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ANAEROBIC BIOLOGICAL DEGRADATION OF CARBAMAZEPINE AT ENVIRONMENTAL CONCENTRATIONS

BIOLOGICZNY ROZKŁAD KARBAMAZEPINY W WARUNKACH BEZTLENOWYCH W STĘŻENIU WYSTĘPUJĄCYM W ŚRODOWISKU NATURALNYM

Abstract

A removal of the antiepileptic drug carbamazepine is frequently observed under anaerobic redox conditions at managed aquifer recharge sites. The biological influence on this effect is widely unknown and therefore it is the focus of this study. Anaerobic degradation batch tests and long-term soil column experiments, conducted within this study, suggest a removal of 2 to 50% induced by microbiological processes. Transformation products and their molecular structures are suggested – one of them is clearly identified as 10,11-dihydro-carbamazepine. This study provides a deeper understanding regarding the biotic reduction of carbamazepine.

Keywords: organic micropollutants, bank filtration, redox potential, reductive transformation

Streszczenie

Eliminacja leku padaczkowego karbamazepiny jest często obserwowana podczas sztucznego wzbogacania wód gruntowych w warunkach beztlenowych. W poniższym artykule przedstawiono badania dotyczące dotychczas niedostatecznie zrozumiałego wpływu faktorów biologicznych na te zjawisko. Przeprowadzone testy naczyniowe (testy batch) w warunkach beztlenowych i testy ciągłe z kolumnami piaskowymi pokazały znaczną eliminację karbamazepiny spowodowanej przez procesy mikrobiologiczne w wysokości 2 do 50%. W artykule zasugerowano produkty transformacji karbamazepiny i ich struktury molekularne – jednoznacznie zidentyfikowana zostala 10,11-dihydro-karbamezepina. Przedstawione badania umożliwiają lepsze zrozumienie biotycznego rozkładu karbamazepiny.

Słowa kluczowe: organiczne mikrozanieczyszczenia, filtracja brzegowa, potencjał redox, transformacja w warunkach redukcyjnych

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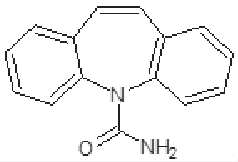
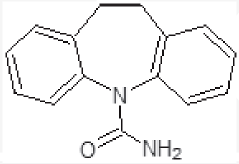
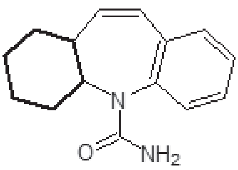
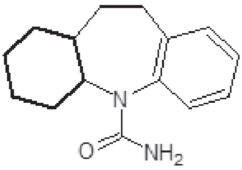
1. Introduction

The antiepileptic drug carbamazepine (CBZ) – used to medicate epilepsy, trigeminal neuralgia and depressions – is released into the anthropogenic water cycle by treated wastewater [1, 2]. In the aquatic environment, several transformation processes, such as photooxidation, sorption, precipitation, biodegradation and redox processes occur, changing the initial concentration of organic micropollutants considerably. For carbamazepine, all these conversion processes are very limited and rather unlikely as reported in literature [2–4]. However, under anaerobic redox conditions (Fe^{3+}/Mn^{4+} reducing, SO_4^{2-} reducing) at bank filtration sites, a removal of CBZ was occasionally observed in the past [5, 6].

Although oxidative transformation pathways of carbamazepine are thoroughly described in literature [7, 8], the fate of carbamazepine under reductive conditions has hardly been investigated so far. With electrochemical experiments, Moses et al. [9] and Atkins et al. [10, 11] described the abiotic reduction of carbamazepine to dihydro-carbamazepine ((2H)-CBZ) (Table 1). Within the study by Koenig et al. [12] using catalytic hydrogenation – besides (2H)-CBZ – eight further transformation products (TPs) were found and identified by high resolution mass spectrometry. Thus, three of them were detected in the effluent of a biologically active column containing zero valent iron to simulate strongly reducing conditions [12].

Table 1

Carbamazepine and selected transformation products (TPs) during reduction; TPs were all determined abiotically by electrochemical reduction [9–12] and catalytic hydrogenation [12]

Carbamazepine $C_{15}H_{12}N_2O$ 236,1 g/mol Consumption 2012: 52 t MEC_{max}/PNEC: 2,4			(2H)-CBZ[9-12] $C_{15}H_{14}N_2O$ 238,1 g/mol 10,11-dihydro-carbamazepine
(6H)-CBZa/b $C_{15}H_{18}N_2O$ 242,2 g/mol 1,2,3,4,4a,11a-hexahydro-5H-dibenzo[b,f]azepine-5-carboxamide			(8H)-CBZ[12] $C_{15}H_{20}N_2O$ 244,2 g/mol 1,2,3,4,4a,10,11,11a-octahydro-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide

In this study, the biological degradation of carbamazepine is investigated using an anaerobic long-term degradation test and lab scale soil columns, which characterize the fate of this compound more realistically. The study contributes to a deeper understanding of the biotically induced reductive transformation of the pharmaceutical carbamazepine, which possibly appears in the anaerobic subsoil at bank filtration sites.

2. Experimental

Anaerobic degradation tests were conducted by modifying the standard rule ISO 11734 [13]. The standard evaluates the "ultimate" anaerobic biodegradability of organic compounds in digested sludge. In triplicates, a test solution (100 mL) containing CBZ (c_0 = 11 µg/L or 8 mg/L CBZ), nutrients, reducing agent (Na_2S) and digested sludge from a waste water treatment plant in Berlin (Germany) was filled into serum bottles under nitrogen atmosphere and incubated at T = 35°C and pH = 7. After 90 days, the temperature was reduced to room temperature. Using a biodegradable organic carbon source (polyethylene glycole PEG 400, 100 mg/L), zero valent iron splints (ZVI; 5 g/L Fe^0) and external hydrogen (3 mL/bottle [14]), the redox potential was reduced. The hydrogen in the headspace was detected by gas chromatography using the GC-14A (Shimadzu, Japan carrier gas: nitrogen) equipped with a packed separation column (CTR I, 182.88 cm, carrier gas: nitrogen, Alltech GmbH, Germany).

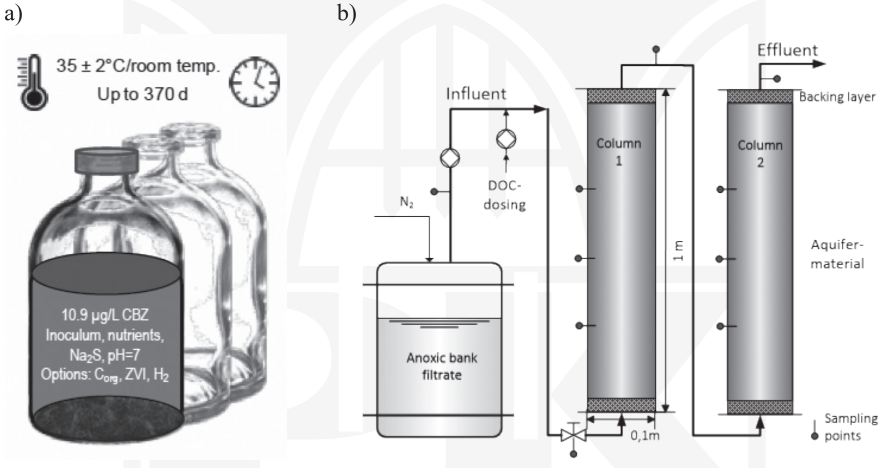


Fig. 1. Experimental setup of the anaerobic degradation batch test (a), scale 1:2 and soil the operated column system (b)

The three soil column systems used in this study consist of two columns in series (Perspex, length: 1 m; $Ø_{inner}$ = 0.09 m; porosity: 32%; pore volume: 7.8 L, residence time: 7.95 d), which are operated upflow with a flux of 250–350 mL/d for more than five years (Fig. 1). The columns are filled with natural aquifer material (grain size ≤ 2 mm) from a drinking water site in Berlin (Germany). The anoxic bank filtrate used for this long-term setup originates from a bank filtration site at lake Tegel in Berlin (Germany; depth: 25 m) and was obtained, transported and stored (at 11°C) gastight in stainless steel barrels. The initial DOC (Dissolved Organic Carbon) concentration (background 5 mg/L DOC; additional 0.5–30 mg/L DOC) was varied, in order to stimulate redox dependent microbiology. Samples from the soil columns were filtered by a 0.45 µm membrane filter (cellulose nitrate), stored in the dark at 6°C and analyzed for CBZ, (2H)-CBZ, DOC and total inorganic carbon (TIC), pH, conductivity, nitrate, iron, manganese and sulfate within three days. Samples from the degradation tests were handled in the same way, if necessary diluted a hundredfold and analyzed only for CBZ, (2H)-CBZ.

DOC and TIC were measured by thermal oxidation using a Vario TOC cube device for DOC and a High TOC II device for TIC (both: Elementar, Germany). The redox potential was determined with a SenTix ORP electrode (WTW, Germany) and converted to the standard hydrogen electrode (DIN 38404). Nitrate was analyzed using the flow injection analysis system FIAstar 5000 from Foss Tecator (Sweden). Iron and manganese were detected by atomic absorption spectroscopy (AAS) depending on their concentration either with a graphite furnace AAS (GF-AAS, SpectrAA-400 Varian, Agilent Technologies, USA) or flame AAS (F-AAS, GBC 906AA; GBC, USA). Sulfate was determined by ion chromatography (DX 500, Dionex, USA), equipped with an AS11 separation column and a conductivity detector. Methane was analyzed by the gas chromatograph 5890 Series II (Hewlett Packard, USA), including a flame ionization detector and equipped with a aluminum sulfate column (30 m × 0,53 mm, Supelco, Sigma-Aldrich, USA).

CBZ and (2H)-CBZ (Sigma Aldrich, USA or Dr. Ehrensdorfer GmbH, Germany) as well as [$^{13}C_6$]-CBZ (Alsachim, France) were analyzed by a LC-MS-MS (TSQ Vantage; Thermo Fisher Scientific, USA) equipped with a Kinetex C18 (2.6 µ) or Luna C18 (3 µ) chromatography column (Phenomenex, USA). The chromatography was applied at a flow rate of 0.2 mL/min and a column temperature of 30 °C (IQL: 50 ng/L CBZ; 50 ng/L (2H)-CBZ). For lower concentrations from the column setup, an online extraction (IQL: 1 ng/L CBZ; 5 ng/L (2H)-CBZ) with a Hypersil Gold enrichment column followed by a Hypersil Gold Phenyl separation column (both Thermo Fisher Scientific (USA) was performed (IQL: 1 ng/L CBZ; 5 ng/L (2H)-CBZ). For both methods, a linear gradient of water (1% methanol, 0.1% formic acid) and methanol starting with 90:10 was chosen. To exclude matrix effects, the samples were spiked with [$^2H_8$]-CBZ (Alsachim, France) before analysis.

3. Results & Discussion

Due to the discharge of treated wastewater into the anthropogenic water cycle, carbamazepine (CBZ) is frequently detected in surface water, with average concentrations between 0.5 µg/L to 1.7 µg/L in urban areas [1, 15]. Therefore, biological processes involving low initial concentrations are the focus of the presented experiments.

3.1. Anaerobic degradation of carbamazepine

Standardized degradation tests allow the general comparison of the biological availability of different substrates under identical testing conditions. By modifying these test conditions, the removal effects are no longer fully comparable. Thus, the degradation may be optimized and a specific removal potential of an organic substrate can be determined.

The modified degradation test with CBZ as substrate is characterized by strongly reductive conditions due to the addition of either an organic carbon source (PEG 400), iron splints (ZVI, inducing anaerobic corrosion and hydrogen production) and hydrogen. The triplicates were sampled over an experimental period up to 370 days. Within 12 days of incubation in all bottles, initial hydrogen concentrations ranging from 5 to 20 mmol/bottle were detected, depending on the testing variation (PEG 400, ZVI, H_2).

Hydrogen was present over the whole experiment and is partly converted to methane by anaerobic microbes, which can be concluded from methane measurements of a batch test at higher CBZ concentration (8 mg/L). A redox potential of –50 ± 40 mV, independent from the applied reductive agent, was detected throughout the experiment.

Figure 2 presents the c/c_0 ratio of CBZ (c_0 = 11 µg/L) in comparison to the formation of its reductive transformation product (2H)-CBZ over the experimental duration of 370 days. The CBZ concentration decreased slightly at all setup variations within the first 150 days, where the triplicates with ZVI showed the highest removal rate of 20% after 135 days.

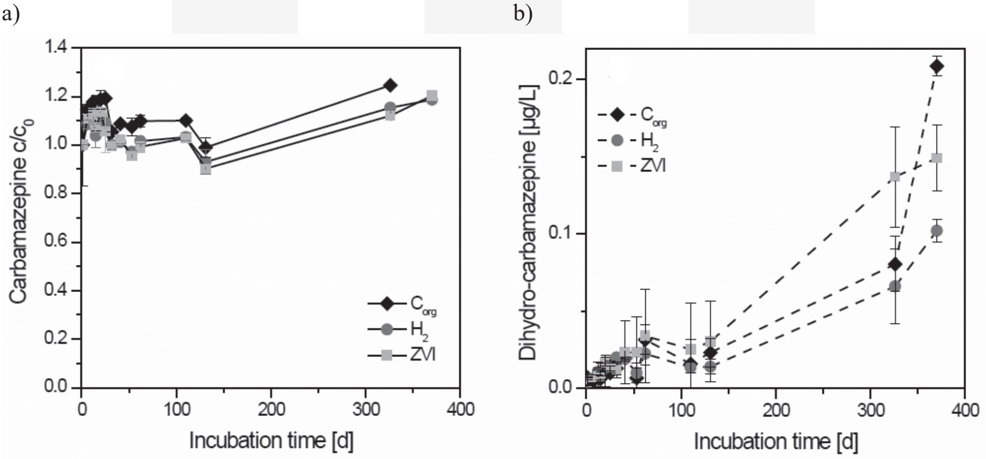


Fig. 2. Microbial degradation of carbamazepine (CBZ) within a modified test standard for anaerobic degradation (ISO 11734): c/c_0-ratio of CBZ (a) and (2H)-CBZ (Dihydro-CBZ) concentration (b); c_0(CBZ) = 10.9 ± 1.3 µg/L; experimental time: 360 days; The addition of zero valent iron (ZVI), external hydrogen or the organic carbon source varied the test conditions

Only after a long incubation time, a significant formation of (2H)-CBZ becomes obvious. After 370 days, up to 0.21 µg/L CBZ were transformed to (2H)-CBZ, representing two percent of the initial CBZ concentration. Several studies determined (2H)-CBZ as the main abiotically formed transformation product, either by electrochemical reduction or catalytic hydrogenation of CBZ [9–12].

However, this study demonstrates the biotic transformation of CBZ to (2H)-CBZ by adapted microbes. Long adaption times and the presence of hydrogen seem to be necessary. ZVI might enhance the reduction by additional hydrogen supply and providing a catalytic surface. Sterile duplicates (CBZ and ZVI) sampled on day 168 showed neither a change of the initial CBZ concentration nor a formation of (2H)-CBZ supporting the findings that the adapted microcosm is responsible for the fate of CBZ.

The other two test conditions (PEG 400 and external hydrogen) showed lower, but also clear conversion rates. With an organic carbon source, the adapted anaerobic bacteria were capable to transform CBZ at environmental concentrations to (2H)-CBZ, which is especially remarkable, since biological degradation processes in the subsoil during bank filtration are mainly influenced by available DOC. In anaerobic aquifers, micro-milieus with strongly reductive conditions can evolve (e.g. in dead end pores of an aquifer) [16, 17] where the observed transformation of CBZ to (2H)-CBZ is conceivable.

The presented findings are supported by the results of the second batch test at a higher initial CBZ concentration of 8 mg/L. The test variations (ZVI, H_2, PEG 400) were equal to the test conditions described before. The highest conversion regarding both – CBZ removal and (2H)-CBZ formation – was observed in bottles with ZVI. Thus, at day 300 50% of the initial CBZ concentration was degraded, of which 20% is transformed to (2H)-CBZ (data not shown, publication in progress). Therefore, the conversion was more effective compared to the first batch test.

However, in both degradation tests under anaerobic conditions, the mass balance of CBZ could not be fully elucidated, implying that the reductive transformation of the compound might involve other, yet undetected TPs and degradation processes.

3.2. Fate of carbamazepine in soil columns

With regard to long hydraulic retention times of bank filtrate in the subsoil, an adapted microcosm is the key factor for the removal of organic micro pollutants. Saturated soil columns simulate relevant transformation processes at bank filtration sites – more or less – near natural. The soil column systems used in this study are operated for five years with anoxic bank filtrate from the lake Tegel in Berlin (Germany), which provides an initial CBZ concentration of 0.65 ± 0.14 µg/L. Due to the long operation time, ad- and desorption effects of CBZ with the column material are assumed to be balanced.

To stimulate microbial growth of redox specific microbes, the redox milieus were controlled by adding DOC to the column influent. Whereas in one column line no DOC was added resulting in anoxic redox conditions ($c/c_0(NO_3^-) \approx 0.3$; neither iron nor manganese release), in two lines the additional DOC was limited to 2 mg/L DOC for four years inducing iron and manganese reduction. Thus, increasing effluent concentrations of 113 ± 40 µg/L for manganese and 4140 ± 1280 µg/L for iron were observed. Both compounds originate from the column filling.

Strongly reducing conditions were then implemented for more than thirteen months by adding high amounts of DOC (30 mg/L). As a result, the iron and manganese concentration proceeded similar to the previously set redox range, and additionally, sulfate is reduced by 80% to 11.6 ± 17.9 mg/L. Small amounts of methane were detected (single measurement) in the effluent proving that the set redox conditions are in the sulfidogenic and partly methanogenic range.

Figure 3 compares the three experimental conditions regarding the fate of CBZ and the known reduced TP (2H)-CBZ. Whereas, at anoxic and iron/manganese reducing conditions neither a removal of CBZ nor a (2H)-CBZ formation were observed, under sulfidogenic conditions (2H)-CBZ is released in the effluent in the range of 30 ng/L, representing 5% of the initial CBZ concentration.

Under sulfate reducing conditions, the isomers (6H)-CBZa and (8H)-CBZ were found in the effluent of the soil columns. Both TPs belong to the reductive reaction pathway presented by Koenig et al. (2015) [12] and represents the six- and eightfold hydrogenated CBZ molecule. The signal intensity is much lower within high-resolution mass spectrometry indicating very low concentrations. Since both TPs were not found in the anaerobic degradation batch tests, abiotic reduction of CBZ might also take place in the soil columns.

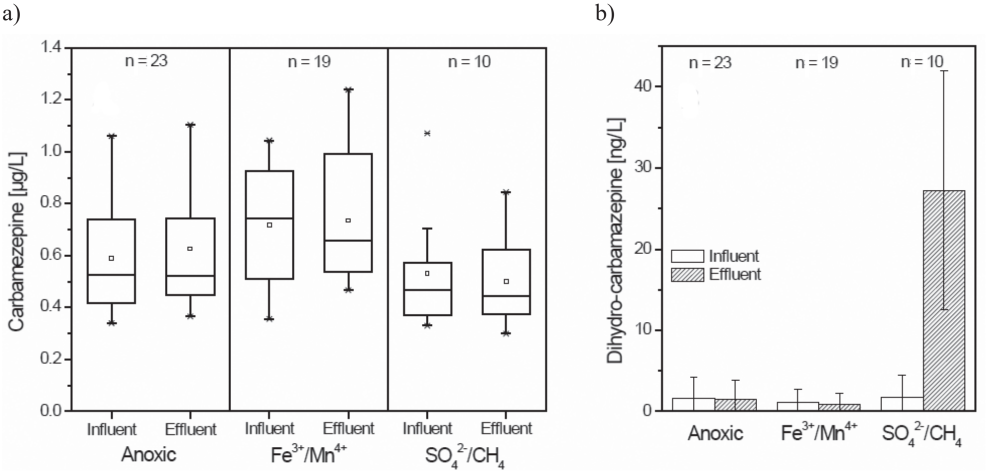


Fig. 3. Fate of carbamazepine (CBZ) and dihydro-CBZ ((2H)-CBZ) under different redox conditions in the soil column system: In- and effluent concentration of CBZ (a) and (2H)-CBZ (b) at anoxic, iron/manganese reducing and sulfogenic/methanogenic redox milieu

Since the c/c_0-ratio of CBZ in this redox milieu is with 0.94 close to 1.00 and the need of adsorption for the reductive transformation was proven for abiotic reduction processes [9–12], the detected (2H)-CBZ is possibly not formed from the CBZ of the current bank filtrate influent. It is rather generated from already adsorbed compound molecules on the column filling. To investigate this aspect, isotope labelled CBZ ($[^{13}C_6]$-CBZ) was introduced into the columns within tracer experiments. Besides the retardation factor for CBZ in the column system, the time shift until the $[^{13}C_6]$-(2H)-CBZ formation occurs can also be estimated.

Whereas conservative tracer experiments with KBr show residence times very close to the calculated residence time of 7.95 d, the injected isotope labelled CBZ interact with the soil material leading to a certain delay of the $[^{13}C_6]$-CBZ signal peak (Figure 4). Using the C_{peak}-method described by Schudel et al. (2002) [18], the specific retardation factor for CBZ is estimated with $R_d = 2.5$. In literature, the R_d values for CBZ during soil passage range from 2.3 to 3.3 [19, 20].

The tracer experiment with the isotope labelled CBZ is still running (04/2016). Remarkably, even after more than 60 days after $[^{13}C_6]$-CBZ-injection, no isotope labelled (2H)-CBZ was observed in the effluent, which indicates that either the injected load of $[^{13}C_6]$-CBZ was too low for the detection of the related transformation product or the necessary time shift for the transformation has not been reached yet. This implies that some isotope labelled CBZ must remain in the column system and be transformed very slowly to the reduced product.

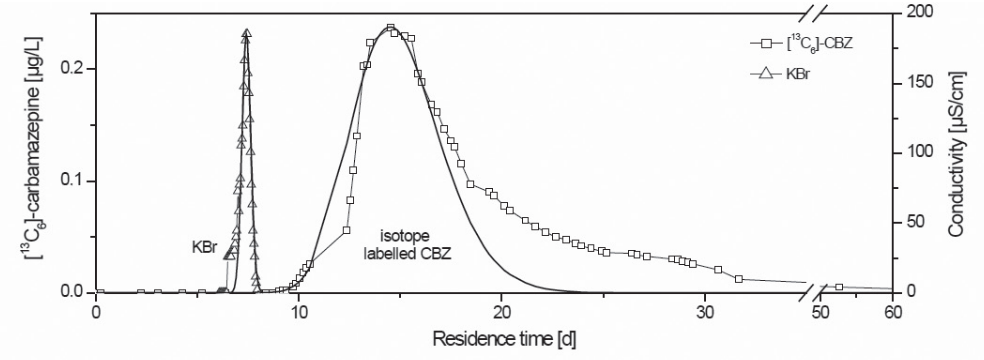


Fig. 4. Residence time peaks of two different tracer experiments with the conservative tracer potassium bromide (KBr, triangles) and the isotope labelled Carbamazepine (CBZ, squares); The respective model curves were determined by an analytical solution of the transport equation suggested by Schudel et al. (2002) [18] which considers advection and dispersion effects

4. Conclusion

The presented study focuses on the biological degradation of carbamazepine under reductive conditions occurring in anaerobic aquifers. In the conducted degradation tests as well as in the soil column experiments, the transformation of carbamazepine was successfully demonstrated at environmental concentrations. The compound was transformed in the range of 2–10% to dihydro-carbamazepine ((2H)-CBZ) in the anaerobic batch tests. The presence of hydrogen appears to be necessary. The microbial transformation of CBZ to (2H)-CBZ was confirmed in the near-natural soil column system at initial CBZ concentrations below 1 µg/L. Thus, under sulfidogenic and methanogenic redox conditions, besides (2H)-CBZ, two further TPs were found in the effluent of the columns. The results of this study show that an adapted microcosm is capable to transform CBZ under reducing conditions. (2H)-CBZ constitutes a central molecule not only in the abiotic – as already shown in literature – but also in the biotic reduction path of CBZ. The experiments imply that other unidentified TPs might be formed, since the mass balance of CBZ in the degradation tests is not closed at all. This aspect and the occurrence of identified TPs at bank filtration sites with anaerobic soil passage require further research and field studies.

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