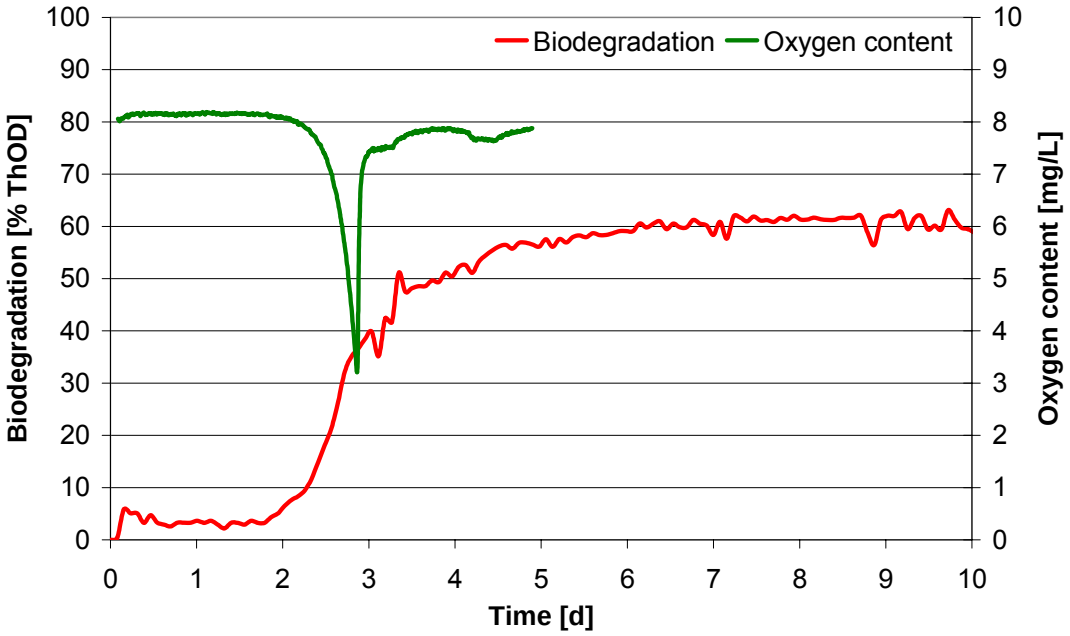


Fig. 4: Biodegradation of 75 mg/L aniline in the new water-sediment system (mean; n = 4) and measurement of oxygen content in the overlying water

3.1.2 Influence of $CaCO_3$ in the sediment

The results show, that the use of a phosphate buffer instead of $CaCO_3$ to adjust the pH of the sediment did not have any effects on the curve progression of the biodegradation of aniline in the water-sediment system (Fig. 5).

Thus, it can be concluded that the produced CO_2 does not react with the $CaCO_3$ in the water body according to the equation $CaCO_3 + CO_2 + H_2O \leftrightarrow Ca(HCO_3)_2$ and is therefore not retained in the water phase. Consequently, the shortened exponential degradation phase in the water-sediment system is not caused by the use of $CaCO_3$ in the artificial sediment. Due to these facts and because of the good reproducibility, the artificial sediment containing $CaCO_3$ will be used in the upcoming degradation experiments.

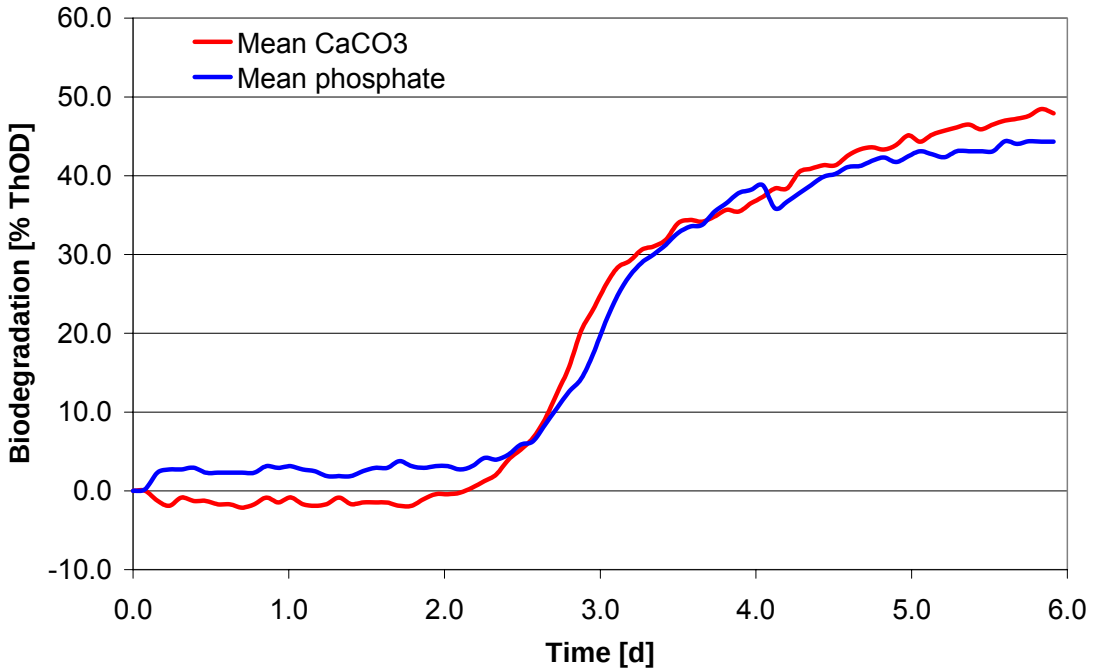


Fig. 5: Biodegradation of 100 mg/L aniline in the new water-sediment system (mean; n = 4) using the artificial sediment (containing $CaCO_3$) and a phosphate buffer (without $CaCO_3$)

3.1.3 Interrelationship between oxygen content and dissolved organic carbon (DOC)

It could be observed, that after two days, 35% of the carbon attributed to aniline was eliminated from the water body, whereas only 2.8% of the test substance were degraded based on the BOD-curve and the bacteria were still in the lag-phase. At the end of the exponential phase at

approximately 35% ThOD, almost 95% of the carbon attributed to aniline was eliminated. Hence, no DOC was available for the bacteria at that time and thus, the exponential growth stopped due to lack of nutrition (Fig. 6). In conclusion, the end of the exponential phase is not caused by an oxygen deficiency, as assumed by Storhas et al. (2000), but by a lack of DOC in the water phase.

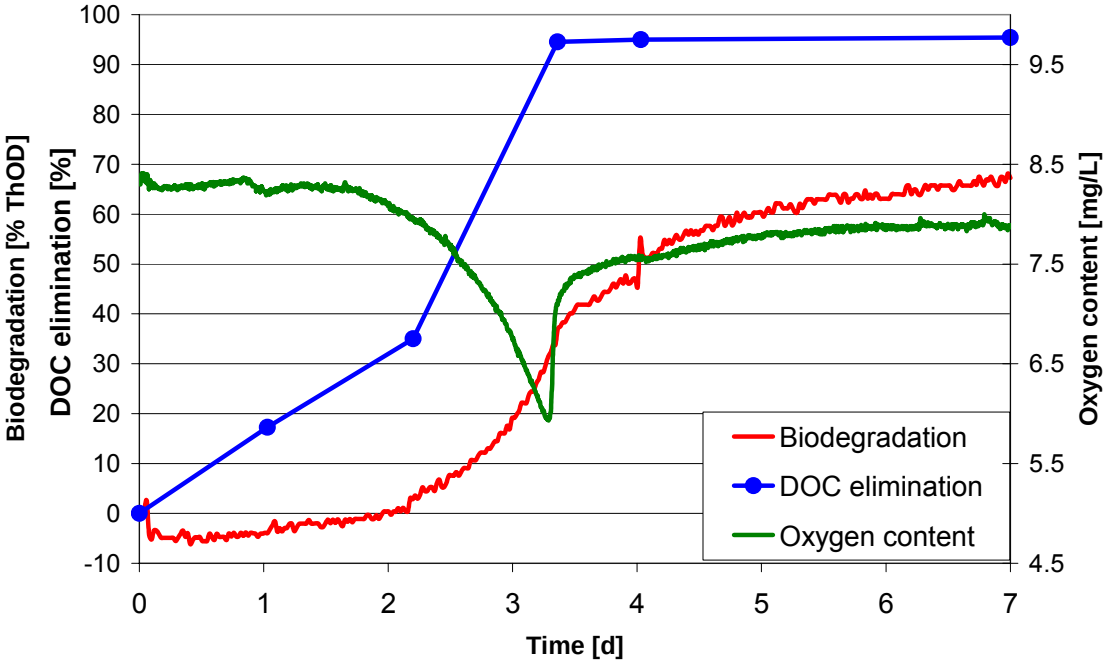


Fig. 6: Biodegradation of 75 mg/L aniline in the new water-sediment system (mean; n = 4), measurement of oxygen content and dissolved organic carbon (DOC) in the overlying water

With regard to the question of the whereabout of the remaining test substance in the water-sediment system, one might suspect, that a considerable amount of the aniline was bound to sediment and pore water leading to a reduced bioavailability. In order to corroborate this hypothesis, an accompanying chemical analysis of the compartments water, sediment and pore water might be helpful.

3.2 Biodegradation test with ivermectin in water and sediment

The degradation curves of ivermectin and the reference substance aniline are presented in Fig. 7.

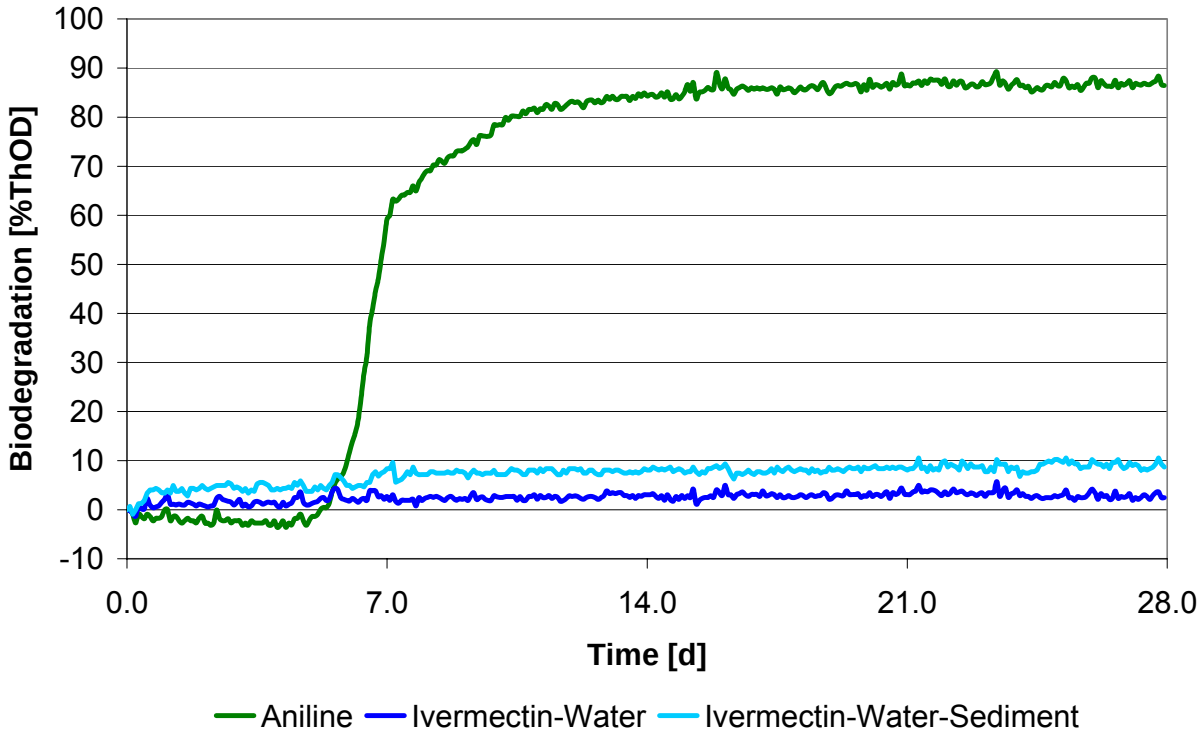


Fig. 7: Biodegradation of ivermectin in the water-only system (mean; n = 4) and in the new water-sediment system (mean; n = 4) and biodegradation of aniline in the water-only system (n = 1)

Whereas the degradation curve of the reference substance aniline shows the characteristic progression, indicating that the used inoculum was in good condition, ivermectin was not degraded, neither in the water-only system nor in the water-sediment system. This corresponds to the biodegradability predicted by BIOWIN models and by CATABOL (Dimitrov and Mekenyan 2006), where ivermectin is classified as recalcitrant and not readily biodegradable with a predicted biodegradation rate of 14.5%.

The biodegradation rates [%ThOD] at different time points during the course of the test are presented in Tab. 3. For more detailed test results see appendix 1.

Tab. 3: Degradation rates at the different time points during the test with ivermectin

Substance	Biodegradation [% ThOD]			
	7.4 d [1]	14 d	21 d	28 d
Aniline – water only	64.1	84.3	86.6	86.4
Ivermectin – water-only	2.0	3.1	3.1	2.5
Ivermectin – water-sediment	6.2	8.1	8.1	8.7

[1] corresponding to the end of the exponential degradation phase for aniline

3.3 Biodegradation test with atenolol in water and sediment

The degradation curves of atenolol and the reference substance aniline are presented in Fig. 8.

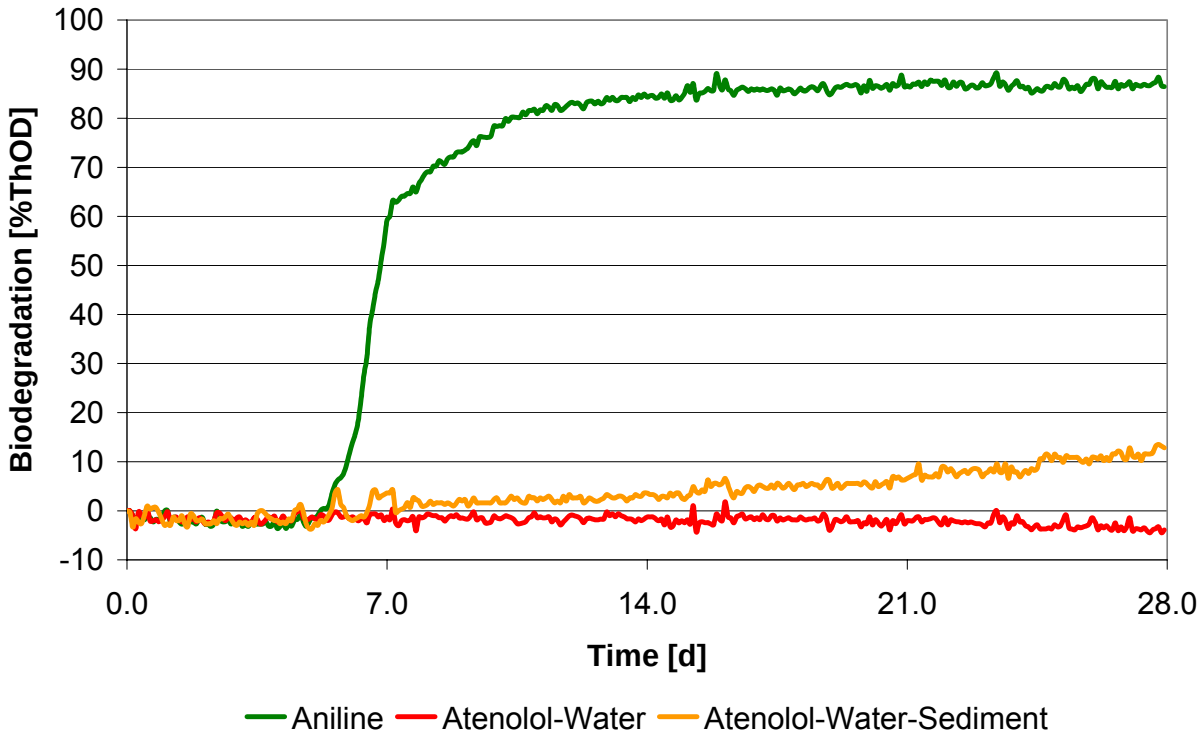


Fig. 8: Biodegradation of atenolol in the water-only system (mean; n = 4) and in the new water-sediment system (mean; n = 4) and biodegradation of aniline in the water-only system (n = 1)

Whereas the degradation curve of the reference substance aniline shows the characteristic progression, indicating that the used inoculum was in good condition, atenolol was not degraded, neither in the water-only system, nor in the water-sediment system. Compared to the biodegradability predicted by BIOWIN models and by CATABOL (Dimitrov and Mekenyan 2006), where atenolol is classified as not readily biodegradable with a predicted biodegradation rate of 33.1% and an ultimate half-life of 2 months, the test results indicate a lower or at least slower biodegradability of atenolol.

The biodegradation rates [%ThOD] at different time points during the course of the test are presented in Tab. 4. For more detailed test results see appendix 1..

Tab. 4: Degradation rates at the different time points during the test with atenolol

Substance	Biodegradation [% ThOD]			
	7.4 d [1]	14 d	21 d	28 d
Aniline – water only	64.1	84.3	86.6	86.4
Atenolol – water-only	0.0	0.0	0.0	0.0
Atenolol – water-sediment	0.3	3.6	6.6	12.9

[1] corresponding to the end of the exponential degradation phase for aniline

3.4 Biodegradation test with 3,5-Dichloroaniline in water and sediment

The degradation curves of 3,5-Dichloroaniline and the reference substance aniline are presented in Fig. 9.

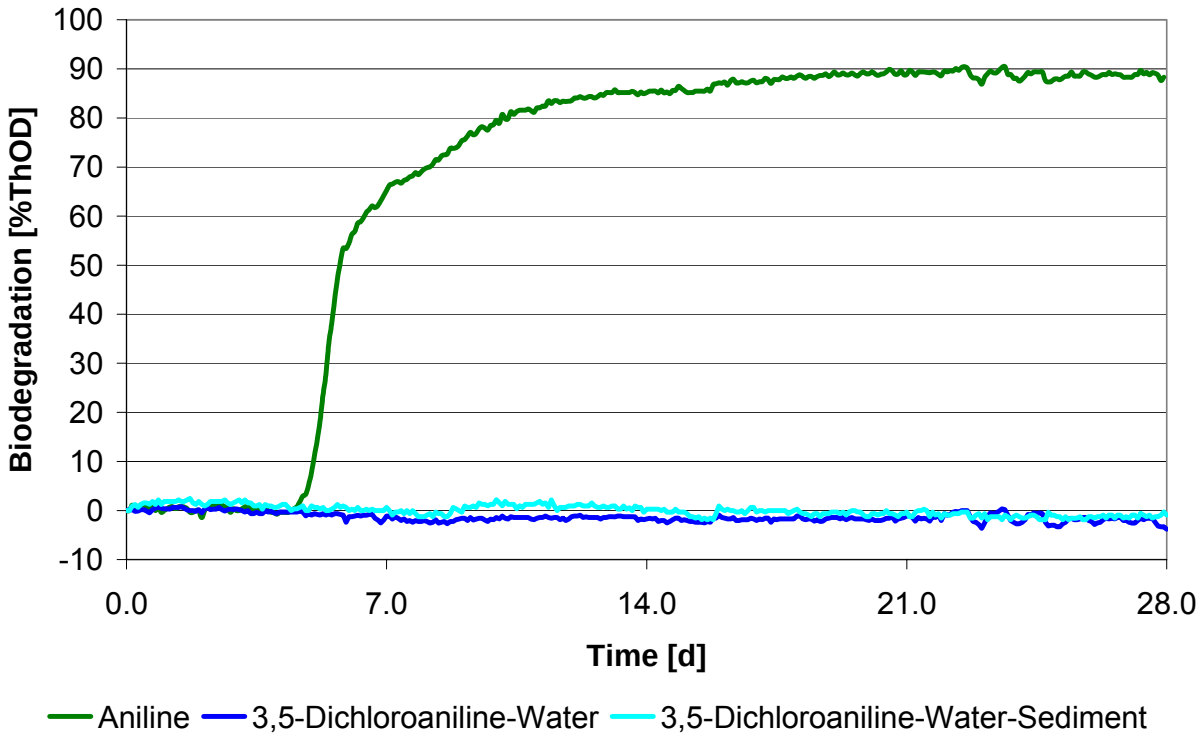


Fig. 9: Biodegradation of 3,5-Dichloroaniline in the water-only system (mean; n = 4) and in the new water-sediment system (mean; n = 4) and biodegradation of aniline in the water-only system (mean; n = 2)

Whereas the degradation curve of the reference substance aniline shows the characteristic progression, indicating that the used inoculum was in good condition, 3,5-Dichloroaniline was not degraded, neither in the water-only system, nor in the water-sediment system. This corresponds to the biodegradability predicted by BIOWIN models and by CATABOL (Dimitrov

and Mekenyan 2006), where 3,5-Dichloroaniline is classified as not biodegradable with a predicted biodegradation rate of 0%.

The biodegradation rates [%ThOD] at different time points during the course of the test are presented in Tab. 5. For more detailed test results see appendix 2.

Tab. 5: Degradation rates at the different time points during the test with 3,5-Dichloroaniline

Substance	Biodegradation [% ThOD]				
	5.8 d [1]	7 d	14 d	21 d	28 d
Aniline – water only	53.4	65.2	85.2	88.9	87.9
3,5-Dichloroaniline – water-only	0.0	0.0	0.0	0.0	0.0
3,5-Dichloroaniline – water-sediment	0.9	0.6	0.3	0.0	0.0

[1] corresponding to the end of the exponential degradation phase for aniline

3.5 Biodegradation test with 2,4-Dinitrophenol in water and sediment

The degradation curves of 2,4-Dinitrophenol and the reference substance aniline are presented in Fig. 10.

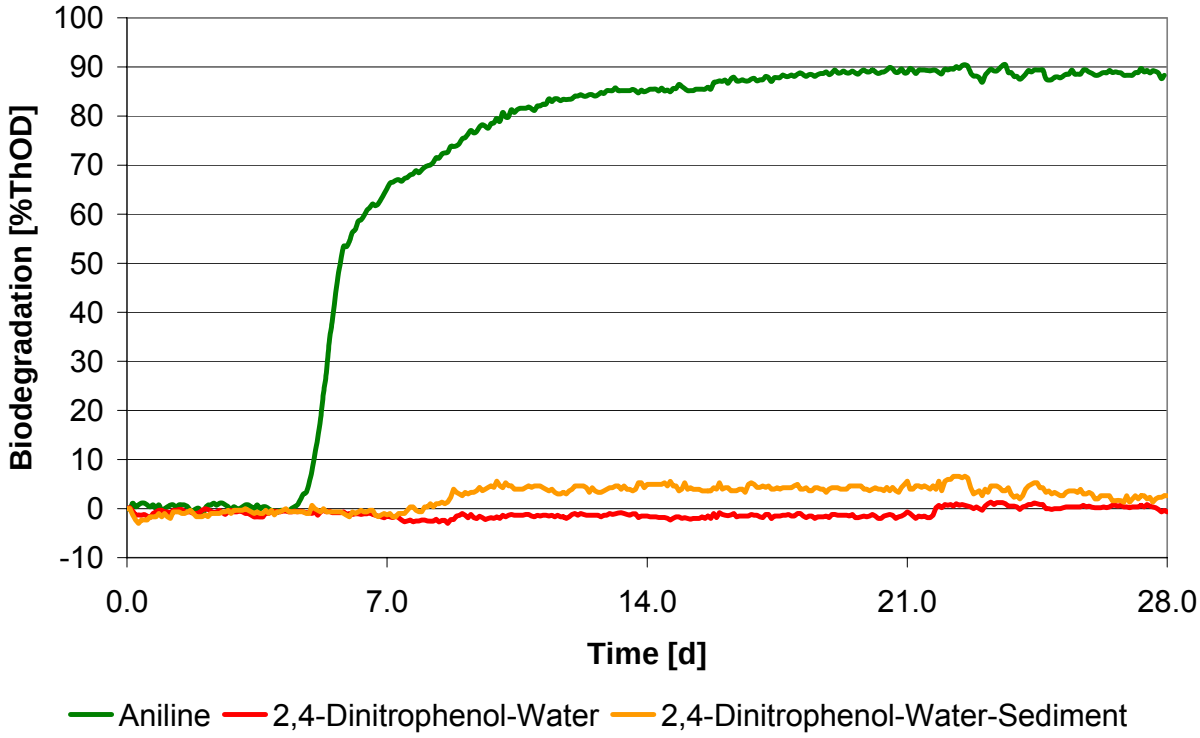


Fig. 10: Biodegradation of 2,4-Dinitrophenol in the water-only system (mean; n = 4) and in the new water-sediment system (mean; n = 4) and biodegradation of aniline in the water-only system (mean; n = 2)

Whereas the degradation curve of the reference substance aniline shows the characteristic progression, indicating that the used inoculum was in good condition, 2,4-Dinitrophenol was not degraded, neither in the water-only system, nor in the water-sediment system. This corresponds to the biodegradability predicted by BIOWIN models and by CATABOL (Dimitrov and Mekenyan 2006), where 2,4-Dinitrophenol is classified as not biodegradable with a predicted biodegradation rate of 0%.

The biodegradation rates [%ThOD] at different time points during the course of the test are presented in Tab. 6. For more detailed test results see appendix 2.

Tab. 6: Degradation rates at the different time points during the test with 2,4-Dinitrophenol

Substance	Biodegradation [% ThOD]				
	5.8 d [1]	7 d	14 d	21 d	28 d
Aniline – water only	53.4	65.2	85.2	88.9	87.9
2,4-Dinitrophenol – water-only	0.0	0.0	0.0	0.0	0.0
2,4-Dinitrophenol – water-sediment	0.0	0.0	4.9	5.6	2.6

[1] corresponding to the end of the exponential degradation phase for aniline

4 Conclusion and Outlook

The observed difference in the fate of the model substance aniline in the standardised water-only and in the new water-sediment system was investigated in experiments, which run under identical conditions in parallel. It could be shown that the end of the exponential phase of biodegradation is caused by a lack of DOC in the water phase instead of an oxygen deficiency. With regard to the differences between the water-only and the new water-sediment system, neither the oxygen deficiency in the water body nor the $CaCO_3$, which is used in the artificial sediment to adjust the pH, is the cause for the shortened exponential phase in the water-sediment system compared to the water-only system.

Experimental biodegradation test for a first set of four test compounds (ivermectin, atenolol, 3,5-Dichloroaniline, 2,4-Dinitrophenol) in water and sediment were performed. None of the test substances were degraded within the test period of 28 days, whereas the reference substance aniline was nearly completely degraded, indicating that the used inoculum was in good

condition. The results correspond to the biodegradability predicted by Dimitrov and Mekenyan (2006) using BIOWIN models and CATABOL.

Within the next period (May 06 - Oct. 07), biodegradation experiments will be continued for further selected substances. To ensure an optimal comparability of the results, each compound is tested using the water-only approach and the new water-sediment test system in parallel. In doing so, existing differences between the test systems will be identified in order to continually improve the comparability of experimental biodegradation data obtained for the different compartments. In this context, major emphasis will be placed on factors, which may influence the results of the biodegradation studies in the water-sediment system (e.g. organic carbon content, pH, temperature, grain size, etc.). If possible, the most important confounding factors will be identified and investigated using a factorial design approach. Besides the experimental part, a systematic search for additional degradation data from literature and competent authorities will be performed for the aquatic compartment.

The results of both, biodegradation experiments and literature search, will provide data which are needed by other partners in WP 2.3 to build a database for biodegradation parameters of organic compounds in water and sediment. Thus, an analysis of degradation parameters across chemicals and media will be possible and improved quantitative structure-property relationships (QSPRs) can be developed.

5 References

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Appendix 1: Detailed test results for ivermectin, atenolol and the reference substance aniline

a) water-only system

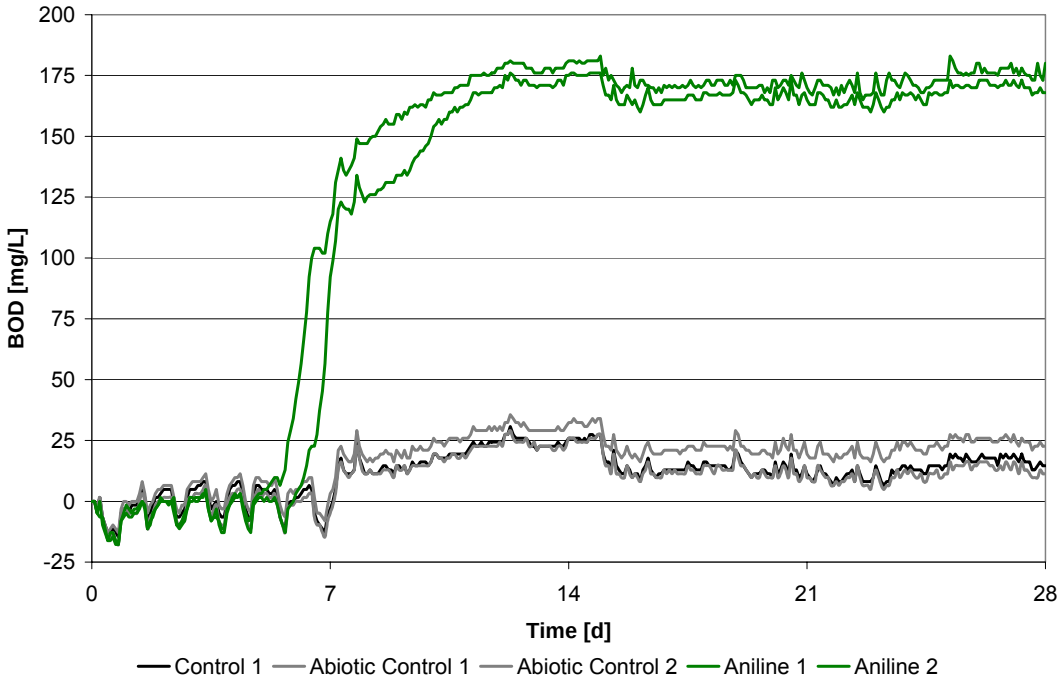

Fig. 11: Biological oxygen demand (BOD) of aniline (n = 2), control and abiotic controls (n = 2) in the water-only system

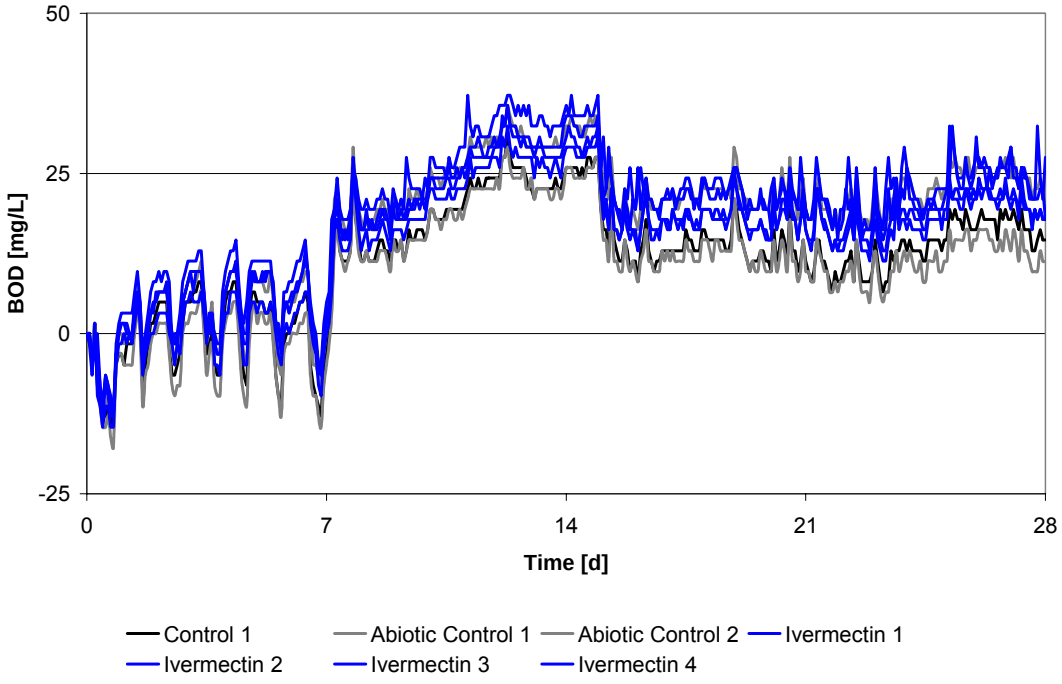

Fig. 12: Biological oxygen demand (BOD) of ivermectin (n = 4), control and abiotic controls (n = 2) in the water-only system

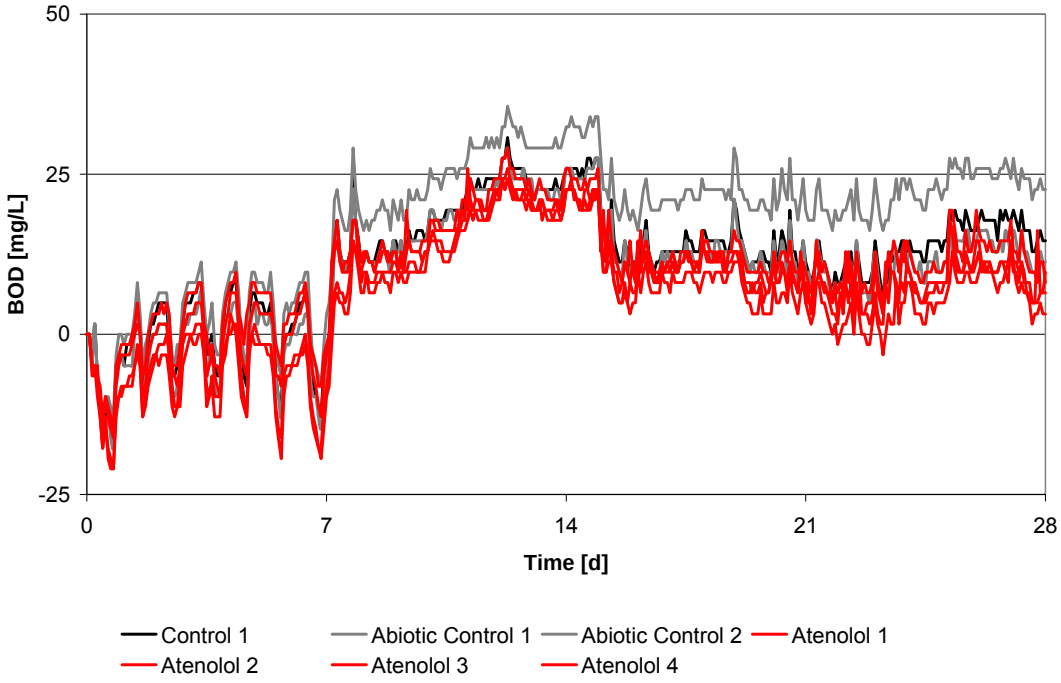

Fig. 13: Biological oxygen demand (BOD) of atenolol (n = 4), control and abiotic controls (n = 2) in the water-only system

Tab. 7: Biological oxygen demand (BOD) at the different time points during the test with ivermectin, atenolol and the reference substance aniline using the water-only system

Replicates	BOD [mg/L]			
	7.4 d [1]	14 d	21 d	28 d
Control 1	12.9	25.9	11.3	14.6
Abiotic control 1	19.4	32.4	19.4	22.6
Abiotic control 2	12.9	25.9	9.7	11.3
Mean ± Std. Dev.	16.2 ± 4.6	29.2 ± 4.6	14.5 ± 6.9	17.0 ± 8.0
Aniline 1	121	175	165	168
Aniline 2	136	181	170	180
Mean ± Std. Dev.	128.5 ± 10.6	178.0 ± 4.2	167.5 ± 3.5	174.0 ± 8.5
Ivermectin 1	14.6	29.1	16.2	17.8
Ivermectin 2	16.2	34.0	19.4	17.8
Ivermectin 3	17.8	30.7	16.2	17.8
Ivermectin 4	19.4	35.6	19.4	27.5
Mean ± Std. Dev.	17.0 ± 2.1	32.4 ± 3.0	17.8 ± 1.8	22.2 ± 4.8
Atenolol 1	11.3	25.9	9.7	8.1
Atenolol 2	12.9	22.6	6.5	9.7
Atenolol 3	6.5	22.6	8.1	6.5
Atenolol 4	4.9	22.6	4.9	3.2
Mean ± Std. Dev.	8.9 ± 3.8	23.4 ± 1.7	7.3 ± 2.1	6.9 ± 2.8

[1] corresponding to the end of the exponential degradation phase for aniline

b) water-sediment system

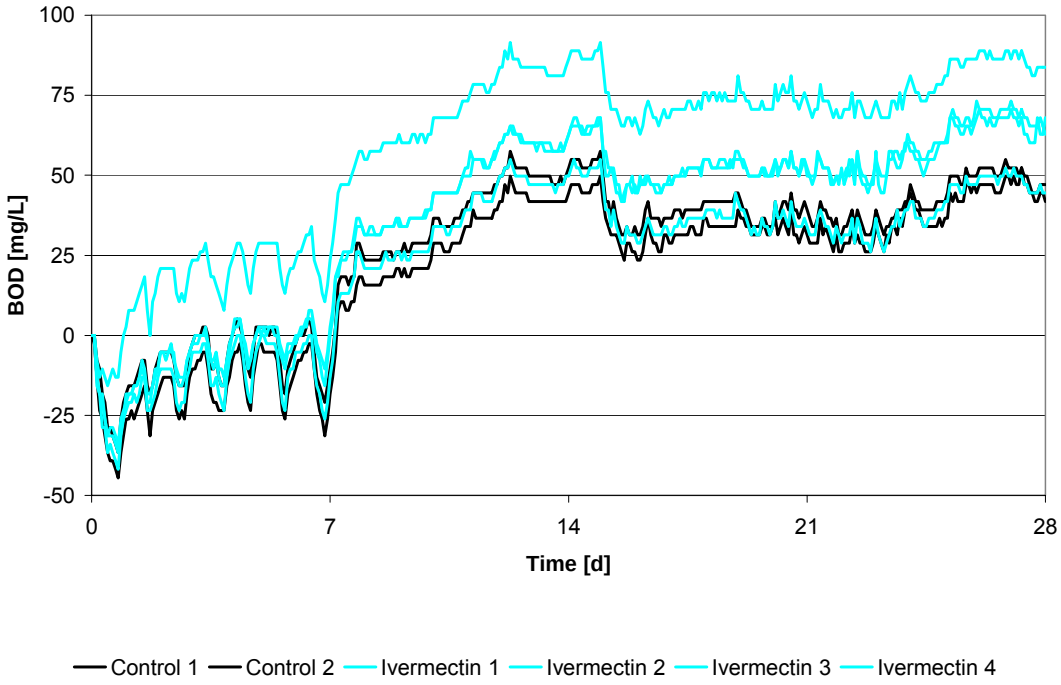

Fig. 14: Biological oxygen demand (BOD) of ivermectin (n = 4) and controls (n = 2) in the water-sediment system

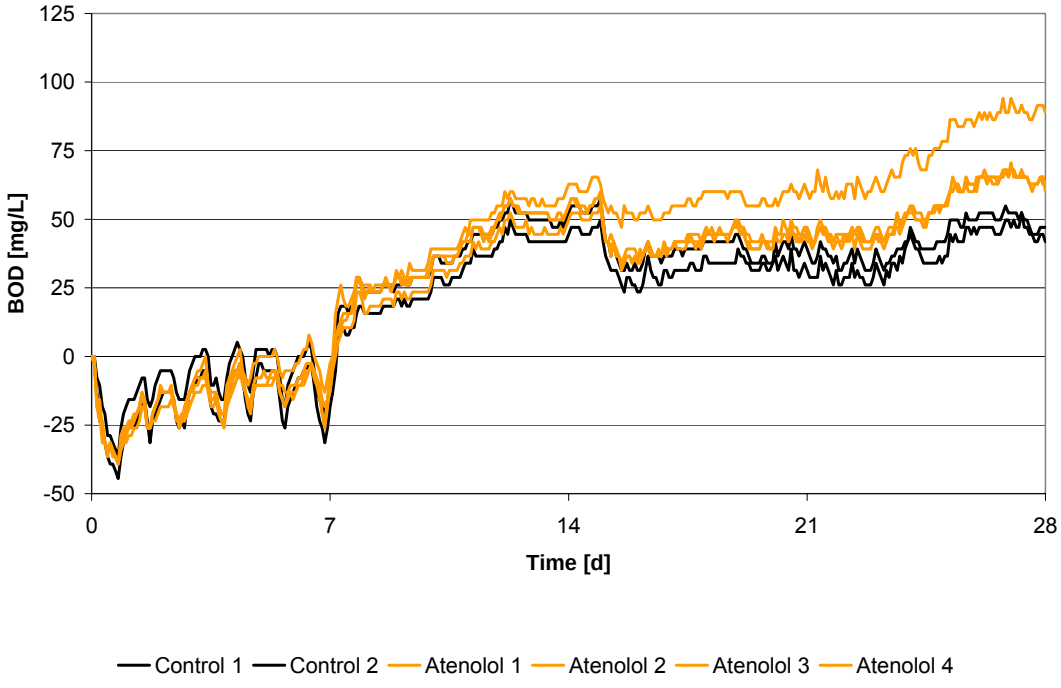

Fig. 15: Biological oxygen demand (BOD) of atenolol (n = 4) and controls (n = 2) in the water-sediment system

Tab. 8: Biological oxygen demand (BOD) at the different time points during the test with ivermectin, atenolol and the reference substance aniline using the water-sediment system

Replicates	BOD [mg/L]			
	7.4 d [1]	14 d	21 d	28 d
Control 1	18.3	52.3	39.2	47.1
Control 2	10.5	44.5	31.4	41.8
Mean ± Std. Dev.	14.4 ± 5.5	48.4 ± 5.5	35.3 ± 5.5	44.5 ±3.7
Ivermectin 1	13.1	49.7	34.0	44.5
Ivermectin 2	47.1	86.3	73.2	83.7
Ivermectin 3	23.5	62.8	49.7	68.0
Ivermectin 4	26.1	62.8	52.3	62.8
Mean ± Std. Dev.	27.5 ± 14.3	65.4 ± 15.2	52.3 ± 16.1	64.8 ± 16.2
Atenolol 1	15.7	54.9	47.1	62.8
Atenolol 2	13.1	54.9	41.8	60.1
Atenolol 3	10.5	49.7	44.5	62.8
Atenolol 4	20.9	62.8	60.1	88.9
Mean ± Std. Dev.	15.1 ± 4.4	55.6 ± 5.4	48.4 ± 8.1	68.7 ± 13.6

[1] corresponding to the end of the exponential degradation phase for aniline

Appendix 2: Detailed test results for 3,5-Dichloroaniline, 2,4-Dinitrophenol and the reference substance aniline

a) water-only system

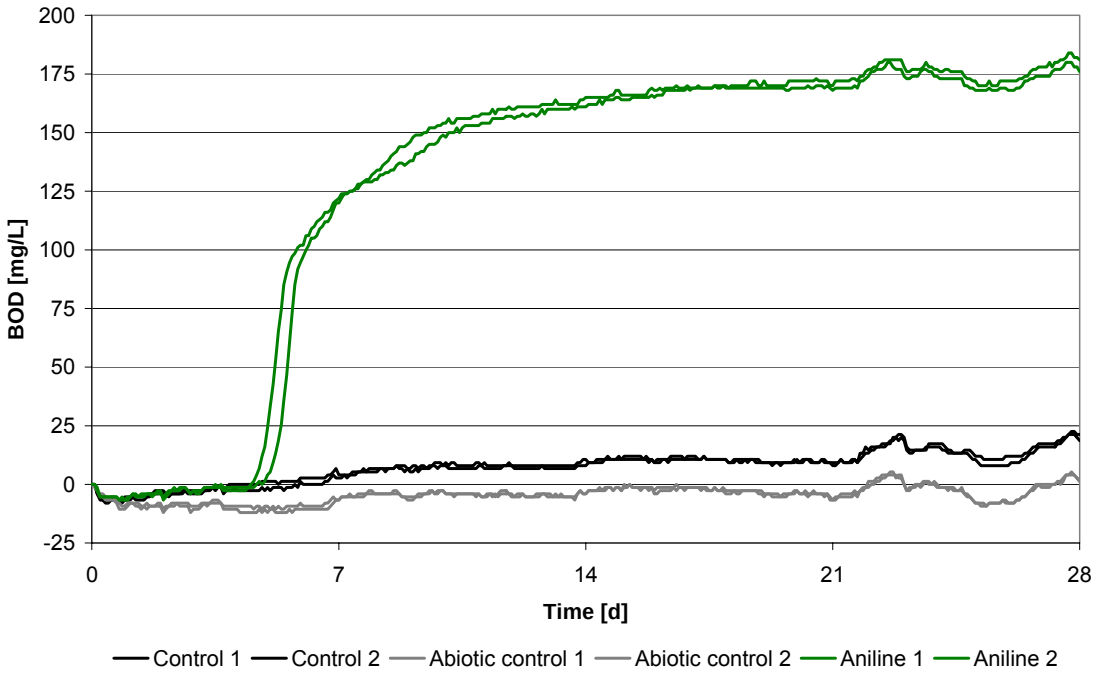

Fig. 16: Biological oxygen demand (BOD) of aniline (n = 2), controls (n = 2) and abiotic controls (n = 2) in the water-only system

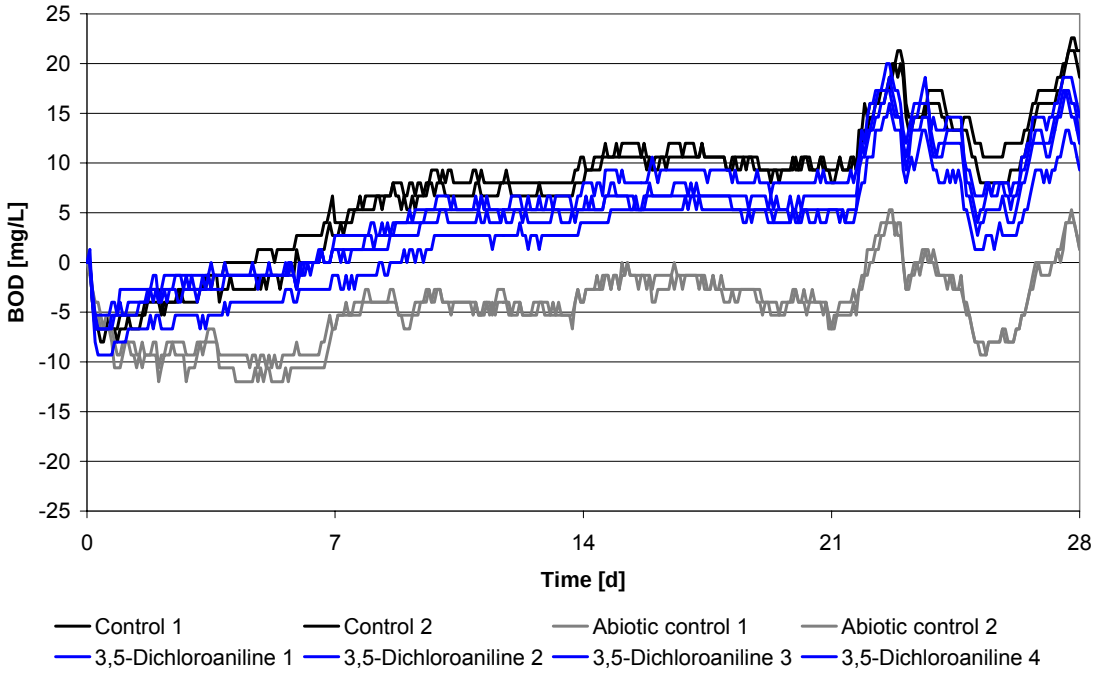

Fig. 17: Biological oxygen demand (BOD) of 3,5-Dichloroanoline (n = 4), controls (n = 2) and abiotic controls (n = 2) in the water-only system

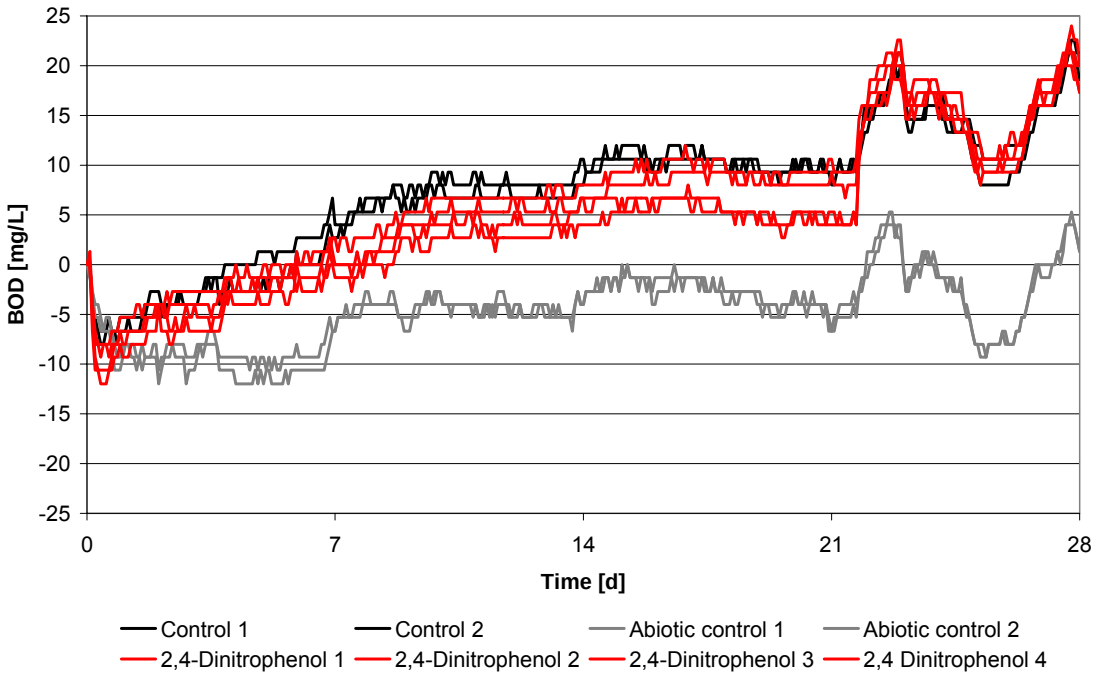

Fig. 18: Biological oxygen demand (BOD) of 2,4-Dinitrophenol (n = 4), controls (n = 2) and abiotic controls (n = 2) in the water-only system

Tab. 9: Biological oxygen demand (BOD) at the different time points during the test with 3,5-Dichloroaniline, 2,4-Dinitrophenol and the reference substance aniline using the water-only system

Replicates	BOD [mg/L]				
	5.8 d [1]	7 d	14 d	21 d	28 d
Control 1	1.3	4.0	10.6	9.3	21.3
Control 2	-1.3	2.7	8.0	8.0	18.6
Mean ± Std. Dev.	0 ± 1.8	3.4 ± 0.9	9.3 ± 1.8	8.7 ± 0.9	20.0 ± 1.9
Abiotic control 1	-9.3	-6.7	-4.0	-6.7	1.3
Abiotic control 2	-10.6	-6.7	-4.0	-6.7	1.3
Mean ± Std. Dev.	-10.0 ± 0.9	-6.7 ± 0	-4.0 ± 0	-6.7 ± 0	1.3 ± 0
Aniline 1	101.0	122.0	161.0	170.0	
Aniline 2	91.8	120.0	165.0	168.0	
Mean ± Std. Dev.	96.4 ± 6.5	121.0 ± 1.4	163.0 ± 2.8	169.0 ± 1.4	178.5 ± 3.5
3,5-Dichloroaniline 1	-1.3	2.7	5.3	5.3	9.3
3,5-Dichloroaniline 2	-1.3	1.3	8.0	8.0	14.6
3,5-Dichloroaniline 3	-1.3	1.3	5.3	6.7	12.0
3,5-Dichloroaniline 4	-4.0	-1.3	4.0	5.3	12.0
Mean ± Std. Dev.	-2.0 ± 1.4	1.0 ± 1.7	5.7 ± 1.7	6.3 ± 1.3	12.0 ± 2.2
2,4-Dinitrophenol 1	0	2.7	8.0	10.6	17.3
2,4-Dinitrophenol 2	-1.3	0	6.7	9.3	17.3
2,4-Dinitrophenol 3	-1.3	0	6.7	5.3	20.0
2,4-Dinitrophenol 4	-2.7	-1.3	4.0	4.0	20.0
Mean ± Std. Dev.	-1.3 ± 1.1	0.4 ± 1.7	6.4 ± 1.7	7.3 ± 3.2	18.7 ± 1.6

[1] corresponding to the end of the exponential degradation phase for aniline

b) water-sediment system

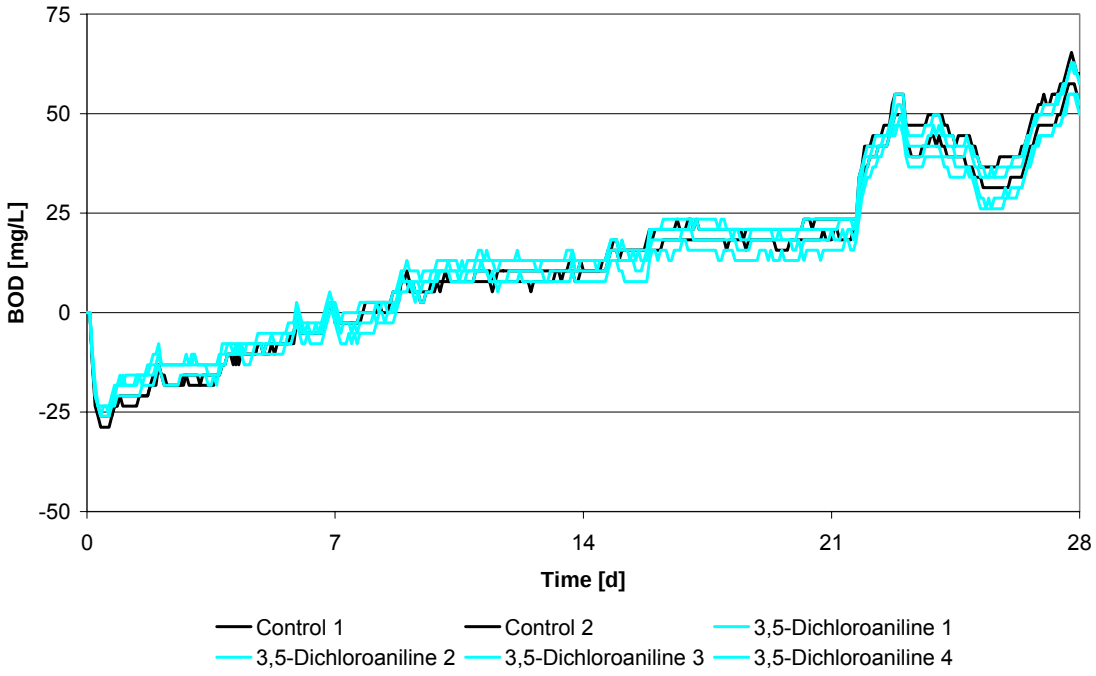

Fig. 19: Biological oxygen demand (BOD) of 3,5-Dichloroaniline (n = 4) and controls (n = 2) in the water-sediment system

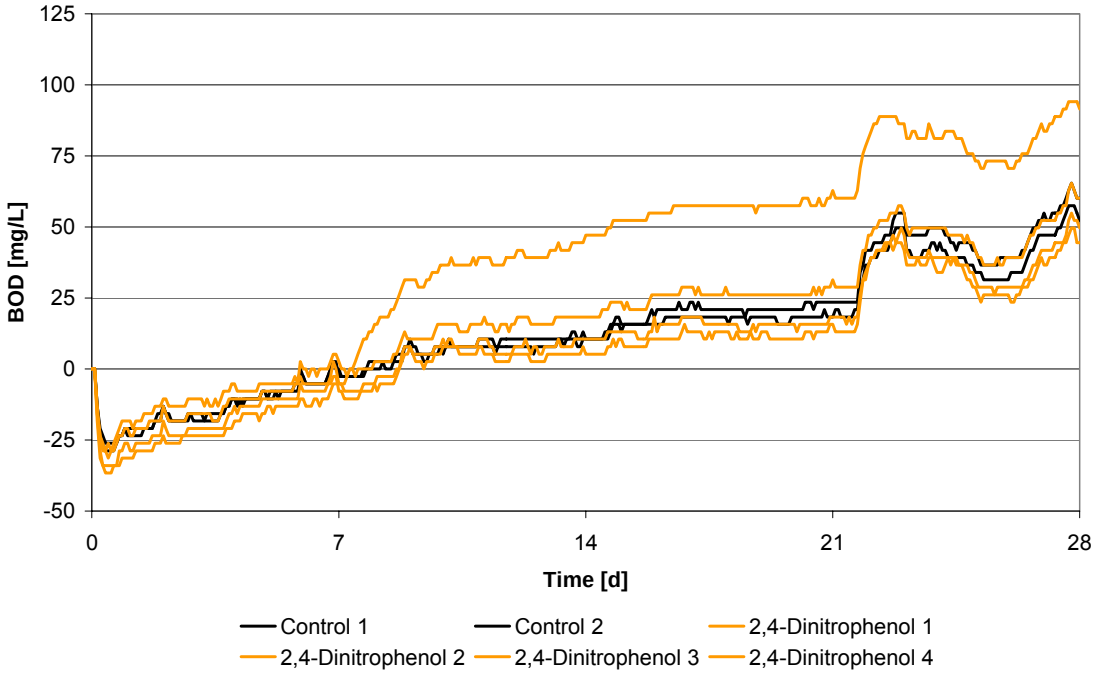

Fig. 20: Biological oxygen demand (BOD) of 2,4-Dinitrophenol (n = 4) and controls (n = 2) in the water-sediment system

Tab. 10: Biological oxygen demand (BOD) at the different time points during the test with 3,5-Dichlororaniline, 2,4-Dinitropheol and the reference substance aniline using the water-sediment system

Replicates	BOD [mg/L]				
	5.8 [1]	7.4 d	14 d	21 d	28 d
Control 1	-7.8	-2.6	10.5	18.3	52.3
Control 2	-5.2	0	10.5	23.5	60.1
Mean ± Std. Dev.	-6.5 ± 1.8	- 1.3 ± 1.8	10.5 ± 0	10.9 ± 3.7	65.2 ± 5.5
3,5-Dichloroaniline 1	-5.2	0	13.1	23.5	57.5
3,5-Dichloroaniline 2	-2.6	2.6	13.1	20.9	57.5
3,5-Dichloroaniline 3	-5.2	-2.6	7.8	15.7	52.3
3,5-Dichloroaniline 4	-5.2	0	10.5	20.9	49.7
Mean ± Std. Dev.	-4.6 ± 1.3	0.0 ± 2.1	11.1 ± 2.5	20.3 ± 3.3	54.3 ± 3.9
2,4-Dinitrophenol 1	-5.2	2-6	18.3	31.4	60.1
2,4-Dinitrophenol 2	-5.2	-5.2	10.5	18.3	49.7
2,4-Dinitrophenol 3	-10.5	-7.8	5.2	15.7	44.5
2,4-Dinitrophenol 4	-13.1	-5.2	47.1	62.8	91.5
Mean ± Std. Dev.	-8.5 ± 4.0	-3.9 ± 4.5	20.3 ± 18.7	32.1 ± 21.6	61.5 ± 21.1

[1] corresponding to the end of the exponential degradation phase for aniline