# Pharmaceuticals and health care products in wastewater effluents: the example of carbamazepine

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**Abstract** Persistence and impact of pharmaceutics in the environment are discussed. The case of carbamazepine (CBZ), a widely used antiepileptic drug detected in rivers, lakes, sludges and even in ground water is examined. CBZ fate was investigated in all possible routes that may follow after it has been discharged to the sewage system: activated sludge, anaerobic digestion sludge, seawater, fresh water and soil. Carbamazepine slowed down, i.e. caused a decrease in the COD consumption rate in the activated sludge process, especially after longer term exposure, while the anaerobic sludge process was unaffected in the operating conditions that were applied. The compound was not degraded under either short term or long term exposure to either aerobic or anaerobic degradation processes. Carbamazepine seemed to biosorb to solid phases (soil, sludge) and this strength of sorption was related to the organic content of the solid phase. These results explain why CBZ is a very persistent xenobiotic compound, as is apparent from its detection in appreciable amounts in various aquatic environments.
**Keywords** Activated sludge; anaerobic digestion; carbamazepine; environment; fate; pharmaceutical; soil

## Introduction

Although pharmaceutical compounds may have a positive and often decisive role on human life, it has recently been realized that they may have a significant negative impact on the environment. A significant amount of the original substance or its metabolites are often excreted with urine or faeces, thus finding their way to raw sewage (Hirsch *et al*., 1999). Given the fact that many pharmaceuticals are often compounds very persistent to biodegradation and to natural physicochemical degradation processes, their presence in the raw sewage may have further implications, such as (a) adverse effect on the microorganism type, number and function and hence on the performance of sewage treatment plants (STP), (b) escape through STP effluent into the aquatic environment affecting marine life and resulting in bioaccumulation, and (c) biosolids from STPs applied on land may provide a path for pharmaceuticals to contaminate ground water and food crops.

Until recently, authorities have assumed that given the fact that the quantities of consumed pharmaceuticals (and other xenobiotic compounds such as PCBs, surfactants etc.) are relatively low, their anticipated impact on the environment is probably negligible (Aherne *et al*., 1985; Warman and Thomas, 1981; Gool van, 1993; Coyne *et al*., 1994; Kerry *et al*., 1995).

Recent research, however, on xenobiotic compounds such as PCBs (Halling-Sorensen *et al*., 1998) demonstrated, that some compounds called endocrine disruptors, have a hormone mimicking effect, even at concentration of a few nanograms per litre. In addition, recently published results clearly demonstrated effects on the endocrine systems of fish exposed to sewage effluent water, due to synthetic contraceptives present in effluents in the low ng/L range (Larsson *et al*., 1999). These findings strongly suggest that the previous assumption – that "no threat for human life and the environment must be expected by the presence of pharmaceuticals at very low concentrations" should be seriously reconsidered (Colburn and Clement, 1992). Furthermore, continuous discharge of generally not

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degraded xenobiotics in the environment will result in their accumulation and their concentration will increase in the future. Therefore, a responsible and environmentally sensitive attitude is only one that is based on the assessment of their presence and influence, as well as the development of the necessary strategies for their abatement.

Carbamazepine (CBZ) is an antiepileptic drug and was taken as an example compound to be studied in this work, with respect to its environmental fate and its effect on wastewater treatment plants. CBZ has frequently been detected in municipal sewage and surface water samples (Ternes, 1998; Heberer, 2002). During sewage treatment, it was found to be removed by less than 10% (Ternes, 1998; Heberer, 2002). As a result, CBZ has been detected at concentrations up to 1,075 ng/L in surface water samples in Berlin (Heberer, 2002). It has also been found in drinking water at a concentration of (30 ng/L).

The persistence of CBZ to photodegradation and its toxicity towards simple living organisms such as algae was studied by Andreozzi *et al*. (2002). They found that CBZ is photodegradable in distilled and river waters and there was no evidence for it being toxic or accumulated in algae. However, there is no systematic approach in the literature on the fate of CBZ in the environment (aquatic or terrestrial) and its effect on biological processes, such as the activated sludge process or anaerobic digestion. Thus, the aim of this work was to study the fate and impact of this commonly used pharmaceutical on the activated sludge and anaerobic digestion processes and its fate if discharged to seawater, fresh water and soil.

## Methods

### Experimental set-up

*Activated sludge process experiments*. The activated sludge process was simulated in a lab scale SBR system (Sequencing Batch Reactor) operated at a hydraulic retention time of 12 h. The SBR was fed with a synthetic medium (SM) containing: 1.94 g sodium acetate, 1.51 g $NH_4Cl$, 0.3 g $MgSO_4 \cdot 7H_2O$/L, 0.04 $CaCl_2 \cdot 2H_2O$/L, 1 drop of a trace metals solution, 7.57 g $K_2HPO_4 \cdot 3H_2O$/L, 2.27 g $KH_2PO_4$/L. The effect of CBZ on the activated sludge process was studied by focusing on the impact it has: a) immediately after it has been added in the SM for the first time (short-term effect), b) after 10 days of SBR operation while treating CBZ containing SM (mid-term effect) and c) while the sludge has been fed with CBZ containing SM for several months (long-term effect).

Short-term effect experiments. Batch tests were conducted using inoculum from an SBR being fed with the SM described above. The tests took place in Erlenmeyer flasks of 250 ml operating volume, where 64 ml of inoculum (5.8 g TSS/L) and 50 ml of SM containing 0 (blank), 2, 4, 6, 8 and 10 mg CBZ/L respectively and 135 ml $H_2O$ (so that the final TSS concentration was 1.5 g/L), were added. The COD concentration was monitored during the first 2 hours of the test and the specific COD consumption rate was calculated.

Mid-term effect experiments. The SBR performance was observed during a cycle of operation (8 h) while it was fed with: a) only SM (containing 0.93 g glucose/L and 0.97 g sodium acetate/L instead of sodium acetate alone) (blank), b) SM + CBZ for the first cycle of operation with CBZ, and c) SM + CMZ after 10 days of operation with CBZ. The CBZ concentration in the SM was 15 mg/L.

*Anaerobic digestion process experiments*. Continuous and batch experiments were conducted in order to study the long and short term effect of CBZ on the anaerobic digestion process, respectively.

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Continuous experiments. Two digesters with a useful volume of 0.5 L each were operated in a draw and fill mode, at a hydraulic and solids retention time of 20 days and under an organic loading rate of 13 g COD/L·d. The digesters were inoculated from a draw and fill anaerobic reactor fed with a glucose based synthetic medium (GSM) that contained 10 g glucose/L, 1.33 g yeast extract /L, 1.33 g casein hydrolysate/L, 10 ml/L of a $(NH_4)2HPO_4$ solution (7.21 g/L) and 10 ml/L of a trace metals solution. The digesters were fed with the same GSM, but one of them also received 10 mg CBZ/L while the other served as blank. The digesters were run for 100 days under these conditions and reached a steady state.

Batch experiments. Batch experiments were conducted to study the immediate effect of the CBZ on the anaerobic sludge by observing the methane production rate in 125 ml serum bottles that contained inoculum from a glucose fed anaerobic digester and a GSM solution (initial COD concentration 750 mg/L). The useful volume was 100 ml. Two series of seven bottles were prepared that contained either the GSM (reference) or a mixture of the GSM with CBZ (10 mg/L). The bottles were sealed and were incubated in 35°C. During sampling, only one bottle from each series was used to measure the biogas production rate, methane content, COD concentration and the CBZ concentration, and then it was discarded.

*"Aquatic" experiments*. The fate of CBZ in the aquatic environment was studied according to OECD method No 306. The experiments were conducted in 1 L bottles containing sea water or fresh water, maintained in abiotic conditions (by adding $HgCl_2$, 100 mg/L) or in conditions favourable for microorganism growth (by supplementing mineral nutrients), kept in the dark or light and contained CBZ (0.5 mg/L) or were kept as blanks. The bottles were placed in a bath to maintain the temperature at 20°C. All tests were run in duplicates.

*"Soil" experiments*. The determination of the sorption coefficient was made according to OECD method No 106. Two types of soils were selected: soil 1 (pH: 4.5–5.5, organic content: 1–2%, clay content: 65–80%, soil texture: clay) and soil 7 (pH: <4.5), organic content: >10%, clay content: <10%, soil texture: sand/loamy sand). For each type of soil, a triplicate was conducted by mixing 25 g soil with 50 ml of an aqueous solution (0.01 M $CaCl_2$) containing 10 mg/L of CBZ. The mixtures were put in flasks and placed in shaking bath at 25°C. Blank mixtures, consisted of CBZ and the $CaCl_2$ solution, were also prepared. Sampling took place 5 min after the CBZ addition and 24 h later, so that an equilibrium state was reached. The distribution coefficient of CBZ (ratio of compound content in the soil phase to its concentration remaining in the solution, when equilibrium has been reached) in each soil type could then be calculated:

$$K_d = \frac{C_s^{ads}(eq)}{C_{aq}^{ads}(eq)} \tag{1}$$

where,
$C_s^{ads}$: the content of the compound adsorbed on the soil (µg/g), and
$C_{aq}^{ads}$: the concentration of the compound in aqueous phase (µg/ml).

### Analytical techniques
CBZ was measured using HPLC (mobile phase: 60% acetonitrile, 40% $NaH_2PO_4$, 0.02 M, pH = 2.7, flowrate: 1ml/min, UV detection at 254 nm, X-Terra™ $RP_{18}$ 5 µm column from Waters). Prior to the analysis in HPLC, the samples were treated using Solid Phase Extraction techniques (Waters HLB® cartridges of 50 mg), so that the samples would be free of other organic compounds that interfered in the analysis or inorganic ions that

decayed the column. The COD, TSS, VSS concentrations were determined according to *Standards Methods* (APHA, 1995). The methane content was determined in a GC with TCD and the biogas volume was measured via liquid displacement.

## Results and discussion

### Activated sludge process experiments

*Short-term effect experiments*. A series of six tests were conducted using inoculum from an SBR operated with SM. CBZ was added at concentrations of 0, 2, 4, 6, 8 and 10 mg/L and the COD concentration was monitored versus time. The COD consumption rates were determined by linear fitting of the experimental data taken within the first 2 h, and were normalised with respect to the VSS concentration (Figure 1). It is clear that CBZ had an immediate effect on the activated sludge process.

*Mid-term effect experiments*. The CBZ mid-term effect on activated sludge was studied by comparing the COD profiles in 3 operating cycles of an SBR: without the presence of CBZ (Figure 2), with CBZ initially (Figure 3) and with CBZ after 10 days of continuous addition of CBZ in the feed (Figure 4). The COD concentration decreased in all cases during the aeration phase, but there was a significant difference in the rate of the decrease.

Since the COD reduction rate changed over time, it was calculated from the first derivative of a second-degree polynomial function (that had been fitted to the experimental data) and was normalised with respect to the VSS concentration (Figure 5). It is apparent that the CBZ negative effect was quite pronounced after 10 days of operation, since it slowed down the COD consumption rate dramatically. However, the overall efficiency of the activated sludge process (within the 8 h of the SBR cycle) was not affected under these operating conditions, since the COD removal was 91% in the first cycle and 95% in the last one.

The fate of CBZ in the initial and final (after 10 d of operation) SBR cycle is shown in Figure 6. It is clear that CBZ was not biodegraded during the SBR operation. Although the CBZ concentration decreased significantly in the first cycle, it increased again in the final cycle and varied near the value of the CBZ concentration in the feed (15 mg/L). The initial decrease of CBZ was thus attributed to biosorption that, after some days of operation, stopped as the sludge was saturated with CBZ. Finally, it should be mentioned that long-term (6 months) draw-and-fill activated sludge experiments showed no evidence of CBZ degradation whatsoever.

### Anaerobic digestion process experiments

The CBZ effect and persistence during anaerobic digestion was investigated by comparing the response of two anaerobic digesters operated with or without CBZ when ran in parallel (Figure 7). CBZ had no effect on the anaerobic process and this was also reflected on
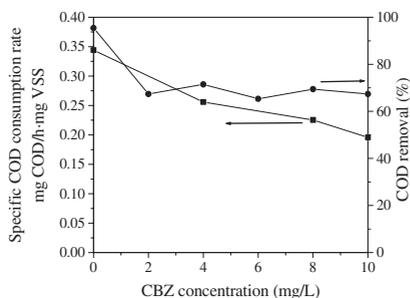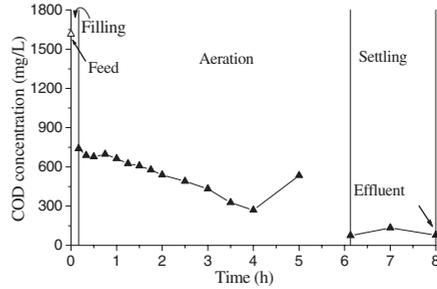
**Figure 1** Short time effect of CBZ on the specific COD consumption rate and COD removal
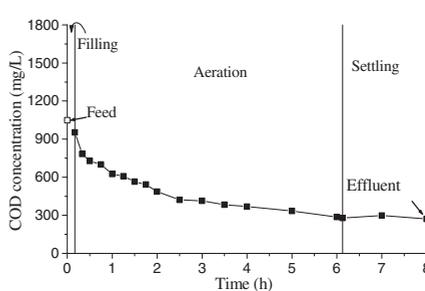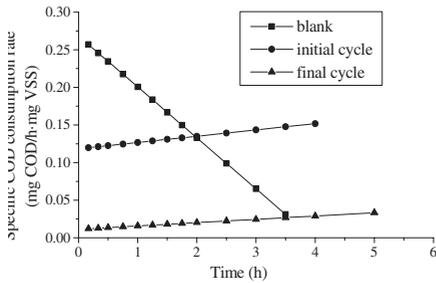


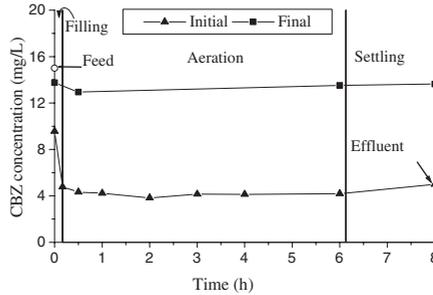**Figure 2** COD profile during a typical SBR cycle (Blank)
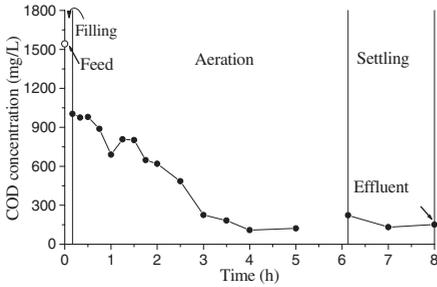
**Figure 3** COD profile during the first SBR cycle with 15 mg/L CBZ (Initial)



**Figure 4** COD profile during a cycle after 10 days of SBR operation with 15 mg/L CBZ (Final)



**Figure 5** Effect of the CBZ on the specific COD consumption rate during SBR aeration phase
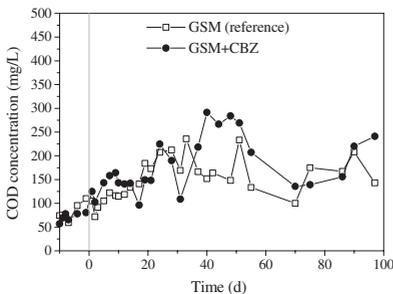


**Figure 6** CBZ concentration during the first and the final cycle of SBR operation with CBZ

all parameters measured but not shown here (biogas production rate, volatile fatty acids concentration, pH). The CBZ concentration in the digester was constant (~8 mg/L) and the difference between CBZ concentration in the feed and the digester is probably attributed to biosorption (Figure 8).

The short time effect of CBZ was also studied by monitoring closely the methane production rate of anaerobic digesters within the first hours after the addition of CBZ at a concentration of 1 mg/L (Figure 9). There was no sign of deterioration in the methane production in the digester that contained the CBZ. There was also no decrease in the CBZ concentration in the digester and thus neither biosorption nor biodegradation took place (Figure 10).

**"Aquatic" experiments**
The fate of CBZ in water was investigated using two types of water (sea and fresh) with the presence of nutrients allowing biodegradation. The biodegradability of a reference
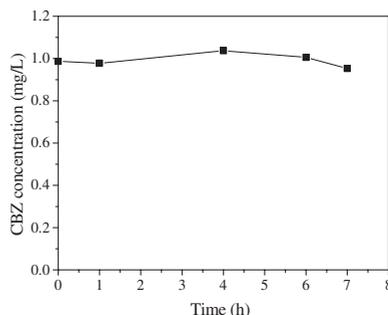


**Figure 7** Effect of the daily CBZ addition (10 mg/L) on effluent COD concentration of anaerobic digester fed with GSM
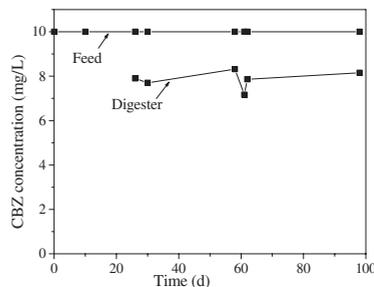


**Figure 8** CBZ concentration during the operation of a digester fed with GSM+CBZ

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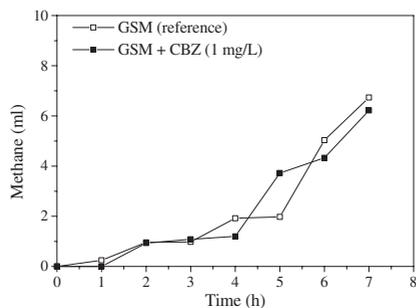
**Figure 9** Effect of CBZ addition (1 mg/L) on a batch anaerobic digester fed with GSM



**Figure 10** CBZ concentration during the batch experiment

compound ($CH_3COONa$) was tested in both sea and fresh water. The duration of the reference tests was 10 days and the concentration of $CH_3COONa$ was decreased from 90.6 to 24.4 mg/L in the seawater (73% reduction) and from 112 to 46 mg/L in the fresh water (59% reduction). However, CBZ was not biodegraded when present in these media at an initial concentration of 0.5 mg/L (Figures 11 and 12).

The effect of light on CBZ fate was also studied in both types of water, but no change in the CBZ concentration was observed. The addition of $HgCl_2$ in half of the tests aimed to ensure abiotic conditions. However, it caused the CBZ concentration to decrease in the case of the fresh water, probably because of physicochemical interactions between the compound and the water composition in the presence of Hg. This was also observed in other tests studying other pharmaceuticals. The substitution of $CHCl_3$ for $HgCl_2$ in these tests solved this problem.

### "Soil" experiments

The fate of CBZ in soil was studied by applying it in two types of soil, with low and high organic content (Table 1). Since CBZ is a hydrophobic compound [log($K_{ow}$) = 2.45, SRC Phys. Prop. DB] it is highly adsorbed onto soil type 2 (high organic content), even within the first 5 min. of the contact between the liquid and solid phase. This was also observed in the mid-term experiments (Figure 6) with the activated sludge, where the CBZ decreased significantly in the first minutes of the first cycle of the SBR operation.

### Conclusions

The experiments conducted in this work confirmed that carbamazepine is a compound persistent to biodegradation in aquatic environment or in biological processes. Wherever a decrease in the carbamazepine concentration was observed, this was due to sorption
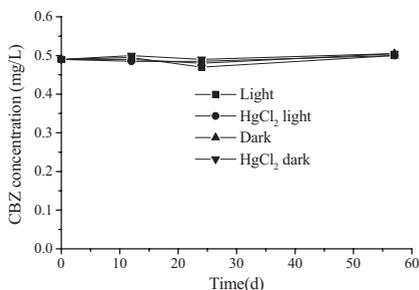


**Figure 11** CBZ fate in seawater when added at 0.5 mg/L
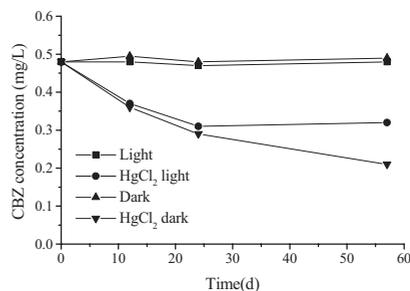


**Figure 12** CBZ fate in fresh water when added at 0.5 mg/L

**Table 1** Sorption of CBZ onto soil type 1 and 7

| Type of soil | Initial concentration of CBZ (mg/L) (mg/L) | Concentration of CBZ after 5 min (mg/L) (mg/L) | Concentration of CBZ after 24 h (mg/L) (mg/L) | Adsorption $A_t$ (%) | Distribution coefficient $K_d$ (ml/g) |
|---|---|---|---|---|---|
| 1 | $9.8 \pm 0.1$ | 6.9 | 5.9 | 40.4 | 1.4 |
| 7 | $9.8 \pm 0.1$ | 3.9 | 3.1 | 68.7 | 4.4 |

phenomena attributed to the hydrophobic nature of the compound. The sorption tendency of the tested compound to the solid phases was more evident in the experiments with soil, where carbamazepine was strongly adsorbed onto the solid phase within the first few minutes of the tests. The adsorption was higher in the case of the soil with higher organic content.

CBZ had a significant negative effect on the specific COD consumption rate in the activated sludge process. However the overall efficiency of the process remained unaffected under the studied conditions. The anaerobic digestion process also seemed to be unaffected by carbamazepine, by monitoring either the process efficiency or the initial methane production rate.

## Acknowledgement

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