Behavior of micropollutants during hydrothermal carbonization of sewage sludge

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Summary

Hydrothermal carbonization (HTC) has become a promising process for treating residual and waste materials. Heating up the aqueous matter to 190 - 250 °C in a closed system at about 15 bar converts the material to biochar. Use of sewage sludge in the HTC process has emerged recently. Compared to sewage sludge the biochar from HTC has an enhanced dewaterability and an increased heating value. Moreover, proponents claim that nutrients enrich in the biochar and pollutants degrade during the process. In this thesis the latter assumption was examined.

Twelve pharmaceuticals from different compound classes and ten perfluorinated compounds (PFC) were selected because of their ecological relevance and persistence. First, liquid chromatography coupled to mass spectrometry (LC-MS) methods were developed for the selected compounds in sewage sludge and biochar from HTC. Matrix effect profiles visualized the sample complexity. Final methods for the analysis of pharmaceuticals included sample lyophilization and pressurized liquid extraction. Extracts were injected directly to LC-MS and quantified via standard addition. Analysis of PFC required an additional clean-up step via solid phase extraction.

Analysis of pharmaceuticals and PFC in sewage sludge from different wastewater treatment plants and their resulting biochars showed that the sewage sludges contained higher amounts of the analytes than their corresponding biochars. Hence, HTC could reduce the micropollutant load in sewage sludge. Although compound loads followed regional trends, the removal rates remained independent from the sewage sludge source.

The mechanisms causing the compound decline were followed in detail by the example of diclofenac. Therefore, the compound was examined in inert HTC experiments, whereby sand replaced the sewage sludge. Non-target analysis using high resolution mass spectrometry (LC-HRMS) helped to identify transformation products. Therewith a degradation mechanism for diclofenac during HTC was postulated. Based on that, results were transferred to the complex sewage sludge matrix. However, behavior of diclofenac during HTC of sewage sludge differed significantly from the inert experiments. The reasons for these discrepancies could not be fully revealed.

Altogether, this thesis presented LC-MS methods to determine micropollutants in sewage sludge and biochar. Sample analysis showed that the HTC could reduce micropollutants in sewage sludge by converting it to biochar. The process was followed mechanistically for diclofenac. Therewith, this work connects developing new analytical LC-MS methods with assessing novel processes like the investigated HTC. Based on that, further studies could investigate the behavior of other compound classes. Further on, the application of the produced biochar in agriculture could be examined.

Zusammenfassung

Die Hydrothermale Karbonisierung (HTC) wird seit einigen Jahren als vielversprechende Methode zur Umwandlung von organischen Reststoffen eingesetzt. Durch das Erhitzen einer wässrigen Suspension auf 190 - 250 °C unter Luftausschluss findet bei einem Druck von ca. 15 bar die Umwandlung zu Biokohle statt. Der Einsatz von Klärschlamm in der HTC wird erst seit Kurzem durchgeführt. Vorteile sind eine verbesserte Entwässerbarkeit und ein höherer Brennwert. Darüber hinaus werden die Möglichkeit zur Nährstoffrückgewinnung und eine Reduktion von Schadstofffrachten postuliert. Letztere Hypothese wurde in dieser Arbeit geprüft. Als relevante Stoffgruppen wurden aufgrund ihrer Umweltrelevanz und Persistenz zwölf Arzneimittel sowie die Gruppe der perfluorierten Tenside (PFT) ausgewählt. Zunächst mussten Methoden der Flüssigchromatographie gekoppelt mit Massenspektrometrie (LC-MS) für die Quantifizierung der Substanzen in den Matrices Klärschlamm und Biokohle entwickelt werden. Matrixeffektchromatogramme machten dabei die Komplexizität der Matrix sichtbar. Die finale Analysenmethode von Arzneimitteln in Klärschlamm und Biokohle basierte auf einer Probenvorbereitung mittels Gefriertrocknung und beschleunigter Lösemittelextraktion. Die Extrakte wurden direkt injiziert und die gesuchten Substanzen mittels Standardaddition quantifiziert. Zur Untersuchung der PFT wurde ein zusätzlicher Aufreinigungsschritt mittels Festphasenextraktion integriert. Bei der Untersuchung von Klärschlämmen und dazugehörigen Biokohlen nach HTC zeigte sich, dass die Klärschlämme eine höhere Belastung mit Arzneimitteln und PFT aufwiesen als die entsprechenden Biokohlen. Daraus ergab sich eine Reduktion der Schadstofffracht durch die HTC. Die Reaktionsmechanismen, die zu dieser Reduktion führten, wurden am Beispiel von Diclofenac genauer untersucht. Hierzu wurde in Inertexperimenten mit Sand das Abbauverhalten der Substanz bei der HTC betrachtet. Non-target Analytik mittels hochauflösender Massenspektrometrie (LC-HRMS) half bei der Identifizierung von Transformationsprodukten. Daraus konnte ein Abbaumechanismus für Diclofenac postuliert werden. Die Übertragung der Ergebnisse aus Interexperimenten auf die komplexe Klärschlammmatrix erfolgte in einem nächsten Schritt. Das Verhalten von Diclofenac in Realproben zeigte allerdings deutliche Diskrepanzen, deren Ursachen nicht vollständig aufgeklärt werden konnten. Insgesamt stellt diese Arbeit die Entwicklung von LC-MS Methoden zur Analyse von Spurenstoffen in Klärschlamm und Biokohle dar. Die Untersuchung von Realproben ergab, dass die HTC geeignet ist, Spurenstoffe aus Klärschlamm zu reduzieren. Für Diclofenac konnte ein Reaktionsmechanismus postuliert werden. Damit verknüpft diese Arbeit die Entwicklung analytischer Methoden für komplexe Umweltproben mit der Bewertung neuartiger Behandlungsverfahren wie der HTC. Darauf aufbauend können weiterführende Studien das Verhalten anderer Stoffgruppen bei der HTC untersuchen oder die Anwendung der produzierten Biokohle in der Landwirtschaft bewerten.

Content

List of abbreviations

4-AA	4-Aminoantipyrine
AFWC	Analytical Forum Water Contaminants
AiF	Arbeitsgemeinschaft industrieller Forschungsvereinigungen, German Federation of Industrial Cooperative Research Associations
AOX	adsorbable organic halogens
CE	collision energy
CEP	collision cell entrance potential
CXP	collision cell exit potential
DIN	Deutsches Institut für Normung, German Institute for Standardization
DM	dry matter
DP	declustering potential
Dr. rer. nat.	Doctor rerum naturalium
EP	entrance potential
EPI	enhanced product ion scan
ESI	electrospray ionization
et al.	et alii or et aliae
FAH	Forschungsvereinigung der Arzneimittel-Hersteller
fig.	figure
GC-MS	gas chromatography - mass spectrometry
GmbH	Gesellschaft mit beschränkter Haftung
GmbH & Co.KG	Gesellschaft mit beschränkter Haftung & Compagnie Kommanditgesellschaft
HPLC	high performance liquid chromatography
HTC	hydrothermal carbonization
IDA	information dependent aquisition
IGF	Industrielle Gemeinschaftsforschung
IUTA	Institut für Energie- und Umwelttechnik e.V. (Institute of Energy and Environmental Technology)
JCGM	Joint Committee for Guides in Metrology
LC	liquid chromatography
LC-HRMS	high resolution mass spectrometry
LC-MS	liquid chromatography - mass spectrometry
LINEG	Linksniederrheinische Entwässerungs-Genossenschaft
LOD	limit of detection

LOQ	limit of quantification
MAA	4-Methylaminoantipyrine
MPFOA	Perfluoro-n[1,2,3,4-$^{13}C_4$] octanoic acid
MPFOS	sodium perfluoro-1-[1,2,3,4-$^{13}C_4$] octanesulfonate
MRM	multiple reaction monitoring
MS	mass spectrometry
MU	measurement uncertainty
m/z	mass-to-charge ratio
n.a.	not available
n.d.	not determined
NER	non-extractable residues
p.	page
PAH	polycyclic aromatic hydrocarbons
PCB	polychlorinated biphenyls
PCDD/F	polychlorinated dibenzodioxins and furans
PE	population equivalent
PFAC-MXA	mixture of perfluorinated compounds
PFBA	perfluorobutyric acid
PFBS	perfluorobutane sulfonate
PFC	perfluorinated compounds
PFDA	perfluorodecanoic acid
PFHpA	tridecafluoroheptanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane sulfonate
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
PFPA	perfluoropentanoic acid
PLE	pressurized liquid extraction
Prof.	Professor
QC	quality control
SD	standard deviation
S/N	signal-to-noise
SPE	solid phase extraction
Suppl.	supplementary material

TEQ	toxicity equivalent
TM	Trademark
TUHH	Technical University Hamburg-Harburg
UV	ultraviolet
vol	volume
WWTP	wastewater treatment plant
XIC	selected ion chromatogram

List of units and symbols

Δ	delta
°C	degree Celsius
cps	counts per second
Da	Dalton
€	Euro
eV	electron volt
g	gram
h	hour
k	factor for the advanced measurement uncertainty
k_d	sorption coefficient
kg	kilogram
kPa	kilopascal
L	litre
log	logarithm
M	mol
m_d	dry mass
m_w	wet mass
min	minute
mg	milligram
MJ	megajoule
mL	millilitre
µL	microlitre
mm	millimeter
µm	micrometer
ng	nanogram
pK_a	acid dissociation constant
ppm	parts per million
psi	pound-force per square inch
rpm	revolutions per minute
t	ton
υ	degree of freedom (student-t distribution)
V	volt

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1. Introduction

1.1 Preface

Increasing urbanization comes along with logistic challenges like the handling of waste, wastewater and sewage sludge. For a long time, sewage sludge was disposed in landfills, incinerated or applied to agricultural fields as a fertilizer.

Since most European countries prohibited disposal in the last decade, this option ceased. However, also the alternatives incineration and agricultural application entail drawbacks: The high water content of sewage sludge causes a low heating value. This hinders direct incineration. Therefore, the sludge has to be dried or co-incinerated, which results in a negative energy balance. Moreover, significant amounts of carbon dioxide emerge, while reusable resources like nutrients and carbon cannot be recycled. Additionally, the ashes still have to be disposed. Agricultural application of the sewage sludge can recycle the plant available carbon and nutrients. This contributes to the concept of sustainable nutrient management, which promotes the reuse of phosphorus sources. However, this pathway has become the target of public criticism due to sanitary and chemical aspects [1]. Sewage sludge is regarded as an important sink for heavy metals and organic pollutants. For example, presence of polycyclic aromatic hydrocarbons (PAH) in soils could be attributed to the agricultural sewage sludge application [2].

The drawbacks of the current handling options have raised the demand for treatment alternatives, which should remove the pollutants and biological risks from sewage sludge and preserve the valuable parts including the plant available carbon and nutrients.

Different options to post-treat the sludge have emerged in the last years. Besides new digestion techniques and post composting of the sewage sludge, thermal treatment methods have emerged like pyrolysis, low temperature pyrolysis, hot water extraction and hydrothermal carbonization (HTC) [3-5]. To that end, the process of HTC got into the focus of attention. Compared to other thermal processes the HTC requires a high water content of the feedstock, which fits well to sewage sludge. Additionally, its low activation energy enables the operator to run small plants cost-effective. The process has been introduced by Bergius [6] to simulate the natural diagenesis of coal. HTC converts organic material into a carbonized product within several hours (h) at elevated temperature of 190 - 250 °C and elevated pressure of 15 - 20 bar. Increasing relevance of the topic can be derived from the number of publications found in the online portal ISI Web of Science with the phrase “hydrothermal carbonization” in the last years shown in figure (fig.) 1.1. Their increasing number demonstrates a progressive interest and engagement in the HTC process technology since 2010. However, research groups investigating the HTC process with sewage sludge are still rare, although their number increased in the last years as well.

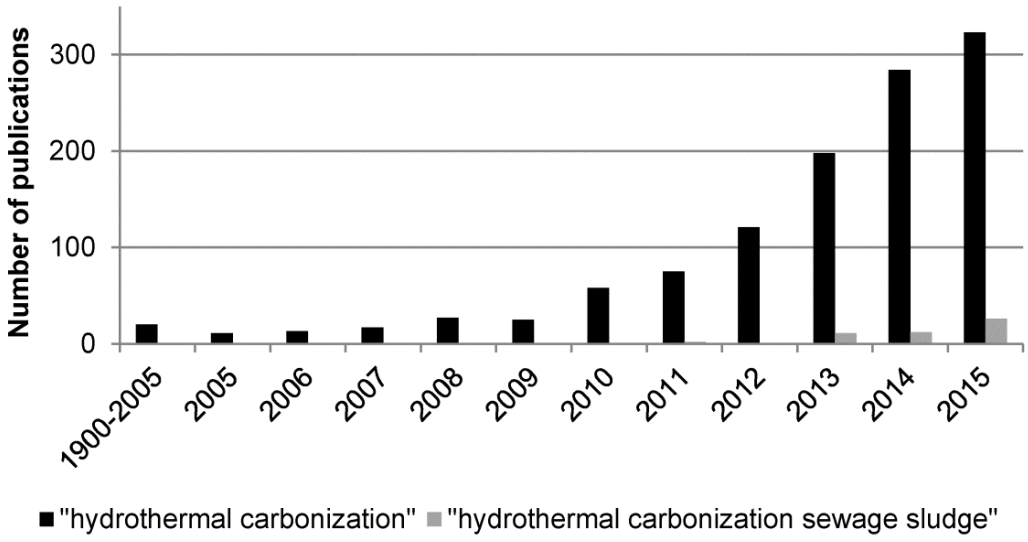

Fig. 1.1 Number of publications with the key phrases "hydrothermal carbonization" and "hydrothermal carbonization sewage sludge found on the online portal ISI Web of Science

The HTC produces a kind of biochar, which is also referred to as hydrochar [7]. It differs from natural biochars which emerges from a slow natural process. Product properties significantly differ from other thermochemical conversion processes [8]. Studies have attributed good fertilizing properties to biochar from HTC including a high nutrient content and low contaminant levels. In fact, the potential to conserve nutrients has been described by Libra et al. [9]. Additionally, the plant available water capacity can be increased by applying biochar from HTC [10]. Weiner et al. [11] have shown that selected compounds degrade during HTC in the presence of a defined reaction media. Complex reaction media like sewage sludge have not been regarded yet.

Alternatively, HTC has been described as a valuable pretreatment technology for subsequent incineration, because it increases the heating value and the dewaterability of sewage sludge [12, 13].

The HTC process is based on carbon and water. Hence, HTC is well suited even for material with low energy and high water content like sewage sludge [14]. The reaction mechanism has been investigated in detail by Funke et al. [15] for the substrates saccharin, lignin and cellulose. Reaction follows several steps: First, the water initiates that the macromolecules hydrolyse at elevated temperature. Then, chemical and physical dehydration occur as the main reaction, which dewater and carbonize the sewage sludge. The carbonization of biomass lowers the H/C and O/C ratios, which is shown in the Van Krevelen diagram in fig. 1.2 [16]. The figure shows that the heating value increases from wood via lignin through to coals. HTC products can be typically found close to natural coal depending on source material and process parameters [9, 17].

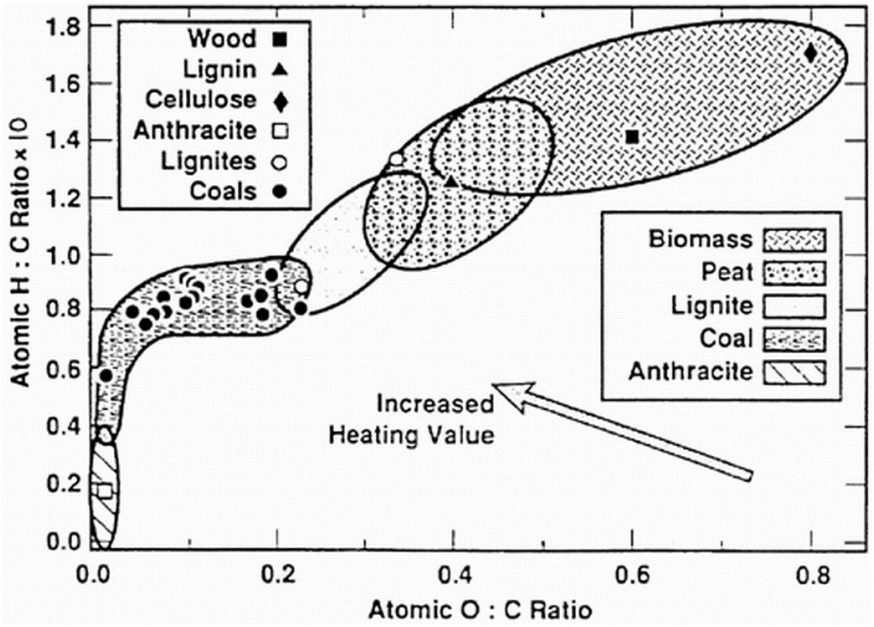

Fig. 1.2 Van Krevelen diagram showing the H/C and O/C ratios for different types of energy sources [16]

Besides the main reactions, HTC also decarboxylates present carboxyl groups. For cellulose and lignite distinct decarboxylation to dehydration ratios have been determined. Other side reactions are polymerization and aromatization. This leads to an increase of aromatic structures [15, 17].
All steps of the process are influenced by variable process parameters, to which temperature, pH, pressure, reaction time and the solid-water ratio belong. A typical HTC might take four h at 210 °C at a pressure of 15 bar. The temperature is the most crucial process parameter. Hydrothermal reaction starts at around 180 °C. Increasing the temperature or extending the heating period accelerates the carbonization and enhances the coal yield. Pressure increases up to 15 - 20 bar in that time while the pH value drops due to formed organic acids [9, 15]. The ratio of biomass to water also influences the reaction. Carbonization only proceeds with biomass enclosed by water. Higher water ratios accelerate the process. However, the ratio of biomass should be high enough to ensure that a solid product results from the process. A higher biomass ratio shifts the reaction towards polymeric structures [15]. Detailed process flow also depends on the carbon source. For example, sources with high fat content like sewage sludge result in lower carbonization than pure sucrose solutions. Feedstock properties influence the carbon and energy content of the product [18].
During HTC, solid, liquid and gaseous products emerge. The solid biochar has been characterized in detail [17, 19]. Its properties mainly depend on the feedstock. About 25% of the biomass is solved in the liquid phase after HTC. Liquid products like organic acids and phenolic compounds accumulate in the process water [20]. During HTC also gaseous products are released: mostly carbon dioxide and in lower amounts methane, hydrogen, carbon monoxide and hydrocarbons.

Although the main mechanisms of transformation during HTC have been revealed for sample substrates, complex matrices might complicate the process. Sewage sludge represents such a complex matrix consisting of a mixture of carbohydrates, proteins, greases, metals, macro- and micropollutants. Detailed mechanisms of these mixtures during HTC still appear like a black box. Basically, increased temperature and pressure splits high molecular weight compounds into smaller ones. This trend can be visualized using liquid chromatography (LC) coupled with a mass spectrometer (MS). Fig. 1.3 compares cloud diagrams of a sewage sludge and a biochar sample in a linear water-acetonitrile gradient run with a scan range of the mass-to-charge ratio (*m/z)* of 50 - 500 in positive electrospray ionization (ESI) mode. The two most intense data points are displayed for each scan.

The cloud diagram of biochar produced by HTC differs significantly from the source sewage sludge material. The sewage sludge diagram shows several peaks within the gradient run. Peaks are distributed evenly over the whole examined mass range throughout the runtime of twenty minutes (min). The fraction of higher mass molecules tends to increase with increasing acetonitrile proportion. The picture represents the complexity of the sewage sludge material which consists of a great variety of different molecular structures.

Contrary, the biochar signals accumulate in a mass range below *m/z* = 250 over the whole runtime. This indicates a higher faction of small analytes compared to the sewage sludge. Molecules with *m/z* above 250 are not visible in biochar because the lower weight molecules exceed them in signal intensity.

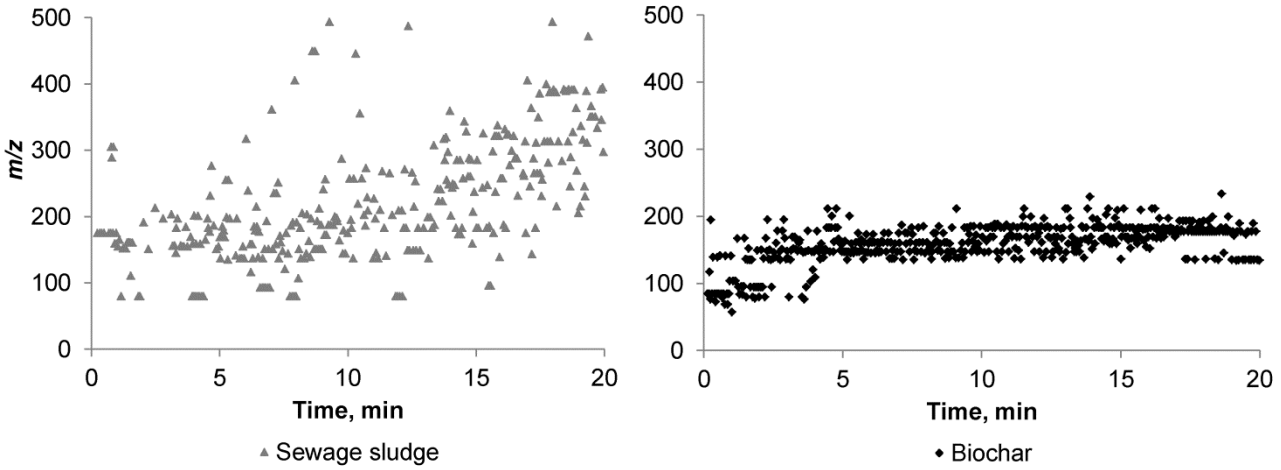

Fig. 1.3 Cloud diagram of sewage sludge (grey triangles) and biochar (black squares), LC-MS scan of *m/z* = 50 - 500, positive ionization mode

Increasing polarity and number of low molecular weight molecules generated by HTC of sewage sludge have also been reported by other groups and analytical techniques. Quitain et al. characterized the potential to produce low molecular weight carboxylic acids from hydrothermal treatment of waste using ion chromatography [21]. Decomposition of aspartic acid to organic acids was followed by Faisal et al. using spectroscopic and electroconductive methods [22]. Products from HTC of olive mill wastewater have been followed with gas chromatography coupled to mass spectrometry (GC-MS) by Poerschmann et al. [20].

Research has been focussed either on general structure changes or on the whereabouts of nutrients like phosphorus or nitrogen. Distinct chemicals have hardly been considered so far.

In pre-investigations of this study we applied existing methods to determine micropollutants in sewage sludge and its corresponding biochar after HTC. The results are summarized in table 1.1, whereby the load of the micropollutants is usually referred to the dry matter (DM) of the sample. Threshold limits are prescribed ty the German sludge ordinance for PAH. For polychlorinated biphenyls (PCB) and polychlorinated dibenzodioxins and furans (PCDD/F), which are presented in ng toxicity equivalents (TEQ) no thresholds have been set by law for biochar. However the International Bicohar Initiative suggests thresholds for these compound classes.

Table 1.1 displays similar values in sewage sludge and biochar for PAH and PCB indicating that these compounds are not affected by HTC. Considering PCDD/F, a significant increase by factor five occurred during HTC. The same trend was described by Tirler et al., who also reported an increase by a factor five with similar process settings [25]. Reza et al. [26] investigated the fate of inorganics and concluded that the HTC can remove the major part of heavy metals from the biomass.

Table 1.1 Pre-investigations of micropollutant load in sewage sludge and biochar from hydrothermal carbonization (HTC) of sewage sludge. HTC was carried out for four hours at 210 °C and 15 bar with sewage sludge from the wastewater treatment plant Hollenstedt, Germany

	Unit	Concentration in sewage sludge	Concentration in biochar	Threshold limit values
Polycyclic aromatic hydrocarbons (PAH)	mg/kg_{DM}	2.02	3.30	20*
Polychlorinated biphenyls (PCB)	mg/kg_{DM}	0.02	0.03	0.20**
Polychlorinated dibenzo-dioxins and furans (PCDD/F)	$ng\ TEQ/kg_{DM}$	3.35 ± 2.3	18.7 ± 1.7	100**

* draft version for biochar specification guidelines, International Biochar Initiative [23]

** German sewage sludge ordinance [24]

Based on the pre-investigations, micropollutants like pharmaceuticals represent a topic of exceptional interest with regard to sewage sludge, because they are one reason to bring forward the prohibition of agricultural sewage sludge application. Also persistent compounds like perfluorinated compounds (PFC) gain attention as they might accumulate in soil bodies or enter adjacent water bodies. Assessing the occurrence of pharmaceuticals and PFC in biochar and investigating their fate during HTC could help to evaluate the biochar product quality. Therefore, methods are required to quantify their occurrence in sewage sludge and biochar after HTC. Liquid chromatography - mass spectrometry (LC-MS) provides a sensitive and selective tool to analyse these micropollutants. However, the matrix effects of the produced biochar have to be taken into account as they might interfere the measurement considerably. Appropriate sample preparation methods and quantification strategies are required.

1.2 Objectives and scope of this thesis

This thesis is based on the previous fundamental HTC research with sewage sludge as source material. Methods are required enabling analysis of selected compounds in sewage sludge and its HTC product biochar. Therefore, chapter 2 starts with LC-MS/MS method development to analyse selected pharmaceuticals in sewage sludge and in biochar obtained from HTC of sewage sludge using LC-MS/MS. Based on the compound choice, analytical challenges like matrix effects are defined and examined. Comparison of different quantification strategies contributes to the research field of analysing complex environmental samples. Finally, a valid and robust method to determine twelve pharmaceuticals in sewage sludge and the resulting biochar is presented in chapter 2.
Thus, the study helps to assess the biochar product quality, which could influence the handling of sewage sludge. In dependence of the results the HTC might produce a material, which is applicable in agriculture regarding its pollutant content. Otherwise, results might promote the further treatment of sewage sludge via incineration. In this context, chapter 3 comprises the application of the developed LC-MS/MS method. HTC is investigated regarding its ability to remove pharmaceuticals from sewage sludge. Removal is determined in experiments with spiked inert material and in native sewage sludge samples. The chapter also discusses the impact of the sewage sludge source as well as the reproducibility of results. These results give answers to the following essential issues: Is HTC suitable to reduce micropollutants from sewage sludge? How critical is the agricultural application of biochar from HTC of sewage sludge? Can we apply HTC biochar onto fields without any concern?
Besides these application-oriented questions, mechanistic investigations provide the possibility to extent the understanding of the HTC process. Especially in complex matrices like sewage sludge, the single process steps have poorly been examined. Assessing transformation products of

selected compounds allows conclusions about reactions and reaction mechanisms. Chapter 4 illuminates the HTC process more detailed with the pharmaceutical agent diclofenac. Diclofenac behavior is investigated during HTC with sand as filling material. The conversion process is followed using non-target LC-MS analyses. Aided by the obtained results fate of diclofenac is also investigated in native sewage sludge and biochar samples. Besides the mechanistic investigations, the general transferability of compound behavior in different sample matrices is considered with sand, spiked sewage sludge and native sewage sludge. These results contribute to the topic of experimental design by addressing the question whether model experiments are comparable with real-world conditions and how far they can reduce the experimental effort.

Process evaluation should not only focus on one group of compounds. Therefore, the perfluorinated compounds are included into the compound spectrum. Chapter 5 presents the LC-MS/MS method development for 10 PFC. In fact, existing LC-MS/MS method for sewage sludge is transferred to biochar analysis. Therewith PFC behavior is followed in inert experiments and in HTC of native sewage sludge. These results extend our knowledge about the biochar product and the process flow.

Chapter 6 summarizes the results of chapter 2 to chapter 5 and gives some general conclusions regarding the contribution of this thesis to the present state of scientific knowledge. The chapter also reveals still open questions based on this thesis and the ongoing research regarding the HTC of sewage sludge. Potential further research topics like the extension of analytical methods or the handling of process water are discussed.

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2. Determination of pharmaceuticals in sewage sludge and biochar from hydrothermal carbonization using different quantification approaches and matrix effect studies

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2.1 Abstract

Producing valuable biochar from waste materials using thermal processes like HTC has gained attention in recent years. However, the fate of micropollutants present in these waste sources have been neglected, although they might entail the risk of environmental pollution. Thus, an HPLC-MS/MS method was developed for 12 pharmaceuticals to determine the micropollutant load of biochar, which was made from sewage sludge via HTC within four h at 210 °C. Pressurized liquid extraction was applied to extract the compounds. Because of the high load of co-extracted matter, matrix effects in HPLC-MS/MS were investigated using matrix effect profiles. Interfering compounds suppressed 50% of the phenazone signal in sewage sludge and 70% in biochar, for example.

The quantification approaches external calibration, internal standard analysis and standard addition were compared considering recovery rate, standard deviation (SD) and measurement uncertainties. The external analysis resulted in decreased or enhanced recovery rates. Spiking before LC-MS/MS compensated instrumental matrix effects. Still, recovery rates remained below 70% for most compounds because this approach neglects sample losses during the extraction. Internal standards compensated the matrix effects sufficiently for up to five compounds. The standard addition over the whole procedure proved to compensate for the matrix effects for 11 compounds and achieved recovery rates between 85% and 125%. Additionally, results showed good reproducibility and validity. Only sulfamethoxazole recovery rate remained below 70% in sewage sludge.

Real sample analysis showed that three pharmaceuticals were detected in the biochar, while the corresponding sewage sludge source contained eight of the investigated compounds.

2.2 Keywords

Hydrochar • Infusion chromatogram • Internal standard • Organic pollutants • Pressurized liquid extraction • Standard addition

2.3 Introduction

Fertilizer of natural origin has gained importance in the course of recent legislative decisions. Upcoming claims for a sustainable phosphorus use prefer the recycling of natural matter like sewage sludge or biochar over synthetic fertilizers [1]. In Germany, 0.57 million tons of sewage sludge were used in agriculture in 2011. Due to lowered threshold values for organic and inorganic contaminants, the application has decreased in the past years [2]. Synthetic fertilizers replaced the sewage sludge [1]. This trend opposes the sustainable use of natural matter.

HTC promises to improve the sewage sludge quality according to legislative requirements. Thereby, wet waste is converted to biochar at high temperature (190 - 250 °C) and elevated pressure within a few h. In contrast to other handling methods, the HTC directly converts materials with a high water ratio like sewage sludge without a pre-drying step, which represents a cost-effective alternative to recycle sewage sludge and promotes the application of natural fertilizers. Bergius [3] introduced the HTC process in 1913 to simulate natural diagenesis of coal. The process is initiated by hydrolysis at elevated temperature. Then, the organic matter can dehydrate, decarboxylate and loose its functional groups. Aromatization and polyreactions mainly occur during the end of the process. Funke and Ziegler [4] elucidated reaction pathways for model compounds. Recently, first results on the behavior of organic compounds during HTC have been published by Weiner et al. [5]. These model studies, however, were conducted with compound concentrations far above environmental levels in presence of sucrose. Results have not been transferred to lower concentrations and other input materials. Therefore, the behavior of contaminants in complex matrices during HTC remains largely unknown.

Although no compulsory system has been established to classify the biochar, the end-user is obliged to avoid contaminated soils. Previous studies showed that sewage sludge serves as a sink for compound classes like PAH, polyfluorinated compounds and pharmaceuticals [6-10], therefore micropollutants should be considered. In particular, pharmaceuticals deserve attention because they might exhibit a toxic potential. Adverse effects to fish and invertebrates have been reported [11].

Research groups highlighted the challenge of quantifying micropollutants in complex environmental samples. The steps of sampling, pretreatment and measurement have been investigated and deserve regard [12-14]. Nevertheless, the target compounds might strongly sorb to the matrix impeding a complete extraction.

High performance liquid chromatography coupled to mass spectrometry (HPLC-MS/MS) in multiple reaction monitoring (MRM) mode excels as a selective and sensitive method to quantify trace compounds in complex samples [15]. However, even this method suffers from matrix effects. In the ion source, the matrix can hinder or accelerate the ionization of the target compounds, which can result in enhanced or reduced signals. The extent of matrix effects depends on sample

composition, analyte and character of co-eluting compounds [13]. However, mechanistic details are not entirely understood. Approaches to investigate matrix effects are manifold. Matrix effect profiles, for example, can visualize matrix effects over a whole chromatographic run by comparing analyte signals, which are injected post-column, of a blank and a matrix-rich sample [16].

The handling of matrix effects is diverse. On the one hand, effects are minimized by separating interfering compounds during sample preparation, for example via solid phase extraction (SPE), microextraction or size exclusion chromatography [14,17,18]. However, this is restricted to a narrow compound range and might fail for multi-methods. Sample dilution also reduces matrix effects, but this procedure accepts a decreased sensitivity [19]. On the other hand, matrix effects can be compensated. The established approaches are the time-consuming standard addition method or the cost-intensive approach with isotopically labeled internal standards [20]. None of the approaches compensating matrix effects can consider natural aging effects, which limits the comparability between spiked and native samples [18]. The expected impact of aging is irreversible adsorption. However, the process is not entirely understood. Degradation might also contribute to aging effects, especially in the biologically active matrix of sewage sludge.

This study investigated matrix effects in sewage sludge and biochar samples using matrix effect profiles to visualize the impact of interfering compounds in HPLC-MS/MS. Based on these studies the different quantification approaches external calibration, standard addition and the use of internal standards were tested to establish a robust method applicable to determine micropollutants in sewage sludge and biochar from HTC using HPLC-MS/MS. We selected 12 pharmaceuticals of several compound classes as target analytes in order to represent a wide range of micropollutants occurring in sewage sludge. Analyses included two analgesics (diclofenac, phenazone), an antirheumatic agent (ibuprofen), an antiepileptic drug (carbamazepine), four antibiotics (sulfamethoxazole, clarithromycin, roxithromycin and erythromycin), two fibrates (bezafibrate and fenofibric acid) and two beta blockers (metoprolol and propranolol).

2.4 Materials and methods

2.4.1 Chemicals

Water for LC-MS, acetonitrile and methanol were achieved from Th. Geyer GmbH & Co. KG (Renningen, Germany). CPS GmbH (Aachen, Germany) supplied cyclophosphamide-d6. Diclofenac-d4, metoprolol-d7 and carbamazepine-d10 were received from CDN isotopes Inc. (Quebec, Canada). Sigma-Aldrich (Taufkirchen, Germany) delivered all other chemicals in highest available degree of purity. Linksniederrheinische Entwässerungs-Genossenschaft (LINEG) provided the sewage sludge from the wastewater treatment plant (WWTP) Rheinhausen. Fisher

Scientific GmbH (Schwerte, Germany) supplied extra pure 20 - 30 mesh sand from Ottawa. Aluminium oxide was obtained from Merck GmbH (Darmstadt, Germany) and ASE Prep DE diatomaceous earth was purchased from Thermo Fisher GmbH (Idstein, Germany).
We prepared stock solutions by weighing 10.00 mg into a volumetric flask and filling it up to 10 mL with ULC/MS grade water/acetonitrile (50/50) to achieve a final concentration of 1.00 mg/mL and stored them for maximal three months at 4 °C. Standards were prepared freshly for each experiment.

2.4.2 HTC experiments

The dry matter content of the sewage sludge was determined by gravimetric after drying at 105 °C to adjust the sludge to 20%$_{DM}$ using ULC/MS grade water. One hundred g of the adjusted sludge was weighed into a laboratory scale limbo high pressure reactor system (Büchi Glas, Uster, Switzerland). While the reactor content was continuously stirred at 500 rpm, experiments proceeded at 210 °C. After keeping the final temperature for four h, the reactor was cooled to room temperature. The bls 2.5 software (Büchi Glas, Uster, Switzerland) recorded reactor temperature, jacket temperature, heating and cooling power, stirring rate and pressure throughout the experiments.

2.4.3 Sample preparation

The reactor content was lyophilized using a freeze-dryer beta 1-16 LDG 2-m system (Martin Christ Gefriertocknungsanlagen GmbH, Osterode, Germany). Pressurized liquid extraction (PLE) with a Dionex ASE 200 system (Thermo Fisher GmbH, Idstein, Germany) was applied to extract the analytes. In fact, 11-mL stainless steel cartridges were prepared with a cellulose filter and a layer of Ottawa Sand (extra pure, 20 - 30 mesh, Fisher Scientific GmbH, Idstein, Germany). Then, 1.00-g sample aliquots were weighted into the cells. Another sand layer and a cellulose filter covered the samples. Methanol served as extraction solvent with the following operating parameters: preheating period 5 min, heating period 5 min, static period 15 min at 100 °C and 100 bar, solvent flush 10% volume and 150 s nitrogen purge. The procedure resulted in a final sample volume of 11 mL, which were evaporated under a gentle nitrogen stream at 50 °C, redissolved in 5 mL water and filtered with 0.45 µm regenerated cellulose syringe filters (Chromafil RC-45/15MS, Machery-Nagel, Düren, Germany).
For standard addition over the whole procedure, appropriate standard amounts were added to the samples prior to the PLE resulting in calibration levels between 10 and 5,120 ng/g$_{DM}$ (2 - 2,024 ng/mL). Data analysis followed three steps using at least five sample aliquots: first, the native sample was checked for an analyte peak. If no peak occurred, the detection limit (LOD)

resulted from the lowest observable standard. If a peak occurred, the calibration range was set to the instrumental linear range of the analyte in a second step. Third, the preselected standards were checked for appropriate accuracy between 70% and 130%.

Standard addition before LC-MS/MS was prepared by resolving the evaporated PLE samples in 2.5 mL water. After filtration, 0.5-mL aliquots were mixed with 0.5 mL appropriate standards to prepare levels ranging from 2 to 1,024 ng/mL.

The external calibration ranged from 2 to 1,024 ng/mL by diluting the stock solution with water.

Internal standards were added to the samples before PLE at a level of 200 ng/g_{DM}.

Quality control samples were prepared by spiking the inert PLE filling material with the analytes to achieve a final concentration of 200 ng in the PLE cartridge.

2.4.4 HPLC-MS/MS analysis

HPLC-MS/MS analysis was conducted with a LC 20 HPLC system consisting of a CBM-20 A communication bus module, a DGU-20 A3 degasser, LC-20 AD pumps, a SIL-20 AC autosampler and a CTO-20 AC column oven (Shimadzu, Duisburg, Germany) coupled to a 3200 QTRAP system (AB Sciex, Darmstadt, Germany). Analyst™ Software 1.5.2 (AB Sciex, Darmstadt, Germany) was used for data analysis. The eluents water (A) and methanol (B) containing 0.1% formic acid, respectively, were applied for chromatographic separation of 20-µL sample aliquots at a flow rate of 0.3 mL min^{-1} on a Waters Atlantis T3 column (100 x 2.1 mm, 3 µm, Waters GmbH, Eschborn, Germany) at 45 °C. The gradient started with 5% B and increased to 27% B within 0.7 min, further increased to 55% after 8 min and kept constant up to 10.5 min. Then, the gradient increased to 100% B after 13.2 min. After 20 min the column was rinsed and re-equilibrated again. All analytes were quantified in MRM mode with the most intense transitions listed in table 2.1. A second MRM transition verifying the compound is additionally indicated as well as compound-specific parameters. Erythromycin was measured as dehydrato-erythromycin. Preceding experiments revealed optimized settings for curtain gas (11 psi), ion source temperature (520 °C), ion source gases (63 and 68 psi), positive electrospray ionization (5,000 V) and collision cell exit potential (4 V).

Table 2.1 Compound-specific quantification parameters during the HPLC-MS/MS measurement

Compound	Quantification transition	Verification transition	Declustering potential	Entrance potenial	Collision cell entrance potential	Collision energy
	m/z	*m/z*	V	V	V	eV
Diclofenac	296 → 214	296 → 250	31	4.0	22	43
Ibuprofen	207 → 161	207 → 119	16	6.0	14	17
Phenazone	189 → 56	189 → 77	51	10	12	45
Carbamazepine	237 → 194	237 → 165	46	5.0	14	27
Sulfamethoxazole	254 → 156	254 → 92	36	5.0	18	21
Bezafibrate	362 → 139	362 → 121	36	4.5	18	33
Fenofibric acid	319 → 139	319 → 233	46	4.0	16	41
Metoprolol	268 → 72	268 → 56	51	5.0	16	33
Propranolol	260 → 116	260 → 56	46	4.0	14	23
Clarithromycin	749 → 83	749 → 158	51	8.5	32	65
Roxithromycin	838 → 158	838 → 83	56	8.0	34	47
Dehydrato-erythromycin	716 → 158	716 → 83	41	6.5	34	41
Diclofenac-d4	300 → 218	-	26	8.5	16	41
Ibuprofen-d3	210 → 164	-	16	8.0	12	19
Cyclophosphamide-d6	276 → 140	-	61	9.5	18	31
Erythromycin-$^{13}C_1$-d3	738 → 162	-	36	6.5	62	39
Carbamazepine-d10	247 → 204	-	20	10	16	30
Sulfamethoxazole-d4	258 → 160	-	20	10	16	30
Metoprolol-d7	275 → 79	-	20	10	17	30

The LOD was calculated at a signal-to-noise (S/N) ratio of 3 and the limit of quantification (LOQ) at a S/N ratio of ten. Instrumental LOD were determined in triplicate. Sewage sludge and biochar limits resulted from $n = 6$ to consider the lower homogeneity of samples. A list of the analytes and their corresponding internal standard is given in the suppl. 7.1. Recovery rates were considered good in a range between 70% and 130%.

The recording of matrix effect profiles followed the concept of Stahnke et al. [16]. In short, a syringe pump injects a constant analyte flux into the MS via a *t*-piece. Simultaneously, a sample is injected via the HPLC, separated on the column and conducted to the MS. This set-up allows observing enhanced or suppressed ionization during the whole run.

2.5 Results and discussion

2.5.1 Sample preparation

PLE parameters were based on existing methods to determine pharmaceuticals in sewage sludge [21-24] and transferred to biochar samples. Mixtures with high water content entail the risk of clogged cells [25]. Therefore methanol served as solvent and obtained good recovery rates (88 - 108%) in pre-experiments. Sand was chosen for filling the PLE cartridges, because Runnqvist et al. emphasized that the filling material should be inert [26].

A static extraction of 15 min has been described as sufficient to approach partition equilibrium [21]. Therefore one 15 min cycle was applied. Pre-experiments with a second PLE cycle could not increase the recovery rates significantly. Comparable results were obtained in a similar study by Göbel el al. [22]. However, they decided to perform three cycles to assure exhaustive extraction. In contrast, we decided to remain at one cycle because the slightly improved recovery rates could not compensate the higher amount of co-eluting matrix in the second cycle.

As recovery rates in pre-experiments did not correlate to the flush volume, the cell flush was set to the minimum of 10%.

A clean-up step can follow after the PLE to separate the target compounds from the coeluted matrix. For sewage sludge methods SPE and size exclusion have obtained good results [8,17]. However, pre-experiments with SPE and nanofiltration in biochar showed a limited transferability. The appendix (suppl. 7.2) entails detailed results of the tested clean-up procedures.

2.5.2 Matrix effects

Injecting complex samples into the HPLC-MS without clean-up requires detailed consideration of possible effects in the instrument. In the ion source, interfering compounds can cause enhanced or suppressed signals leading to misinterpreted results. Therefore, the complex matrices of sewage sludge and biochar were considered by matrix effect profiles, which visualize the ionization efficiency of the target compounds. Fig. 2.1 exemplarily illustrates a matrix effect profile for phenazone. Matrix effect profiles of the other compounds are included in the appendix (suppl. 7.3).

The matrix effect profile in fig. 2 1 compares sewage sludge and biochar in the chromatographic run. The reference line, which is normalized, results from injecting water, which gives a stable signal during the increasing gradient indicating a consistent ionization. The y-axis indicates if the signal is enhanced or suppressed, for example: a doubled signal appears at a height of 100%, while -100% indicates that the analyte is fully suppressed.

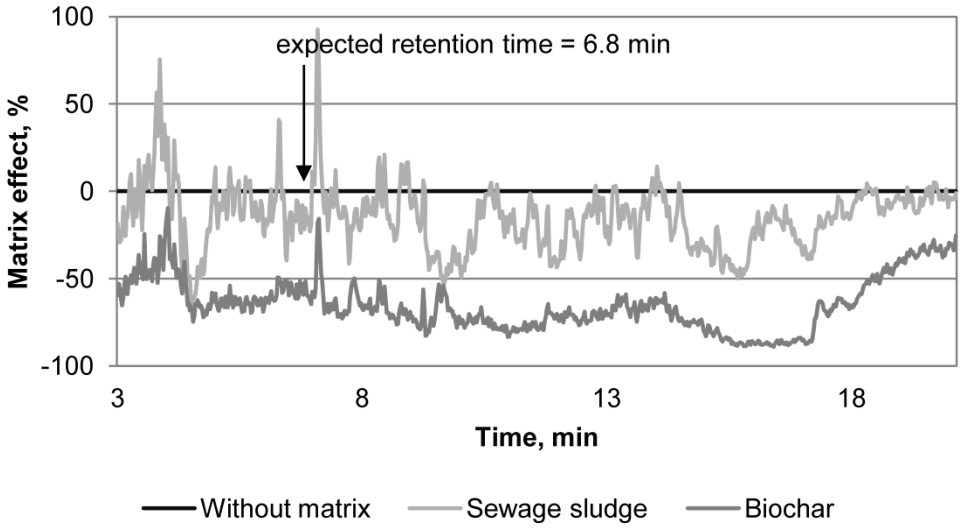

Fig. 2.1 Matrix effect profile of phenazone (selected ion chromatogram, XIC, *m/z* = 189 → 56) with water as the reference line. The bright grey line indicates signal suppression and enhancement for sewage sludge and the dark grey line reflects signal suppression in the measurement of biochar

In sewage sludge samples, co-eluting substances suppress the phenazone peak to some extent nearby the expected retention time at 6.8 min. The effect profile is considerably unstable over the whole run. Therefore, no improvement can be expected from modifying the chromatographic method. Small changes in sample composition or small retention time shifts entail the risk of great impacts on the ionization efficiency. In biochar, signal is reduced to great extent compared to pure water, while it remains more stable during the run than in sewage sludge. Stahnke et al. demonstrated the relationship of ionization efficiency and different plant materials when analyzing pesticides in fruits and vegetables [16]. Although their study revealed recovery rates below 50%, none of the effect profiles demonstrated as severe signal suppression over the whole run as biochar. This confirms the challenge to transfer analytical methods from sewage sludge to biochar. Stahnke et al. concluded that matrix effects differ more strongly between matrices than between analytes [16], which could be confirmed in this study. In both materials general similarities were observed. In biochar, for example, strongest suppression for all investigated compounds occurred at around min 15, while the sewage sludge matrix effect chromatograms often showed an enhancement at around min 7. Despite this, characteristic differences were obvious for some compound classes. The pattern of the antibiotics clarithromycin, roxithromycin and erythromycin showed a double peak between min 17 and 18. Also ibuprofen and sulfamethoxazole patterns clearly differed from the other compound classes. However, the analgesics, the antiepileptic drug, the fibrates and the beta blockers can be summarized. For these compound classes one representative matrix effect profile would be enough to evaluate their signal suppression and enhancement.

Considering the great impact of small retention time shifts on signal intensity, which is visible in fig. 2.1, the analytes were checked for varying retention time in matrix-free samples, sewage sludge and biochar samples. The shifts in the quality control and biochar samples remained below 1.0% within one series. In sewage sludge only propranolol (2.1%) exceeded the 1.0% limit. Regarding the differences between the three sample types, slight retention time shifts varying from 1.8% (ibuprofen) up to 7.9% (erythromycin dehydrate) were observed. Inferred from these results, real samples should be carefully considered with regard to false positive results caused by ghost peaks eluting at a similar retention time.

2.5.3 Method validation

Fig. 2.2 compares the different quantification approaches to determine the twelve pharmaceuticals in sewage sludge and biochar. The recovery rates result from subtracting the native amount of the samples from the concentrations found for the spiked samples.

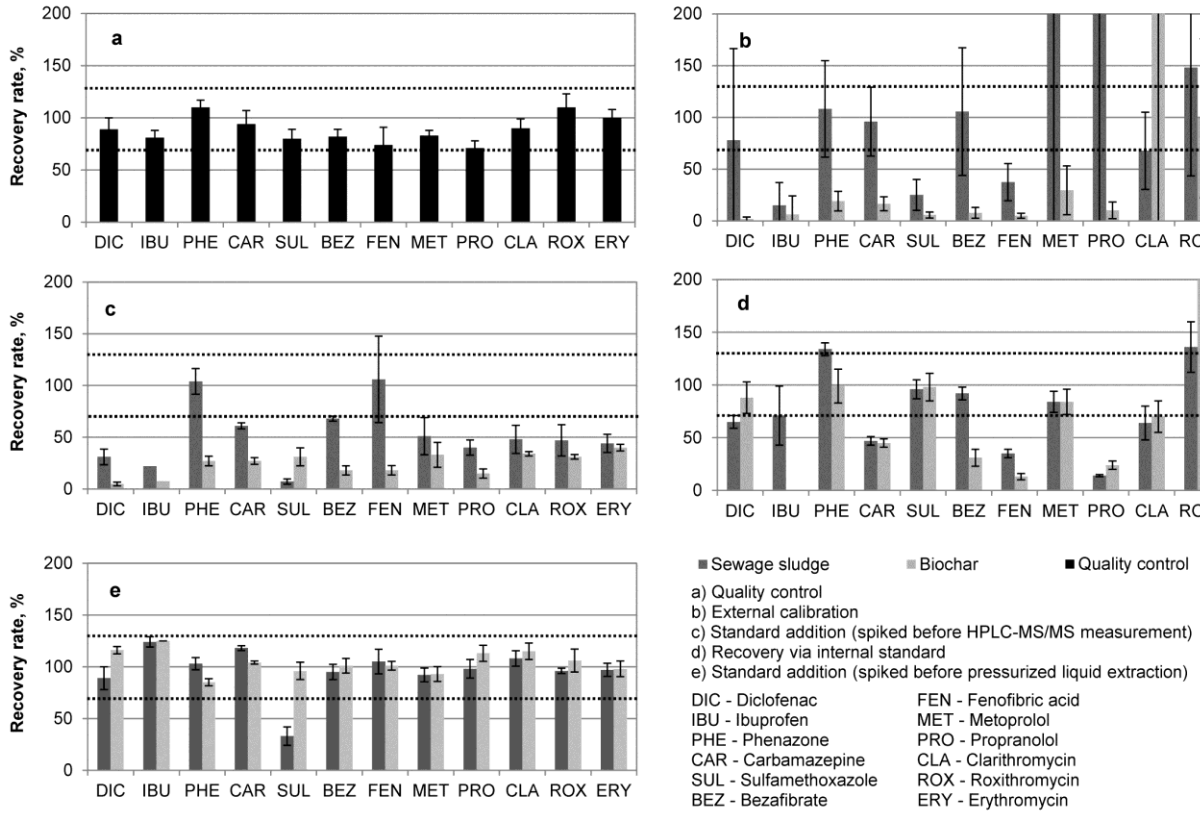

Fig. 2.2 Comparison of different quantification approaches to determine the recovery rates of selected pharmaceuticals in sewage sludge and biochar, for a) n = 4, for b) - e) n = 3

Fig. 2.2 a presents the recovery rates for quality control samples without matrix. The investigated pharmaceuticals achieved recovery rates of 71 - 110%. Hence, none of the analytes degrades thermally during the PLE. The SD ranged from 5 to 17%.

Fig. 2.2 b displays the recovery rates for sewage sludge and biochar obtained by use of external calibration, whereby neither losses during sample preparation nor matrix effects during mass spectrometric analysis were considered. Deficiency of this approach has already been emphasized by Göbel et al., who reported reduced absolute recovery rates for sulfonamides and macrolides in activated sludge, although samples were cleaned-up by SPE [22]. Additionally, Ternes et al. stated reduced recovery rates of 25 - 78% after SPE without correcting for sample losses in the analysis of acidic and neutral pharmaceuticals in activated and digested sludge [23]. In the present study, the results cover a wide range of reduced recovery rates as for ibuprofen (26%) and sulfamethoxazole (25%) in sewage sludge and diclofenac (1.9%) and fenofibric acid (5.0%) in biochar as well as enhanced recovery rates, for example for metoprolol (215%) and propranolol (257%) in sewage sludge and clarithromycin (253%) in biochar. Moreover, SD, which is above 30% for all compounds and sometimes exceeds 100% in both matrices, indicates low reproducibility. Nevertheless, good recovery rates were obtained for the five compounds diclofenac (78%), phenazone (108%), carbamazepine (96%), bezafibrate (106%) and erythromycin (108%) in sewage sludge. In biochar, only the average recovery rate of 99% for roxithromycin appears to be good. However, also the compounds with acceptable recovery rates show high SD of up to more than 100%. Therefore the approach of external calibration proved its deficiency in determining pharmaceuticals in sewage sludge and biochar.

The recovery rates resulting from analysis via standard addition before HPLC-MS/MS are shown in fig. 2.2 c. This approach considers instrumental matrix effects. However, losses during sample preparation are neglected. In sewage sludge, recovery rates of around 50% are achieved for most of the analytes. Ibuprofen (22%) and sulfamethoxazole (7.2%) deviated to lower values. Full recovery was obtained for phenazone (104%) and fenofibric acid (106%). Biochar results showed reduced recovery rates for the pharmaceuticals varying from 4.9% (diclofenac) to 40% (erythromycin), which suggests an incomplete extraction. Ternes et al. also assigned decreased recovery rates in sewage sludge to the extraction step [23]. Ding et al. and Radjenovic et al. could attribute reduced recovery rates in sewage sludge to the sample preparation in general [21,24]. In contrast to the use of external calibration no upward outlier occurs indicating that instrumental effects caused the enhanced signals. Furthermore, SD shows that the results obtained by standard addition before LC-MS/MS are reproducible. Nevertheless, this approach requires the use of a correction factor to consider the reduced recovery rates, which might become a problem, when the sample composition varies.

Fig. 2.2 d displays the recovery rates obtained from application of isotopic labelled internal standards, which represents a method to compensate matrix effects [22-24]. In sewage sludge the

use of internal standards resulted in good recovery rates of 71%, 96%, 92% and 84% for ibuprofen, sulfamethoxazole, bezafibrate and metoprolol, respectively. The other eight compounds remained out of the accepted recovery range between 70 and 130%, while diclofenac (65%), phenazone (134%), clarithromycin (64%) and roxithromycin (136%) were close to this range. Radjenovic et al. observed similar results with the internal standard analysis of trimethoprim, which failed to compensate suppressed signals in sewage sludge [24]. Also Wang et al. reported that matrix effects are not always entirely compensated by internal standards [27]. The results of insufficient comparability between internal standard and analyte might be explained by the high amount of matrix in the samples in consequence of skipping a clean-up procedure. In fact, matrix effects are evident on every MRM transition. Because of the different MRM transitions of compound and its internal standard the matrix effects might differ as well. This is exemplarily shown for metoprolol in Fig. 2.3. While interferences affect the whole extracted ion chromatogram *m/z* 268 → 72 for metoprolol, the mass trace *m/z* 275 → 79 of its deuterated standard metoprolol-d7 remains unaffected by matrix effects.

In biochar the recovery rates determined with internal standards show the applicability of this approach for diclofenac (88%), phenazone (99%), sulfamethoxazole (98%), metoprolol (84%) and clarithromycin (70%). The seven other compounds analyzed with internal standards miss the targeted recovery range. Ibuprofen could not be detected in the biochar, because its internal standard remained below the LOD. The SD of the internal standard approach varies from 4.5% (phenazone) to 39% (ibuprofen) in sewage sludge and from 4.9% (erythromycin) to 26% (bezafibrate) in biochar.

Being the most time-consuming approach the standard addition, which was prepared before PLE and is shown in fig. 2.2 e, compensates all sample preparation losses and all instrumental matrix effects without a complex clean-up procedure. This results in good recovery rates ranging from 85% to 125% for all investigated pharmaceuticals in sewage sludge and biochar except sulfamethoxazole (33%) in sewage sludge. The low SD, however, allows using a correction factor. The SD of the other compounds remains below 12% in sewage sludge and below 11% in biochar, which shows reproducible results in both matrices. Contrary to our results, Göbel et al. did not observe any difference between spiking before and after PLE in sewage sludge [22], which might result from a different choice of solvent mixture, a shorter static extraction time or the final number of cycles during the PLE. The different spiking procedure compared to the present study cannot explain the different results. Although Göbel et al. added the standards directly after PLE while we added the standards after evaporating and filtering the samples, pre-experiments proved that neither the evaporation step nor the filtration step caused any sample losses.

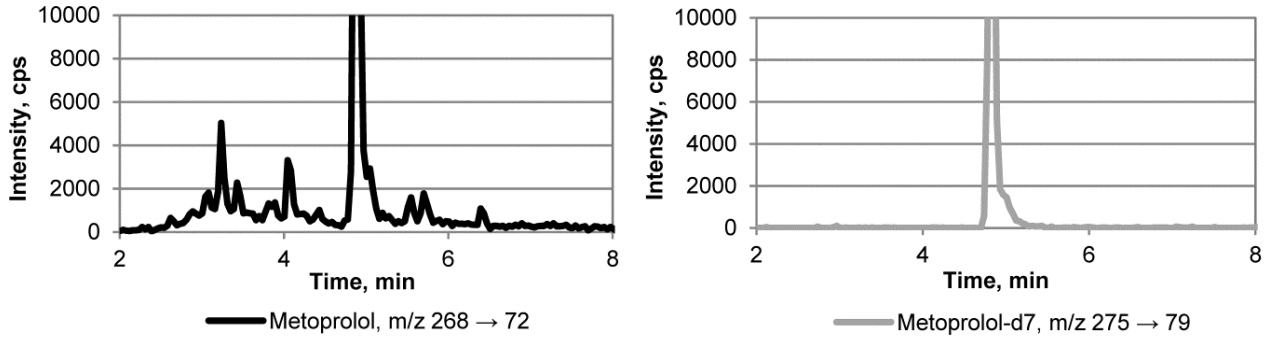

Fig. 2.3 Extracted ion chromatograms of metoprolol (*m/z* 268 → 72) and its deuterated standard metoprolol-d7 (*m/z* 275 → 79), which are varyingly strong affected by matrix effects

Besides the variation, which is expressed as SD, the four approaches were tested regarding their expanded measurement uncertainty (MU) according to the principles of the Joint Committee for Guides in Metrology (JCGM) 100:2008 [28]. The MU represents one measure to evaluate the validity of a measurement.

The approaches external calibration and use of internal standards require the type A MU, which includes repeated analyses of a sample. Results of the standard MU are provided in the appendix (suppl. 7.4). The quality control samples were determined with n = 4, their advanced MU with k = 3.2 is almost twice the SD. The approaches external calibration and internal standard (n = 3) result in 2.5 times higher advanced MU compared to the SD, because the low number of repeated measurements requires a high k = 4.3. However, both parameters, the SD and the MU, depend on each other.

Type B MU, which considers calibration data, fits to the standard addition approaches (suppl. 7.5) and avoids the high experimental effort for repeated analyses. Type B approach, however, fails for external calibration and internal standards, because uncertainties would result in underestimated values, which derive from correlating matrix free standards with matrix containing samples. In contrast, this correlation fits to the standard addition method, because the calibration already contains the same matrix as the samples. The results of the standard addition before LC-MS/MS show that MU exceeds 50% and more for nine samples, which indicates a low validity and proves the deficiency of this approach. Contrary, the MU derived from the standard addition over the whole procedure shows that the MU remains below 20% for nine samples. Consequently, spiking before PLE helps to obtain valid results.

However, the presented approaches are limited to the extractable part of the compound. The determined value might differ from the true amount in the sample, because aging effects might hinder the full extraction of the analyte [29]. Nevertheless, studies claimed that sequestration also reduces leaching and bioavailability, which makes the non-extractable part negligible [30,31].

Table 2.2 lists the validation data of the final method of choice, the standard addition before PLE,

for sewage sludge and biochar. Instrumental LOD refers to the best of three independent measurements and the linear range depicts the instrumental range of each calibration graph with a good linearity ($r^2 > 0.99$). LOD and LOQ in sewage sludge are best of six samplings. Biochar data derive from six production charges. However, limits may vary for other sludges and biochars. Generally, LOD and LOQ are elevated in sewage sludge and biochar compared to the low instrumental limits. Diclofenac, for example, achieves an instrumental LOD of 0.5 ng/mL, while in sewage sludge and biochar the LOD amounts to 3.2 ng/g (equal to 0.64 ng/mL) and 11 ng/g (equal to 2.2 ng/mL), respectively. The enhanced LOD might be caused by higher noise and suppressed signals in the MS analysis. LOD and LOQ of all six sewage sludge and biochar samples are shown in the appendix (suppl. 7.6). These data emphasize, that the limits cover a great range due to varying matrix effects.

Table 2.2 Validation data of 12 pharmaceuticals, which were extracted from sewage sludge and biochar using pressurized liquid extraction and quantified via standard addition over the whole procedure; indication of best values of n = 3 (instrumental) and n = 6 (in sewage sludge and biochar)

	Instrumental linear range	Instrumental limit of detection	Limit of detection		Limit of quantification	
			Sewage sludge	Biochar	Sewage sludge	Biochar
	ng/mL	ng/mL	ng/g_{DM}	ng/g_{DM}	ng/g_{DM}	ng/g_{DM}
Diclofenac	0.5 - 250	0.5	3.2	44	11	150
Ibuprofen	25 - 250	14	20	260	68	850
Phenazone	0.1 - 100	0.1	1.2	17	4.1	57
Carbamazepine	0.1 - 500	0.03	0.9	6.8	2.8	23
Sulfamethoxazole	0.5 - 1,000	0.1	0.4	20	1.2	68
Bezafibrate	0.3 - 500	0.04	1.4	1.8	4.7	6.1
Fenofibric acid	1 - 1,000	0.4	1.4	10	4.6	35
Metoprolol	2.5 - 1,000	0.1	9.1	33	30	110
Propranolol	0.3 - 250	0.1	0.9	4.3	2.8	14
Clarithromycin	2.5 - 50	0.1	1.5	8.6	5.1	29
Roxithromycin	0.3 - 1,000	0.1	4.1	2.9	14	10
Erythromycin	0.5 - 1,000	0.1	2.3	6.7	7.7	22

2.5.4 Method application

We applied the validated method to sewage sludge received from the WWTP Rheinhausen and the corresponding biochar produced from this sewage sludge.

Eight of the twelve investigated pharmaceuticals were detected in the sewage sludge. Bezafibrate and the antibiotics sulfamethoxazole, clarithromycin and erythromycin remained below the LOQ. Previous studies reported concentrations of these compounds varying from the LOQ up to 50, 180, 500 and 1800 ng/g_{DM}, respectively, in sewage sludge [7-10,22,24,32,33]. The beta blocker metoprolol was detected in highest concentration (430 ± 50 ng/g_{DM}). In similar studies, the sludges contained lower amounts of metoprolol (< 5-100 ng/g_{DM}) [7,32,33]. Likewise, diclofenac (250 ± 30 ng/g_{DM}), ibuprofen (160 ± 20 ng/g_{DM}), carbamazepine (150 ± 10 ng/g_{DM}) and propranolol (110 ± 50 ng/g_{DM}) occurred in considerable amounts, while phenazone (21 ± 8 ng/g_{DM}), fenofibric acid (67 ± 9 ng/g_{DM}) and roxithromycin (35 ± 9 ng/g_{DM}) concentrations were low. Other groups reported comparable concentration ranges [7,9,10,23,24,32].

In biochar from HTC of sewage sludge only three of twelve compounds were detected, namely phenazone (130 ± 10 ng/g_{DM}), metoprolol (220 ± 10 ng/g_{DM}) and propranolol (26 ± 9 ng/g_{DM}). The elevated LOQ in the biochar inhibits a quantitative comparison of sewage sludge and biochar loads for some of the pharmaceuticals. However, reduced concentrations were determined for metoprolol and propranolol, which degraded by 49 and 77%, respectively. This indicates the potential of the HTC to reduce the load of extractable micropollutants during the conversion of sewage sludge to biochar.

2.6 Conclusion

A robust and reliable LC-MS method was developed for the quantification of twelve pharmaceuticals in sewage sludge and biochar. Sewage sludge and biochar samples were characterized regarding matrix effects in HPLC-MS/MS. Matrix effect profiles demonstrated suppressed and enhanced sections throughout the chromatograms indicating that matrix compounds affect the analyte ionization. Severe signal suppression was observed for biochar samples. During method development we evaluated and compared the approaches external calibration, use of internal standards, standard addition before HPLC-MS/MS and standard addition over the whole procedure. The latter approach obtained best recovery rates by compensating all losses during sample preparation and matrix effects. Therefore this approach served to build up a robust method to quantify twelve pharmaceuticals in sewage sludge and biochar. The final method was applied exemplarily and revealed that pharmaceuticals might be susceptible to degrade or to bind irreversible to the organic matter during HTC.

However, subsequent studies should focus on the predominant binding or degradation processes

during the HTC. Further studies could compare biochars from different sewage sludges and HTC plants. Moreover, an extended compound spectrum might enable to assess the biochar more comprehensive. In fact, experimental work on the formation of dioxins and PAH has been started and will be put into the context of other combustion processes.

2.7 Acknowledgement

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3. Pharmaceutical load in sewage sludge and biochar produced by hydrothermal carbonization

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3.1 Abstract

We investigated the removal of pharmaceuticals in sewage sludge by HTC, which has emerged as a technology for improving the quality of organic waste materials producing a valuable biochar material.

In this study, the HTC converted sewage sludge samples to a biochar product within four h at a temperature of 210 °C and a resulting pressure of about 15 bar. Initial pharmaceutical load of the sewage sludge was investigated as well as the residual concentrations in biochar produced from spiked and eight native sewage sludge samples from three wastewater treatment plants. Additionally, the solid contents of source material and product were compared, which showed a considerable increase of the solid content after filtration by HTC.

All pharmaceuticals except sulfamethoxazole, which remained below the LOQ, frequently occurred in the investigated sewage sludges in the $\mu g/kg_{DM}$ range. Diclofenac, carbamazepine, metoprolol and propranolol were detected in all sludge samples with a maximum concentration of 800 $\mu g/kg_{DM}$ for metoprolol. HTC was investigated regarding its contaminant removal efficiency using spiked sewage sludge. Pharmaceutical concentrations were reduced for seven compounds by 39% (metoprolol) to ≥ 97% (carbamazepine). In native biochar samples the four compounds phenazone, carbamazepine, metoprolol and propranolol were detected, which confirmed that the HTC process can reduce the load of micropollutants. In contrast to the other investigated compounds phenazone concentration increased, which was further addressed in thermal behavior studies including three structurally similar potential precursors.

3.2 Keywords

HTC • Hydrochar • Micropollutants • Sewage sludge conversion • Waste treatment • Pyrazolones

3.3 Introduction

The proper handling of sewage sludge has gained attention in times of increasing energy costs

and stricter legislative demands. In 2005, the German government banned sewage sludge deposition leaving the alternatives to incinerate the sludge or to apply it in agriculture. However, both choices face major drawbacks. On the one hand incineration is restricted to dried sewage sludge demanding lots of energy to separate the water. In addition, this alternative accepts the loss of nutrients like nitrogen and phosphorus. On the other hand agricultural application of sewage sludge is limited by regulations for heavy metals and pathogens and suffers from the low social acceptance [1]. Although the nutrients nitrogen and phosphorus are recycled many contaminants may be released into the environment contributing to contamination of soils and adjacent water bodies [2]. In this context, pharmaceuticals are of particular concern because studies showed low effect concentrations and the evolution of multi-resistant pathogens [2].

Recently, the technology of HTC has been established as an improved sewage sludge handling method. HTC generates biochar from wet biomass at elevated temperature and pressure [3]. Bergius [4] introduced the process in 1913 to simulate natural diagenesis of coal. HTC is feasible for biomass with poor fuel properties like sewage sludge and entails benefits in subsequent processing [5]. In fact, HTC increases the specific heat content of sewage sludge, which is valuable for mono-incineration [6]. Alternatively, governmental efforts support the recycling of nutrients, which favors the application of biochar in agriculture. The loss of functional groups increases the hydrophobic character of the biochar enhancing its dewaterability, which reduces drying and transport costs. Escala et al. [7] estimated thermal and electric energy savings using the HTC instead of conventional drying methods for sewage sludge on a laboratory scale. In their experiments about 60% of thermal energy and 65% of electric energy was saved. Additionally, the high carbon efficiency of the process minimizes greenhouse gas emissions and contributes to a positive carbon footprint [8].

However, little is known about the behavior and whereabouts of micropollutants during the HTC of sewage sludge yet. For model substances, a reaction-process dependency regarding the micropollutant behavior has been shown [5]. Weiner et al. [9] reported that selected micropollutants degrade at HTC conditions in water and sucrose solution. However, these results have not been transferred to sewage sludge yet. Recently, we [10] developed a method to determine pharmaceuticals in sewage sludge and biochar.

Based on the described method we investigated the fate of 12 representative pharmaceuticals during the HTC comparing two HTC reactors and sewage sludges from three German WWTP including repeated sampling campaigns. Analyses comprised diclofenac, ibuprofen, phenazone, carbamazepine, sulfamethoxazole, clarithromycin, roxithromycin, erythromycin, bezafibrate, fenofibric acid, metoprolol and propranolol. Furthermore, the three pyrazolones propyphenazone, 4-aminoantipyrine (4-AA) and 4-methylaminoantipyrine (MAA) were investigated in additional experiments.

3.4 Materials and methods

3.4.1 Chemicals

Diclofenac, ibuprofen, phenazone, propyphenazone, 4-AA, MAA, carbamazepine, sulfamethoxazole, bezafibrate, fenofibric acid, metoprolol, propranolol, clarithromycin, roxithromycin and erythromycin were purchased from Sigma-Aldrich (Taufkirchen, Germany) in highest available purity. Table 3.1 list the physico-chemicals characteristics of the investigated pharmaceuticals. Th. Geyer GmbH & Co. KG (Renningen, Germany) delivered LC-MS water, acetonitrile and methanol. Fisher Scientific GmbH (Schwerte, Germany) supplied the extra pure mesh sand from Ottawa, Canada. Stock solutions were prepared in water/acetonitrile (50/50, *v/v*). Storage at 4 °C did not exceed three months. Standards were prepared for every experiment by diluting the stock solutions with LC-MS water.

Table 3.1 Physico-chemical characteristics of the 12 investigated pharmaceuticals. Decomposition temperature and log K_d are collected from literature (given in brackets), pK_a is collected from the online database drugbank

	M	Sum formula	Decomposition temperature	pk_a	log K_d
	g/mol		°C		
Diclofenac	296.15	$C_{14}H_{11}Cl_2NO_2$	280 [11]	4.15	1.82 [12]
Ibuprofen	206.28	$C_{13}H_{18}O_2$	190 [13]	4.9	1.58 [12]
Phenazone	188.23	$C_{11}H_{12}N_2O$	300 [14]	1.4	n.a.
Carbamazepine	236.27	$C_{15}H_{12}N_2O$	198 [15]	n.a.	1.55 [12]
Sulfamethoxazole	253.28	$C_{10}H_{11}N_3O_3S$	190 [16]	1.97	1.36 [12]
Bezafibrate	361.82	$C_{19}H_{20}ClNO_4$	250 [17]	3.83	1.5 [18]
Fenofibric acid	360.83	$C_{20}H_{21}ClO_4$	n.a.	n.a.	n.a.
Metoprolol	267.36	$C_{15}H_{25}NO_3$	n.a.	9.7	1.9 [18]
Propranolol	259.34	$C_{16}H_{21}NO_2$	252 [19]	9.42	2.52 [20]
Clarithromycin	747.95	$C_{38}H_{69}NO_{13}$	245 [21]	8.99	2.7 [18]
Roxithromycin	837.05	$C_{41}H_{76}N_2O_{15}$	230 [21]	9.08	2.6 [18]
Erythromycin	733.93	$C_{37}H_{67}NO_{13}$	190 [22]	8.88	2.1 [18]

n.a. = not available

3.4.2 Sewage sludges

LINEG, Ruhrverband and Hamburg Wasser enabled sewage sludge sampling at the WWTP Rheinhausen, Rahmedetal and Hollenstedt. The samples consisted of centrifuged secondary sludge. Hollenstedt (9,500 population equivalent, PE) located southwest of Hamburg treats wastewater from a mainly rural area. In contrast, mixed rural, urban and industrial influences are present in Rahmedetal (64,000 PE) in North Rhine-Westphalia. The catchment area of Rheinhausen (220,000 PE) located in the western Ruhr catchment shows mainly urban and industrial impacts. The WWTP represent classical profiles of different catchment influences. Additional sewage sludge parameters are given in the Supp. 7.7. Samples were taken after the final drying step in five sampling campaigns distributed over two years. Five samples originated from Hollenstedt, two from Rheinhausen and one from Rahmedetal.

3.4.3 Biochar produced by HTC of sewage sludge

HTC was conducted in high pressure systems (Büchi Glas Uster, Switzerland) with reactor volumes of 0.2 L and 5 L. The sewage sludge was adjusted to 20%$_{DM}$ using water. Then, the mixture was filled into the reactor. The system was closed and heated to 210 °C while a stirrer continuously mixed the content at 500 rpm. After keeping the final temperature for four h, the reactor was cooled down to room temperature. The product from HTC of sewage sludge is called biochar or hydrochar. Bls 2.5 software (Büchi Glas Uster, Switzerland) controlled and recorded the experimental parameters reactor temperature, jacket temperature, heating power, stirring rate and pressure. Afterwards, the produced biochar was stored at 4 °C until analysis.

3.4.4 Experimental design

First, the HTC process was evaluated considering its dewaterability dependent on the runtime with sewage sludge from the WWTP Hollenstedt. In fact, sewage sludge and biochar were compared regarding their solid content after filtering the samples with a 1-µm glass fibre filter (Macherey-Nagel, Düren, Germany). The solid fraction resulted from $\frac{m_d}{m_w} \times 100$ with the dried mass m_d and the wet mass m_w. Proponents claim dewaterability as one of the major advantages of the process, although only one study has described it yet without relation to the runtime [7].

Second, pharmaceutical load in sewage sludge (n = 8) was investigated and related to literature data. This should reveal that pharmaceutical load of the applied sewage sludges is representative, which provides a solid basis to evaluate their behavior during HTC.

Third, compound behavior during HTC was exemplary traced in spiked experiments. Therefore, 12 investigated compounds were spiked to a sewage sludge with 200 µg/kg$_{DM}$ for each compound and intermixed for 24 h at 500 rpm. Afterwards, HTC transformed the mixture to biochar within a

4-h HTC. These experiments were conducted to investigate the potential of HTC to remove considerable amounts of the pharmaceuticals.

Fourth, the spiked sewage sludge experiments were transferred to native samples (n = 8). Therefore, the sewage sludges underwent HTC to produce biochar. Afterwards, residual pharmaceutical concentrations were determined and related to the corresponding sludges determining removal rates.

The last experimental set examined pyrazolones in more detail to gain a deeper process understanding, because of unexpected results for phenazone in the previous investigations. Pyrazolone stability was investigated in so-called inert experiments (c_0 = 500 µg/kg_{DM}), in which Ottawa sand replaced the sewage sludge to guarantee monitoring of compounds behavior without interfering reactions from sewage sludge. Besides this, pyrazolones occurrence in native biochar was compared to its corresponding sewage sludge. This might identify possible sources or reasons for phenazone increase during HTC.

3.4.5 Sample extraction, preparation and HPLC-MS/MS analysis

Sewage sludge and biochar samples, which were produced as described in section 3.4.3, were lyophilized using a freeze-dryer beta 1-16 LDG 2-m system (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). In fact, the drying method pre-freezed the samples at -20 °C and dried them at 1.030 kPa and 10 °C. After attaining the product temperature the freeze-dryer was adjusted to 0.001 kPa for two h.

Standard addition was applied in the range of 10 - 5,120 µg/kg_{DM} to cover a wide calibration range, which comprises all target amounts in sewage sludge. The single calibration steps were 10 - 20 - 40 - 80 - 160 - 320 - 640 - 1,028 - 2,056 - 5,120 µg/kg_{DM}. Sample aliquots of 1 g were mixed with Ottawa sand, transferred to 11-mL stainless steel cartridges, which were filled with Ottawa sand and extracted using PLE (Dionex ASE200, Thermo Fisher GmbH, Idstein, Germany) according to an existing method [10] for the pharmaceuticals except propyphenazone, 4-AA and MAA. In short: The solvent methanol extracted the compounds in one cycle within 15 min at 100 °C and 100 bar. Afterwards, the cartridge was flushed with 10% methanol and purged with nitrogen for 150 s. The extract was evaporated and resolved in 5 mL LC-MS water and filtrated (0.45 µm regenerated cellulose syringe filters, Chromafil RC-45/15MS, Macherey-Nagel, Dueren, Germany). Recovery rates ranged from 33% for sulfamethoxazole to 124% for ibuprofen in sewage sludge. In biochar, recovery rates between 93% (Metoprolol) and 125% (Ibuprofen) were achieved. For the pyrazolones study, PLE parameters were adapted, because initial experiments showed low recoveries at high PLE temperature. Static extraction was extended to 35 min at a temperature of 50 °C and a pressure of 40 bar. All extracts were evaporated, resolved in 5 mL LC-MS water and filtrated. The appendix gives detailed information about the recovery rates of the analytical

methods (suppl. 7.8 and suppl. 7.9).

Samples were analyzed using an LC 20 HPLC (Shimadzu, Duisburg, Germany) connected to a 3200 QTRAP (AB Sciex, Darmstadt, Germany) MS. A Waters Atlantis T3 column (100 x 2.1 mm, 3 µm, Waters GmbH, Eschborn, Germany) was applied for chromatographic separation. Chromatographic and MS parameters are given elsewhere [10] except for the pyrazolones. The appendix lists all chromatographic and mass spectrometric parameters (suppl. 7.10-7.12).

The LOD and LOQ resulted from a S/N ratio of three and 10 of the peak response, respectively. If the sample contained the compound of interest, the amount present in the non-spiked sample served to determine the LOD and LOQ. The standard addition method involves determining LOD and LOQ for each experiment. In sewage sludge, best LOQ ranged from 1.2 $\mu g/kg_{DM}$ for clarithromycin to 28 $\mu g/kg_{DM}$ for ibuprofen. Biochar LOQ ranged from 2.3 $\mu g/kg_{DM}$ (roxithromycin) to 64 $\mu g/kg_{DM}$ (diclofenac). All LOQ are given in the appendix (suppl. 7.8 and suppl. 7.9).

The calculated concentration refers to the extractable part of the compound. Non-extractable residues (NER) might remain in the sewage sludge and biochar because aging effects might hinder exhaustive extraction [23]. Moreover, the experimental setup impedes to differentiate whether the compound degrades or converts to NER during the HTC. However, PLE parameters exceed environmental temperature and ambient pressure. Therefore, they are supposed to surpass the natural leaching process. The NER formation is considered as detoxification pathway [24].

3.5 Results and discussion

3.5.1 Dewaterability of sewage sludge and biochar

A previous study already reported enhanced dewaterability after HTC [7]. However, the HTC runtime necessary to improve separation of solid and liquid phase was not investigated.

Experiments showed that the received sewage sludge had an average solid content of 23% in the filter cake with minor deviations (3%). Up to one h HTC runtime was insufficient to increase the dewaterability. Longer runtimes of three or six h enabled hydrolysis reactions which increased subsequent dewatering. The described process could be compared to the thermal hydrolysis of sewage sludge [25], which improves solid-liquid-separation by use of high temperature to break the cell structure of organic compounds. A 4 h-HTC at 210 °C increased the solid content to 39% ± 7%. Detailed results are given in the appendix (suppl. 7.13). Overall, results show that the process of HTC with a runtime of four h is appropriate to improve sewage sludge drying and might provide an alternative for conventional sludge drying technologies like filter press or centrifugal systems.

3.5.2 Occurrence of pharmaceuticals in sewage sludge

The occurrence of pharmaceuticals in sewage sludge is well-known [26]. However, studies usually focus on a certain compound class, which hampers conclusions about the general contaminant load. Therefore, this study combined pharmaceuticals of different compound classes in one method.

In order to evaluate the sewage sludges applied for HTC with regard to their pharmaceutical load, eight samples from the three WWTP were analyzed for the selected compounds. Fig. 3.1 displays the range of detected pharmaceuticals in this study (black bars) and compares these results with data from Germany, Spain, China, France, Japan and Estonia (grey bars) [20, 26-39].

The comparison shows that most investigated compounds occur in the same concentration range as previously reported. Diclofenac results corroborates a previous German study [39], while other countries reported lower amounts [20, 26, 27, 34-38]. The ibuprofen level corresponds to several Spanish studies [34, 37, 38], while another study reported a 20-fold higher concentration [20] in primary sludge. This can be explained by the good microbial degradability of ibuprofen during wastewater treatment [20], since in this and comparable studies secondary sludge was used. Phenazone was detected in six of eight samples during this study with an average concentration of 30 µg/kg_{DM}. This confirms previous findings from Spain, France and Japan [35, 36, 38]. Carbamazepine concentration ranged between < LOQ and 410 µg/kg_{DM} in this study. Martin et al. [20] reported similar loads, other studies reported lower values [26, 27, 34-38]. Sulfamethoxazole remained below the LOQ of 15 µg/kg_{DM} in this study, while another German study reported up to 113 µg/kg_{DM} [29]. Bezafibrate was detected in the same concentration range as previously described [27, 34, 35, 38]. The reported loads for fenofibric acid and metoprolol remained below the amounts detected in this study [26, 27, 34, 35, 38]. However, only two studies considered fenofibric acid and three studies included metoprolol in their monitoring. The results of the antibiotics clarithromycin, roxithromycin and erythromycin are difficult to evaluate, because previous investigations showed considerable differences, even within one country [26, 27, 29-31, 33, 35-38]. Seasonal diseases increase the demand for antibiotics. The reported ranges fit therefore to the detected amounts in this study.

The three investigated WWTP showed no significant variations regarding their pharmaceutical load despite their different catchment areas.

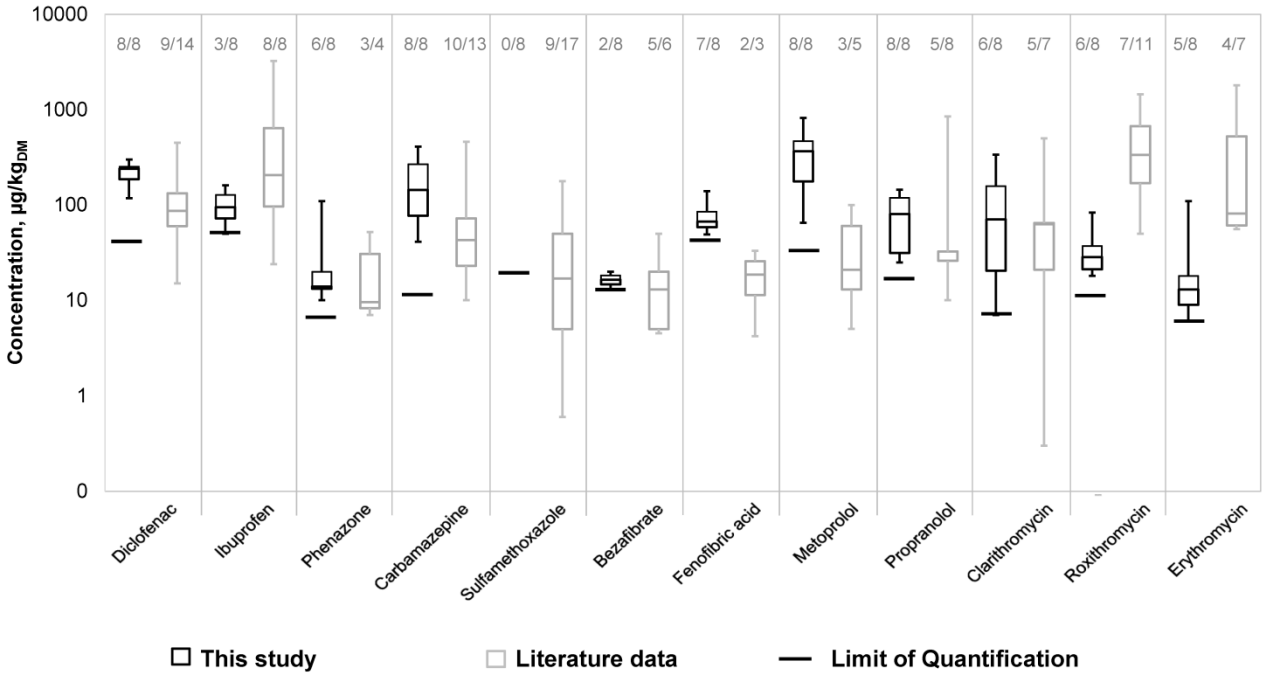

Fig. 3.1 Boxplot of selected pharmaceuticals in sewage sludge determined in this study compared to previous studies from Germany, Spain, China, France, Japan and Estonia [20, 26-39] (all values above the limit of quantification were considered). Data above each box denote the number of positive results and the number of studies considered (which are influenced by the limit of detection in the specific studies)

3.5.3 Removal rates of pharmaceuticals during the HTC with spiked sewage sludge

Removal efficiencies for 12 investigated compounds were obtained from experiments with spiked sewage sludge. To that end, a mixture of the pharmaceuticals (c = 200 µg/kg_{DM}) was added to the sewage sludge. The concentrations of the spiked sewage sludge containing the native amount and the spiked 200 µg/kg_{DM} were determined and considered in the calculation of the removal rates during the HTC. Table 3.2 displays the initial load of the spiked sewage sludge, its corresponding biochar, and the resulting removal rates for each of the investigated compounds. No removal rates are given for diclofenac, because matrix effects caused a high LOQ in this biochar sample impeding to derive removal rates. Sulfamethoxazole showed decreased recovery in sewage sludge, which was reported previously [10].

Table 3.2 Concentrations of ten pharmaceuticals in spiked sewage sludge (c_0 = 200 µg/kg_{DM}) from the wastewater treatment plant Rheinhausen, its corresponding biochar and the derived removal rates during hydrothermal carbonization (HTC) including the expanded measurement uncertainty according to the principles of the JCGM 100:2008 [40]

	Measured concentration in spiked sewage sludge	Concentration after HTC	Removal during HTC
	µg/kg_{DM}	µg/kg_{DM}	%
Ibuprofen	350 ± 33	130 ± 15	63
Phenazone	210 ± 33	230 ± 6	No removal
Carbamazepine	560 ± 23	< 20	> 98
Bezafibrate	180 ± 8	< 40	> 89
Fenofibric acid	340 ± 23	< 20	> 97
Metoprolol	650 ± 96	400 ± 23	39
Propranolol	360 ± 120	70 ± 14	81
Clarithromycin	220 ± 55	< 20	> 95
Roxithromycin	190 ± 63	< 10	> 97
Erythromycin	180 ± 24	< 10	> 98

Residual amounts of ibuprofen, phenazone, metoprolol and propranolol were detected after HTC. Weiner et al. also reported residual ibuprofen concentrations after HTC in sucrose solution [9]. However, their described degree of conversion (30 - 50%) remains below the 63% conversion detected in this study, which might be caused by the different source material.

Slightly enhanced concentration after HTC was detected for phenazone (statistically not significant). An increase might be caused by precursors or structurally related compounds present in the native sewage sludge. Further results regarding the pyrazolones are shown in section 3.5.5.

Removal rates for the other compounds ranged from 39% (metoprolol) to > 98% (carbamazepine and erythromycin). Carbamazepine, bezafibrate, fenofibric acid, clarithromycin, roxithromycin and erythromycin were below the LOQ in biochar. The high removal rates indicate that HTC is appropriate to reduce the micropollutant load in sewage sludge.

An explanation for the removal might be a limited thermal stability of the active pharmaceutical ingredient. Pharmaceuticals show a wide range of thermal stability. Reported decomposition temperatures of the investigated compounds vary from 190 °C for ibuprofen [13] to more than 300 °C for phenazone [14]. The range corresponds to the common HTC temperature of 200 - 250 °C. However, decomposition temperature alone cannot explain the removal behavior as ibuprofen would already start to decompose at 190 °C [13]. Therefore higher removal rates would be expected. Propranolol would decompose at a temperature of 252 °C [19]. Nevertheless, more than 80% of the compound was removed during HTC. Temperature peaks within the reactor or

catalytic effects could explain this discrepancy. The two examples show that decomposition temperature does not correspond to the removal behavior during HTC. The accompanying factor of elevated pressure (about 15 bar) during HTC might also affect the micropollutant removal. Additionally, the pharmaceuticals might sorb to the sewage sludge, bind reversibly or form complexes. The thermal behavior of the surrounding sewage sludge has shown to be very heterogeneous during pyrolysis processes. Decomposition of degradable organic matter and dead bacteria starts at around 200 °C, while non-biodegradable material remains stable up to more than 300 °C [41].

Alternatively, other physico-chemical properties might explain the removal behavior. Therefore, removal rates were correlated to compound's pK_a, but no correlation was visible. Metoprolol and clarithromycin, for example, although having a similar pK_a of 9.7 and 8.99, significantly deviate in their residual concentration. Correlation of removal rates and log K_d also failed. Ibuprofen and carbamazepine having log K_d of 1.58 and1.55, respectively, differ in their removal rates during HTC by more than 20 percentage points. Detailed results for all investigated compounds are shown in the appendix (suppl. 7.14).

3.5.4 Removal rates of pharmaceuticals during HTC of native sewage sludge

Investigation of removal behavior of pharmaceuticals during HTC was transferred from spiked to native samples. To our knowledge, no study has yet monitored the micropollutant load of biochars produced from sewage sludge by HTC for different sampling campaigns and sludge sources to prove the expected compound removal.

Therefore, table 3.3 compares the removal rates during HTC calculated from concentrations in native sewage sludges and their corresponding biochars (n = 8).

Values below the LOQ in sewage sludge were excluded from the total number of experiments resulting in the number of samples considered. The removal rates of phenazone, carbamazepine, metoprolol and propranolol are averaged values from all experiments containing the compounds in sewage sludge and biochar. The compounds diclofenac, clarithromycin, roxithromycin and erythromycin remained below the LOQ in biochar. Therefore, the calculated removal rates result from the determined amounts in sewage sludge and half of the LOQ in biochar. Enhanced LOQ in biochar caused by the complex matrix hindered determining removal rates for ibuprofen, sulfamethoxazole, bezafibrate, and fenofibric acid. Hence, they were excluded from the table.

Table 3.3 Removal rates during hydrothermal carbonization derived from determined concentrations in native sewage sludges and the corresponding biochars

Analyte	Number of samples considered	Minimal removal, %	Maximal removal, %	Averaged removal, %
Diclofenac	5	> 57	> 78	> 69
Phenazone	6	↑*	↑*	↑*
Carbamazepine	1	87	87	87
Metoprolol	6	15	64	42 ± 16
Propranolol	3	64	77	70 ± 7
Clarithromycin	3	> 61	> 94	> 79
Roxithromycin	7	> 29	> 95	> 68
Erythromycin	4	> 55	> 84	> 68

* ↑: no compound removal, enhanced concentrations in the biochar samples.

Concentrations of all pharmaceuticals were reduced by HTC except phenazone, which is addressed separately in section 3.5.5. The antibiotics and diclofenac showed a decrease by more than two-thirds. Carbamazepine, metoprolol and propranolol were removed by 87, 42, and 70%, respectively. The results indicate that thermal conversion of sewage sludge might not only hygienize the sludge but also remove organic pollutants like pharmaceuticals.

Eight of the 12 investigated pharmaceuticals remained below the LOQ in all eight investigated biochar samples. Three compounds were detected frequently. Fig. 3.2 shows their concentration range. Phenazone occurred in seven biochar samples. Its concentration varied between 35 and 195 µg/kg_{DM}. Metoprolol was also frequently detected at concentrations exceeding the other investigated pharmaceuticals (up to 510 µg/kg_{DM}). Three biochar samples contained propranolol at concentrations ranging between 9 and 46 µg/kg_{DM}. Additionally, one sample contained carbamazepine (55 µg/kg_{DM}) while it remained below the LOQ in the other seven biochars.

Comparing the two reactor systems (0.2 L and 5 L), no significant difference was observed for the pharmaceutical load in the produced biochar.

Overall, results derived from native samples correspond to the spiked experiments. For the antibiotics clarithromycin, roxithromycin and erythromycin removal rates in the spiked sewage sludge exceed those in real samples. However, this is attributed to the low levels detected in native sewage sludge. Metoprolol showed 42% removal in native samples, which matches the removal rate in the spiked experiment (39%). Similarly 70% propranolol removal in native samples fits to 81% in the spiked sludge. Carbamazepine result shows the same trend (87% compared to > 98% removal).

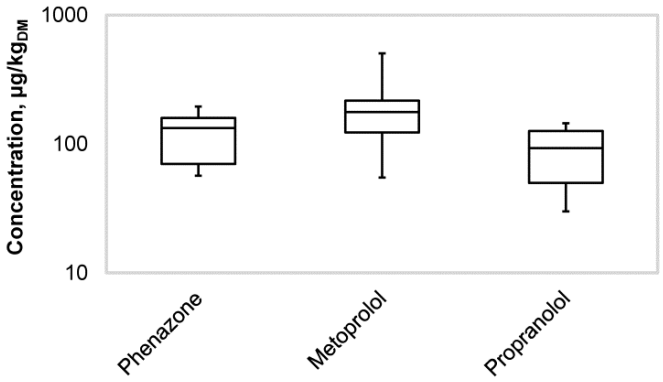

Fig. 3.2 Concentration ranges of the three repeatedly detected pharmaceuticals in biochar produced from sewage sludge via hydrothermal carbonization, n = 8 (all values above the limit of quantification were considered)

3.5.5 Phenazone and its precursors

In contrast to the other pharmaceuticals, phenazone load increased during the HTC in spiked experiments as well as in native samples. Absolute amounts of increase (25 µg/kg$_{DM}$ in spiked samples compared to 77 µg/kg$_{DM}$ in native samples) were in the same order of magnitude. This indicates, that additional compounds present in the native sewage sludge might cause the increase of phenazone concentration. Eichelbaum et al. [42] studied the phenazone metabolites 4-hydroxy-antipyrine and 3-hydroxymethylantipyrine in pharmaceutical stability tests. These precursors might degrade to phenazone during the HTC again. Otherwise, chemically similar compounds, which could also occur in sewage sludge, like aminophenazone, salipyrine or metamizole might degrade to phenazone. Further on, a high thermal stability up to 300 °C of phenazone, which was reported by Fulias et al. [14] in thermogravimetric analysis, hinders subsequent degradation reactions.
Behavior of phenazone during HTC was elucidated in experiments including phenazone and three other pyrazolones, namely propyphenazone, 4-AA and MAA. Their structure is shown in fig 3.3. The precursors are structurally similar to phenazone and serve to replace it in pharmaceutical applications.

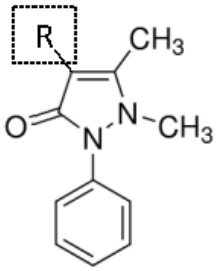

Fig. 3.3 Structures of pyrazolones. Phenazone: R = –H. Propyphenazone: R = –CH-(CH$_3$)$_2$. 4-aminoantipyrine: R = –NH$_2$. 4-methylaminoantipyrine: R = –NH-CH$_3$

In sewage sludge from Hollenstedt propyphenazone and 4-AA amounted to 2 and 100 µg/kg_{DM}, respectively. Concentration of 4-AA in biochar was considerably lower (45 µg/kg_{DM}). Abstraction of the amino group in 4-AA might occur and could explain the increase of phenazone concentration during the HTC of sewage sludge.

Stability of the four pyrazolones at HTC conditions was also investigated in inert sand material. The initial concentration was adjusted to 500 µg/kg_{DM}. Propyphenazone showed good thermal stability. After HTC about 80% of the compound remained recoverable. In contrast, the other investigated potential precursors 4-AA and MAA were reduced by more than 98 and 83%, respectively. Phenazone itself slightly decreased to 80% of the input concentration. The consistent amount of phenazone indicates that 4-AA and MAA do not only lose their functional amino group, but degrade further. Otherwise, phenazone concentration would have increased. Further decomposition like ring-opening mechanisms are supposed as described for the reaction of phenazone and ozone [34].

Inert experiments differ from findings in sewage sludge. Thus, the reaction process in sewage sludge remains unknown. Catalytic effects could promote the conversion of the precursors to phenazone by reversible chemical bonds mentioned above.

Alternatively to the chemical precursors, also human metabolites like 4-hydroxy-antipyrine or 3-hydroxymethyl-antipyrine may cause the phenazone increase during HTC. Reactions at the relevant side chain were already described in microbial degradation studies [35]. However, these assumptions need to be investigated in future studies.

3.6 Conclusion

HTC proved to be a conversion process for sewage sludge, which enhances its dewaterability considerably for reactions exceeding one h. HTC proved to be an effective option to improve the quality of sewage sludge regarding its pharmaceutical load. Most of the investigated compounds were removed or at least their concentrations reduced during the HTC process. Remaining residuals of pharmaceuticals in biochar after HTC were reported for the first time. Compared to sewage sludge, enhanced phenazone concentrations were detected in biochar. Therefore, the HTC process and the biochar product should be investigated in more detail to assess the product quality comprehensively.

3.7 Acknowledgement

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4. Fate and behavior of diclofenac during hydrothermal carbonization

submitted to: C. vom Eyser, K. Palmu, T. C. Schmidt, J. Tuerk, Chemosphere, 2015.

4.1 Abstract

HTC has become an esteemed method to convert sewage sludge into biochar. Besides dewatering and desinfection the process is suggested to reduce the micropollutant load, which would be beneficial for the use of biochar as fertilizer. This study was designed to examine reduction of micropollutants and formation of transformation products during HTC using the example of diclofenac. We investigated compounds' removal at HTC conditions in inert experiments and in real samples. Results showed that HTC temperature (> 190 °C) and pressure (~ 15 bar) have the potential to fully degrade diclofenac in inert experiments and spiked sewage sludge (> 99%) within one h. However, interfering effects hinder full removal in native samples resulting in 44% remaining diclofenac. Additionally, a combination of suspected-target and non-target analysis using LC-MS/MS and LC coupled to high resolution MS (LC-HRMS) resulted in the determination of six transformation products. These products have been reported in biochar from HTC for the first time, although other studies described them for other processes like advanced oxidation. Based on the detected transformation products, we proposed a degradation mechanism reflecting HTC reactions such as dehydroxylation and decarboxylation.

4.2 Keywords

Degradation mechanism • Pharmaceuticals • Sewage sludge • Transformation products

4.3 Introduction

Sewage sludge occurs as a product at WWTP. In Germany, sewage sludge is incinerated or applied in agriculture, since disposal is prohibited [1]. In 2010, 53% of the 1.89 million tons sewage sludge were incinerated and 30% served as soil amendment [2]. In other European countries the amount used as fertilizer varies between 0% (The Netherlands) and 90% (Luxembourg) [3]. However, both handling strategies suffer from disadvantages. Agricultural usage attains a low consumer acceptance because of its non-sterile character [3]. Consequently, upcoming regulations require steps to disinfect agriculturally applied sewage sludge [4]. Moreover, strict limitations

regulate the exposure of heavy metals and organic pollutants, because already low constant input of pollutants might induce negative effects such as the growth of resistant species in the presence of antibiotics [5, 6]. Despite this, the agricultural pathway causes costs of about 150 €/t_{DM}, which is cheaper than sludge incineration (about 250 €/t_{DM}) [7]. Beside the high incineration costs, nutrients like phosphorus remain in the ashes for disposal, which contradicts the concept of sustainable phosphorus management [8].

In recent years, research has discovered approaches to improve sewage sludge handling. Pre-treatment procedures like wet air oxidation or thermal hydrolysis were tested in different scales [9, 10]. HTC has turned out as a promising tool, which combines advantages for further utilization pathways. The process converts wet biomass to biochar at elevated temperature (190 - 250 °C) and pressure within a few h [9]. HTC has originally been applied to simulate natural coalification and has recently become interesting in the wastewater sector, because HTC even works with low-energy biomass like sewage sludge due to the catalytic effect of water facilitating hydrolysis, ionic condensation and cleavage [11].

The biochar is beneficial to incinerate, because its specific heating content (12.03 MJ/L) exceeds sewage sludge values (3.17 MJ/L) [12]. Alternatively, the disinfecting character of HTC promotes to apply biochar in agriculture and to recover valuable nutrients like nitrogen and phosphorus. Despite the promising features of biochar, conversion mechanisms during HTC are still unknown in detail. Libra et al. investigated the predominant reactions of model compounds during HTC [9]. Cellulose and lignin mainly dehydrate and decarboxylate, while they lose functional groups. Later, molecules recombine to aromatic and more complex structures again. However, reaction mechanisms of complex biomasses like sewage sludge including the fate of micro pollutants like pharmaceuticals and personal care products still remain unknown. Weiner et al. have investigated organic pollutants after applying HTC conditions in sucrose solution [13]. Further studies have shown the potential of HTC to reduce selected pharmaceuticals in sewage sludge [14]. Nevertheless, mechanistic details appear like a black box. The incomplete knowledge about the process details makes it difficult to evaluate the biochar product. Although the occurrence of non-regulated micropollutants in biochar might not hinder its use as a fertilizer, they impact the product quality because high concentrations entail the risk of adverse effects in soil flora and fauna.

Therefore, this study was designed to get a deeper insight into the degradation characteristics of micropollutants during HTC. In fact, we investigated the behavior of the representative micropollutant diclofenac during HTC in spiked sand, spiked sewage sludge and native sewage sludge to derive its degradation efficiency. Moreover, transformation products of diclofenac were investigated to elucidate the reaction mechanism. This approach should help evaluating the behavior of the investigated and similar compounds during HTC of sewage sludge.

The active pharmaceutical ingredient diclofenac served as the model compound, because it occurs ubiquitously. In Germany, about 90 t were consumed in 2008 [15]. Removal in WWTPs is low,

almost no biodegradation occurs [16]. Diclofenac sorbs to different sewage sludges with coefficients (log K_d) of 1.2 - 2.7 L/kg [17]. Environmental studies reported 1 - 1.6 µg/L in WWTP effluents and up to 400 µg/kg_{DM} in sewage sludge [18-20]. Toxic effects in fish can already occur at the level of 1 µg/L in the water body [21] resulting in a predicted no effect concentration of 0.1 µg/L, which is therefore suggested as environmental quality standard [22].
Analysis of diclofenac is hindered by ion suppression during LC-MS/MS measurement [18, 23]. This might also appear during the analysis of coal-like products as they are known to hinder analysis by adsorbing organic substances [24, 25]. Therefore we evaluated the LC-MS method and considered model experiments as well as real samples.

4.4 Materials and methods

4.4.1 Chemicals

LC-MS grade water, acetonitrile and methanol were purchased from Th. Geyer GmbH & Co. KG (Renningen, Germany), diclofenac sodium and formic acid from Sigma-Aldrich (Taufkirchen, Germany) in the highest available purity. Extra pure 20 - 30 mesh sand from Ottawa, Canada (Fisher Scientific GmbH, Schwerte, Germany) served as inert filling material. We prepared stock solutions of 1 g/L diclofenac with water/acetonitrile (50/50). Calibration standards were prepared freshly for each experiment using the according amounts of stock solution and LC-MS water.

4.4.2 Sewage sludge characteristics

The WWTP Hollenstedt, located in the south-west of Hamburg (Germany), provided the sewage sludge. Sewage sludge parameters are shown in table 4.1. Rural area mainly influences the composition of the wastewater.

Table 4.1 Sewage sludge parameters from the wastewater treatment plant Hollenstedt (Germany)

Dry matter	20%
pH	6.6
Organic substance	84%
Mineral substance	16%
Total nitrogen	78 kg/t
Phosphorus	58 kg/t
Organochlorides (AOX)	110 mg/kg
Hydrocarbons	3,770 mg/kg

4.4.3 HTC experiments

Experiments were carried out by weighting 50 g sand or sewage sludge + 17 mL water into a 200 mL laboratory scale limbo high pressure reactor system (Büchi Glas, Uster, Switzerland). In spiked experiments, 0.77 mL diclofenac stock solution (c = 1 g/L) was added to achieve an initial concentration of 15.4 mg/kg_{DM} in sand and 77 mg/kg_{DM} in sewage sludge, respectively. The reactor was closed and stirred at least one h at 500 rpm before adjusting the temperature to 190 - 210 °C for different runtimes. The bls 2.5 software (Büchi Glas, Uster, Switzerland) recorded reactor temperature, jacket temperature, heating and cooling power, stirring rate and pressure.

4.4.4 Sample extraction and preparation

After conducting the experiments with spiked sand the supernatant was separated from the sand. The supernatant was measured directly via standard addition while the sand residue was prepared like the sewage sludge and biochar. These samples were freeze-dried and extracted via PLE as described in detail by vom Eyser et al. [26]. In short: 11-mL cartridges were filled with aliquots of 1 g biomass and Ottawa sand served to fill up the cartridge. Samples were extracted in one 15 min cycle using methanol at 100 °C and 100 bar. The cartridges were flushed with 10% of the cell volume and purged with nitrogen for 150 s. Extracts were evaporated and dissolved in 5 mL LC-MS water. All extracts and filtrates were filtered with Chromafil RC 0.45 µm syringe filters (Macherey-Nagel, Düren, Germany) before LC-MS/MS measurement.

4.4.5 HPLC-MS/MS analysis

4.4.5.1 Quantification

HPLC-MS/MS analysis was carried out using a LC 20 HPLC system consisting of a CBM-20A communciation bus module, a CTO-20AC column oven, a DGU-20A3 degasser, LC-20AD pumps and a SIL-20AC autosampler (Shimadzu, Duisburg, Germany) coupled to a 3200 QTRAP system (Sciex, Darmstadt, Germany). Analyst™ Software 1.5 (AB Sciex, Darmstadt, Germany) recorded LC-MS data. Chromatographic separation follows a 20 min gradient using water + 0.1% formic acid and acetonitrile + 0.1% formic acid. 20 µL were injected on a 100 x 2.1 mm, 3 µm Atlantis T 3 column (Waters GmbH, Eschborn, Germany) at a flow rate of 0.3 mL/min and a temperature of 45 °C.

The MS was adjusted to ESI at 5,000 V in positive mode. MRM mode with the transitions and transition specific instrumental parameters listed in table 4.2 quantified diclofenac. Instrumental settings were: curtain gas 11 V, ion source temperature 520 °C, ion source gas one 63 psi and ion source gas two 68 psi.

Table 4.2 Instrumental parameters for mass spectrometric detection of diclofenac. ESI = Electrospray Ionization; DP = declustering potential; EP = entrance potential; CEP = collision cell entrance potential; CE = collision energy; CXP = collision cell exit potential

MRM	DP V	EP V	CEP V	CE eV	CXP V
296 → 214	31	4	22	43	4
296 → 151	31	4	22	81	4

4.4.5.2 Suspected target and non-target measurements

The QTRAP system was also used for elucidation of HTC degradation products in inert experiments using a non-target method. In fact, the system was used to gather enhanced product ion scan of potential products, which might help to derive structural information. Chromatographic settings were adopted from the MRM method adjusting the MS to Information Dependent Acquisition (IDA) mode. The mass range of *m/z* = 50 - 500 was scanned with a threshold of 1,000 counts, a mass tolerance of 0,25 Da and low collision energy (10 eV). Enhanced product ion scans (EPI) recorded for the two most intense mass transitions with curtain gas adjusted to 30 V, ion source temperature to 550 °C, ion source gases one and two to 30 psi each, ion transfer voltage to 5000 V, declustering potential to 20 V, entrance potential to 10 V and rolling collision energy to 35 ±15 eV.

The Exactive^{TM} Plus (Thermo Fisher Scientific, Bremen, Germany) served for high resolution suspected target and non-target measurements. The suspected target approach was based on findings from 27 studies that considered diclofenac degradation products from different processes and detected 74 producs [27-53].

In non-target experiments products with *m/z* = 50 - 750 were generated at 35 eV collision energy and recorded via an all ion fragmentation scan using the exact mass with a mass resolving power of 70,000 and the isotopic pattern. The Sieve^{TM} software (version 2.1) compared obtained results to literature data by aligning a provided bibliography to a list of hits. Peaks were processed in positive and negative ionization mode with a threshold of 100,000 counts per second and a S/N ratio of ten or more. Peaks occurring in reference samples were excluded.

4.5 Results and discussion

4.5.1 Method validation

Table 4.3 presents the LOD, the LOQ and recovery rates of diclofenac in spiked sand, sewage sludge and biochar samples. The LOD results from a S/N ratio of 3, the LOQ from a S/N ratio of ten.

Table 4.3 Diclofenac limits of detection and quantification in spiked sand, sewage sludge and biochar samples, indication of best values. Recovery rates of sample preparation (n = 5)

	Inert experiments	Sewage sludge	Biochar
Limit of detection	0.03 µg/L	0.6 µg/kg_{DM}	1 µg/kg_{DM}
Limit of quantification	0.1 µg/L	1.8 µg/kg_{DM}	3 µg/kg_{DM}
Recovery rate	104 ± 2.4%	84 ± 5.3%	105 ± 11%

Increased and varying limits in sewage sludge and biochar result from matrix effects. These effects require the standard addition method to quantify diclofenac.

Diclofenac distribution in the reactor was checked after spiking and stirring for 24 h at 500 rpm sampling the sewage sludge in six fractions (fraction 1 at the top, fraction 6 at the bottom of the reactor), which were extracted and measured separately. The overall recovery rate of 90 ± 20% showed no systematic deviation between the different samples. Therefore, diclofenac seems to distribute homogeneously inside the reactor.

4.5.2 Target analysis

4.5.2.1 Inert experiments

Inert experiments were carried out with diclofenac in sand with an initial concentration of 15.4 mg/kg_{DM}. This experimental setup helps to investigate the behavior of diclofenac under HTC conditions by avoiding influences of the matrix with replacement of the sewage sludge with sand. Fig. 4.1 displays diclofenac fate at varying HTC runtimes (5 min to 1 h). It shows that diclofenac was fully recovered after 24 h stirring without HTC (t = 0). The compound is distributed between liquid (84%) and adsorbed (16%) phase. A similar fraction of sorbed diclofenac (16 - 23%) was reported for grape bagasse [54] and low to moderate values in sandy soils [55].

Diclofenac was degraded within a few min in inert experiments with Ottawa sand and water. Tudja et al. reported that diclofenac starts degrading at 200 °C in thermal behavior studies [56], Additionally, Funke et al. have already observed that the carbonization process is accelerated by water [11], which might explain the decay at 190 °C.

Diclofenac degraded by 95% within 10 min, after 1 h it was completely removed (> 99%). Degradation proceeds equally in the aqueous and adsorbed phase. Pseudo first order reaction was observed with a rate constant k = 1.01 and 0.994 as coefficient of determination.

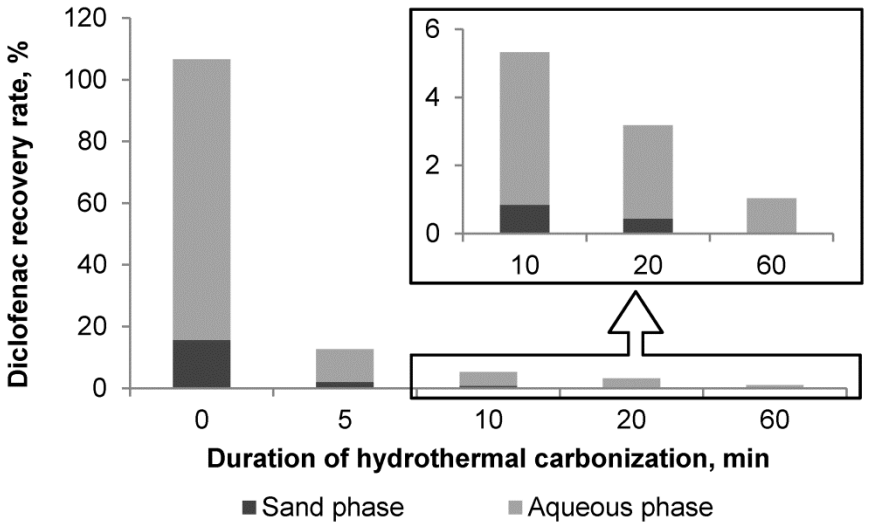

Fig. 4.1 Fate of diclofenac in the presence of inert Ottawa sand and water at 190 °C, 10 - 15 bar, 500 rpm and varying runtimes, c_0 = 15.4 mg/kg_{DM}. The sum of the sand phase and the aqueous phase results in the total diclofenac

4.5.2.2 Spiked sewage sludge

Fig. 4.2 presents the fate of diclofenac during the HTC of spiked sewage sludge (c_0 = 77 mg/kg_{DM}) from the WWTP Hollenstedt. Diclofenac was removed by 98% within 5 min runtime. Short heating to 190 °C (1 min) was enough to reduce diclofenac by 40% and further decrease was observed to the last sample taken after 60 min. Residual 0.2 mg/kg_{DM} remained in the biochar, which corresponds to 0.3% of initially added compound.

The experiment in sewage sludge considered the total concentration without differentiating between liquid and solid phase, because the applied 20%$_{DM}$ sewage sludge was hardly dewaterable. Ternes et al. already investigated diclofenac sorption to sewage sludge and reported log K_d values between 1.2 and 2.7 depending on the sludge characteristics [17]. These values suggest that the adsorbed part in sewage sludge exceeds the sorbed part in sand.

Despite the differences in the extent of sorption, one can transfer results obtained in inert experiments to spiked sewage sludge. Reaction also proceeds via pseudo first order while the rate constant of k = 0.70 is lower than in inert experiments and the coefficient of determination only amounts to 0.894, which indicates the influence of other reaction partners.

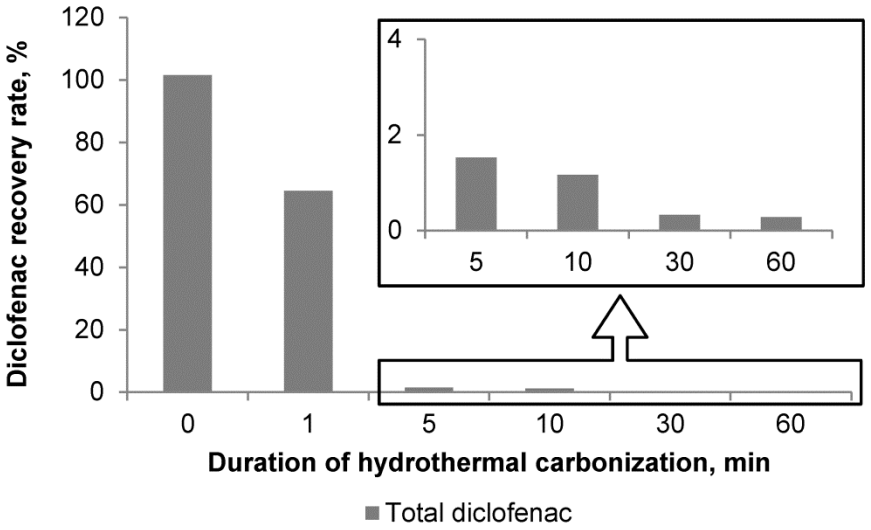

Fig. 4.2 Fate of diclofenac during the HTC of sewage sludge from the wastewater treatment plant Hollenstedt at 190 °C, 10 - 15 bar, 500 min^{-1} and varying runtimes, c_0 = 77 mg/kg_{DM}

4.5.2.3 Real samples

Based on the results in spiked sand and spiked sewage sludge, the same experiment was carried out with a native sample to investigate transferability. To that end, diclofenac concentration in sewage sludge was compared to its corresponding biochar, which was produced via a 4 h-HTC at 210 °C. The investigated sewage sludge contained 270 ± 40 $\mu g/kg_{DM}$. Other surveys reported comparable sewage sludge loads in Germany, Spain and France [18-20]. HTC reduced the diclofenac content to 120 ± 40 $\mu g/kg_{DM}$ in biochar, which corresponds to 56% compound removal.
Diclofenac decay in spiked sand and sewage sludge differs significantly from native samples. While Diclofenac was reduced by more than 99% in spiked samples, biochar from native sewage sludge still contained 44% of the initial load, although HTC was performed with longer runtimes and at higher temperature. Although the results confirm a general transferability of compound reduction from spiked to native samples, they also suggest that diclofenac in spiked samples might be to higher extent still solved in the solution or only attached to the surface of particles. Diffusional limitations, which also lead to kinetic limitations, might hinder strong adsorption and diffusion into the particles. In contrast, in real samples the pressure and temperature exposure could not lead to a full removal because the active ingredient might be shielded by the matrix through absorption or complexation. The described process of adsorbed molecules intercalating narrow spaces has been mentioned in pore hysteresis [57]. Native contaminants age with the sample matrix leading to sorption binding sites, which become less accessible by time. Burford et al. also reported a different extraction yield of spiked and environmental samples [58]. They concluded that neither the extraction procedure nor sample aging after spiking compensates for this phenomenon.
Fractions sorbed to non-accessible binding sites are also called NER. The target approach

impedes to differentiate between NER formation during HTC and compound degradation. Therefore, non-target and suspected target approaches are essential to illuminate the removal mechanism.

4.5.3 Non-target analysis

Diclofenac transformation products were investigated using a non-target screening and a suspected-target approach.

Six products, which have previously been reported in biological and chemical diclofenac transformation like ultraviolet (UV) treatment, were identified in the biochar via the suspected-target screening (table 4.4). No additional products were identified during the non-target analysis.

Table 4.4 Transformation products detected via a suspected target screening from hydrothermal carbonization of diclofenac, c_0= 15.4 mg/kg_{DM} in inert experiments and c_0 = 77 mg/kg_{DM} in sewage sludge

	Experimental mass, *m/z*	Calculated mass, *m/z*	Ionization mode	Experiment	Δ ppm	Formula	Transformation process in literature	References
D-I	311.0110	311.0116	ESI (+)	Inert	1.9	$C_{14}H_{11}NO_3Cl_2$	Photo-catalytic, UV, UV/H_2O_2, O_3, bacterial, electro-chemical, ultrasonic, ClO_2	[28-31, 33, 36-38, 40, 42, 43, 45, 48, 50-52]
D-II	326.0044	325.9987	ESI (-)	Inert + spiked sewage sludge	17.5	$C_{14}H_{10}NO_4Cl_2$	UV, Photo-catalytic	[43, 46]
D-III	308.9964	308.9959	ESI (-)	Inert	1.5	$C_{14}H_9NO_3Cl_2$	Photo-catalytic, bacterial	[29, 30, 38, 43, 52]
D-IV	281.0011	281.0010	ESI (-)	Inert	0.4	$C_{13}H_9NO_2Cl_2$	Photo-catalytic, O_3, electro-chemical, ClO_2, UV	[27, 30, 33, 43, 45, 47, 49, 51]
D-V	258.0323	258.0322	ESI (+)	Inert	0.5	$C_{14}H_9NO_2Cl$	UV, UV/H_2O_2	[27, 39, 52]
D-VI	230.0401	230.0373	ESI (-)	Inert + spiked sewage sludge	12.4	$C_{13}H_9NOCl$	UV	[27]

The most frequently reported product from other studies, product D-I, hydroxy-diclofenac (*m/z* = 311), was detected after inert HTC experiments. However, intensity was rather low, which might indicate high reactivity. The product is supposed to form from diclofenac. Sein et al. described hydroxyl-diclofenac formation via aminyl radicals, which originated from diclofenac reacting with ozone [48]. During HTC other reaction partners might form reactive intermediates. For example, highly reactive hydroxymethylfurfural emerges from HTC of cellulose material [11]. The exact position of hydroxylation at each of the aromatic rings in diclofenac remains unknown, although the most probable site of attack is at position C-4 (see fig. 4.4, product D-I') due to the *para* position to the -NH substituent at the more electron-rich aromatic ring and the large HOMO coefficient [43]. However, the obtained MS^2 spectra give no clear answer to this hypothesis. The most intense fragment (*m/z* = 242) merely confirms that the molecule contains two chlorines. Radical attack at position C-3, C-5 or at the other aromatic ring (see fig. 4.4, product D-I'') has also been reported for oxidative reactions [36, 50].

Product D-I might further react to D-II (*m/z* = 326) by adding another hydroxyl group to the molecule, either at the same ring as the first hydroxyl group or at the dihalogenated ring. Lower retention than diclofenac suggests a high polarity of the product D-II. The reaction might proceed via a diclofenac-2,5-iminoquinone intermediate as it was described for the reaction with ozone [48]. However, the intermediate was neither detected in inert experiments nor in spiked sewage sludge. Instead, product D-II was detected in large quantity in spiked sewage sludge. In contrast, the detected amount in inert experiments was low. This suggests that product formation benefits from the many -OH groups provided by the sewage sludge matrix.

D-III might be obtained from the intermediate product D-I by loss of two hydrogens resulting in diclofenac-2,5-quinone imine. Kosjek et al. reported that the most probable structure is a benzoquinone imine species [38]. This product occurred in highest amounts in inert experiments indicating a good stability. The compound has also been reported during oxidative processes and biotransformation [30, 38, 48, 52]. However, D-III was not detected in sewage sludge. On the one hand, sewage sludge might enhance further transformation. On the other hand formation of product D-II might be accelerated instead of product D-III.

Product D-IV was detected in inert experiments. It is supposed to contain a keto group attached to the aromatic ring structure. Additionally, a hydroxyl group replaces the carboxyl group following an oxidative decarboxylation reaction resulting in the product with *m/z* 281. The fragmentation pattern shows intense fragments at *m/z* 265, 247 and 135 (fig. 4.3). Fragment 247 indicates the abstraction of one chlorine. Fragment 135 indicates that the keto group is positioned at the other aromatic ring. This confirms the suggestion of initial hydroxylation at the more electron-rich aromatic ring for the intermediate product D-I.

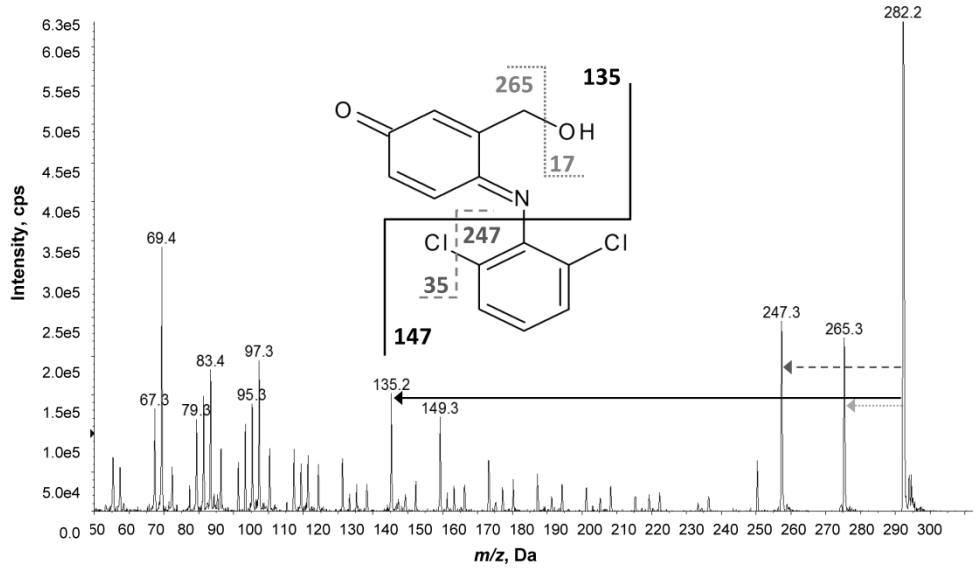

Fig. 4.3 ESI(+)-MS² spectrum of product D-IV produced during hydrothermal carbonization of diclofenac in inert experiments

Besides the described reaction pathway, dechlorination leads to cyclisation of the monohalogenated carbazole leading to the cyclized dehydrochlorination product D-V, *m/z* 258. This reaction has previously been described in phototransformation and UV/H_2O_2 of diclofenac [43, 44]. Though, dehalogenation reactions usually occur in reductive environment. While most studies report that the reaction requires microbial mediation, also abiotic reductive dechlorination pathways have been reported for diclofenac [59, 60]. The high electro-negativity of the leaving group stabilizes the molecule's transition state, which is followed by addition of an electron provided by the water [44]. The carbon centered radical then undergoes intra-molecular transformation resulting in product D-V. Its detection in inert experiments only and in rather low intensity indicates a low stability.

Product D-V might further degrade to D-VI via replacement of the carboxyl group by a hydroxyl group. Although intensity of the product D-VI is low, it was detected in inert experiments and in biochar from spiked sewage sludge. Aguera et al. have described this pathway in more detail for diclofenac under sunlight exposition [27]. However, the other intermediates described in their study were not detected under HTC conditions.

Fig. 4.4 summarizes a proposed degradation mechanism. The proposed positions of the substituents have not been verified.

The main transformation products described in experiments with spiked sand and spiked sewage sludge provide a first insight into the degradation mechanisms of organic pollutants during HTC. However, these transformation products were rather detected with low intensities compared to diclofenac. This hampers their detection in real samples. Neither the degradation products from inert experiments nor any other products were detected in real samples.

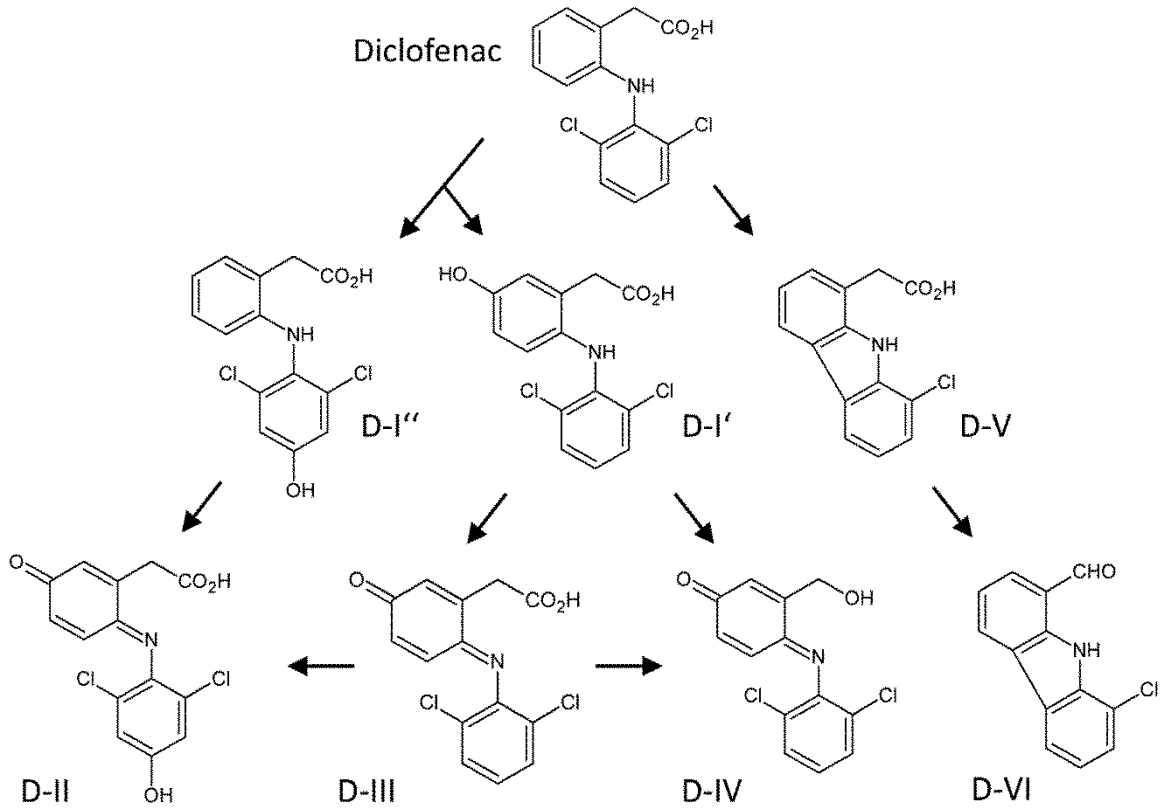

Fig. 4.4 Proposed transformation pathway of diclofenac during hydrothermal carbonization. The proposed positions of the substituents have not been verified

4.6 Conclusion

Diclofenac was fully removed at HTC conditions in inert experiments and spiked sewage sludge. Considerable compound reduction was also achieved in native sewage sludge. A suspected-target approach identified six transformation products in inert experiments, two products also occurred in spiked sewage sludge. Based on the detected products, a reaction pathway was postulated for diclofenac removal during HTC for the first time.

Overall, the study showed that degradation is the main mechanism to remove organic trace compounds during HTC. Typical HTC reactions were identified and might be transferred to other compounds in a next step.

Transformation products frequently show high mobility in aquatic systems. Therefore, the produced biochar should be comprehensively evaluated considering the risk of transformation products to contaminate soil and water bodies.

4.7 Acknowledgement

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5. Occurrence of perfluorinated compounds in sewage sludge and biochar produced by hydrothermal carbonization

considered to submission in Chemosphere

5.1 Abstract

HTC has emerged as a process, which reduces organic pollutants in sewage sludge by elevated temperature (> 190 °C) and elevated pressure (~ 15 bar) within 4 h producing biochar. This study developed an HPLC-MS/MS method to investigate the behavior of perfluorinated compounds (PFC) during the HTC of sewage sludge, because of the widespread environmental distribution of PFC and their high persistence. The internal standard method was compared with standard addition for quantifying the PFC. Behavior during HTC was examined in inert experiments with sand and real sewage sludge samples.

The internal standard approach revealed severe matrix effects for the sulfonated compounds resulting in enhanced recovery rates (1,600 - 1,900%). The standard addition approach yielded good recovery rates for sewage sludge samples (61 - 100%) and biochar (62 - 83%) except for perfluorobutane sulfonate (PFBA). Inert experiments showed full removal of perfluorooctanoic acid (PFOA) at 210 °C, while perfluorooctane sulfonate (PFOS) load was halved, which was corroborated in real sewage sludge, where HTC reduced the PFC load by 70%.

5.2 Introduction

Anthropogenic sources cause the widespread occurrence of persistent PFC in soils and water bodies [1-3]. Besides industrial sources, where PFC can be treated on-site, diffuse sources contribute to the ubiquitous distribution. The compounds may also enter the wastewater through domestic households and are transferred to WWTP. The major part of the PFC remains stable in the sewage sludge [4, 5]. Nevertheless, parts of the PFC enter the water cycle. There, occurrence of perfluorooctane sulfonate (PFOS), for example, is regulated to 0.65 ng/L by the environmental quality standards of the European Water Framework Directive. The actual PFOS load is considerably higher, studies reported 7.5 - 449 ng/L in rivers and WWTP effluents [6, 7].

Agricultural application of the sewage sludge, which contains PFC in the $\mu g/kg_{DM}$ range [7], comes along with the entry of PFC into the soil and adjacent rivers [8]. The topic of PFC has gained particular attention as adverse health effects of PFC have been reported [9]. Although they are not metabolized, transport in the body leads to their detection in serum, kidney and liver, where they

accumulate. Links between PFC exposure and mortality or cancer have been described [9].
Therefore, the German fertilizer regulation included the PFC as the sum of perfluorooctanoic acid (PFOA) and PFOS into their regulation. PFC in sewage sludge for agriculture are limited to 100 µg/kg_{DM}, a declaration is mandatory above 50 µg/kg_{DM} [10]. However, regulation only covers PFOA and PFOS, the other PFC remain disregarded yet, although they show similar environmental and toxicological behavior [9, 11].
Various studies developed methods to determine PFC in sewage sludge, which was reviewed recently by Arvaniti and Stasinakis [5]. PFC loads in sewage sludge range between 0.01 µg/kg_{DM} and 10,000 µg/kg_{DM}, depending on the site of the WWTP and the composition of the wastewater [5]. Typically, PFOS concentration exceeds that of the other PFC and digested sludge contains more PFC than primary sludge [12, 13].
In recent years, HTC has emerged as a method to treat sewage sludge with high temperature (190 - 250 °C) at elevated pressure (~ 15 bar) in the absence of oxygen to improve its fertilizing properties. On the one hand, the material is disinfected by HTC. On the other hand, the process has shown the potential to reduce environmental contaminant loads [14] but the fate of PFC during the HTC process has not been investigated yet.
Therefore, this study was designed to investigate the load of PFC in sewage sludge and biochar from HTC. Based on these experiments the potential to reduce PFC in agricultural fertilizers by converting sewage sludge into biochar by use of the HTC process was evaluated.

5.3 Materials and methods

5.3.1 Chemicals

Water for LC-MS, acetonitrile, and methanol were achieved from Th. Geyer GmbH & Co. KG (Renningen, Germany). Wellington (Guelph Ontario, Canada) supplied Perfluoro-n[1,2,3,4-$^{13}C_4$] octanoic acid (MPFOA) and sodium perfluoro-1-[1,2,3,4-$^{13}C_4$] octanesulfonate (MPFOS) and a PFC mixture (PFAC-MXA) containing perfluorobutyric acid (PFBA), perfluoropentanoic acid (PFPA), perfluorohexanoic acid (PFHxA), tridecafluoroheptanoic acid (PFHpA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), PFOA and PFOS. Single compound standards of PFOA and PFOS were purchased from Sigma-Aldrich (Taufkirchen, Germany). Fisher Scientific GmbH (Schwerte, Germany) supplied extra pure 20 - 30 mesh sand from Ottawa, Canada. Sewage sludge was received from the WWTP Hollenstedt located in the south-west of Hamburg, Germany.

5.3.2 Hydrothermal carbonization

For the HTC process 80 g sewage sludge were adjusted to 20%$_{DM}$ using HPLC/MS water and filled

into a 0.2 L high pressure reactor (Limbo, Büchi Glas, Uster, Switzerland). After heating for one h the reactor attained the final temperature of 210 °C. The temperature was maintained for four h and cooled down to room temperature again. The bls 2.5 software (Büchi Glas, Uster, Switzerland), recorded all adjustments including a continuous stirring rate at 500 rpm and the pressure, which raised up to 15 bar within the run.
In inert experiments sewage sludge was replaced by Ottawa sand. The experiments were carried out as described above at target temperatures of 180 °C, 210 °C and 240 °C.
In control experiments, the compounds were added to the material inside the reactor, which was stirred for 24 h without performing the HTC.

5.3.3 Extraction and clean-up

After carbonizing the sewage sludge the biochar was filtered to separate the liquid phase. Extraction of sewage sludge and biochar was carried out using PLE according to Llorca et al. [13]. Briefly, 1.00 g of the sample was weighted into an 11 mL cartridge and filled with Ottawa sand. At a temperature of 70 °C and a pressure of 100 bar, the PFC were extracted with 20 mL methanol within two cycles of seven min with 100% flush volume and 60 s nitrogen purge. The extracts were dried under a gentle nitrogen stream at 40 °C and reconstituted with 50 mL water followed by 5 min in the ultra-sonic bath and another filtration step.
SPE was applied to clean-up the samples following the German Institute for Standardization (DIN) 38414-4 [15]. Strata X-AW 33 µm polymeric weak anion cartridges (Phenomenex, Aschaffenburg, Germany) were conditioned using 5 mL 0.1% NH_3 in methanol and 5 mL methanol. Subsequently, equilibration of the material was done with 5 mL water before the extracted samples were applied. Afterwards, three washing steps minimized matrix interferences: First, 5 mL water, second, 5 mL 1% formic acid in acetone:acetonitrile (1:1) and third, 5 mL methanol was applied. The target analytes were eluted from the cartridge using 3 x 3 mL 0.1% NH_3 in methanol with subsequent drying under nitrogen at 40 °C. The samples were dissolved in 1 mL methanol and filtered prior to the HPLC-MS/MS analysis. With this procedure 1 g sample results in 1 mL extract. Therefore, the unit ng/mL equals the corresponding unit $\mu g/kg_{DM}$.

5.3.4 HPLC-MS/MS measurement

Samples were analyzed using an LC 20 HPLC (Shimadzu, Duisburg, Germany) connected to a 3200 QTRAP (AB Sciex, Darmstadt, Germany) MS. A Nucleodur Sphinx column (150 x 2 mm, 3 µm, Macherey-Nagel, Düren, Germany) was applied for chromatographic separation. The solvents water and methanol contained 0.01 M ammonium acetate and 0.1% acetic acid. 20 µL of the sample was separated in a 10-min linear gradient at a flow rate of 0.3 mL/min and an oven

temperature of 40 °C. The MS system was operated in the MRM mode with optimized settings for curtain gas (10 psi), ion source temperature (600 °C) and ion source gases 1 (80 psi) and 2 (40 psi) using negative electrospray ionization (-4,200 V). MRM transitions are depicted in table 5.1.

Standard addition and internal standards method were compared for quantification. To that end, we added known amounts of PFC or the internal standards, respectively, to the samples prior to PLE. MPFOS served for quantification with internal standards of PFBS, PFHxS and PFOS. The other compounds were quantified by use of the internal standard MPFOA.

Table 5.1 Compound specific MRM parameters for the mass spectrometric detection of the selected perfluorinated compounds

Compound	Quanti-fication transition	Verification transition	Declus-tering potential	Entrance potenial	Collision cell entrance potential	Collision energy	Collision cell exit potential
	m/z	*m/z*	V	V	V	eV	V
PFBA	213 → 169	none	-20	-3.0	-14	-12	-2
PFPA	263 → 219	263 → 69	-20	-2.0	-14	-12	-4
PFHxA	313 → 269	313 → 91	-20	-2.0	-16	-12	-2
PFHpA	363 → 319	363 → 169	-25	-2.5	-18	-14	-4
PFOA	413 → 369	413 → 169	-20	-3.5	-18	-14	-10
PFNA	463 → 419	463 → 219	-25	-2.5	-20	-14	-12
PFDA	513 → 469	513 → 219	-25	-4.0	-24	-16	-14
PFBS	299 → 80	299 → 99	-60	-3.5	-24	-52	-8
PFHxS	399 → 80	399 → 99	-60	-4.0	-20	-66	-8
PFOS	499 → 80	499 → 99	-85	-4.0	-24	-60	-2
MPFOS	503 → 80	503 → 99	-85	-4.0	-31	-88	-2
MPFOA	417 → 372	417 → 169	-20	-3.5	-28	-14	-10

5.4 Results and discussion

5.4.1 Method validation

Blank samples were checked for PFC contamination, which might occur in lines, connectors and other components of the sample preparation and the analytical system. Nine investigated PFC remained below the LOQ in the method blank. Only PFBA showed an instrumental blank concentration of 1.5 ng/mL, which is assumed to originate from the tubes. Therefore, the instrumental LOQ for PFBA was set to 2 ng/mL.

In sewage sludge and biochar, co-eluting matrix constituents interfered with the determination of the PFC leading to higher LOQs. For example, the instrumental LOQ of PFHxS (0.3 ng/mL) was considerably lower than 3.5 ng/mL in sewage sludge (corresponding to 3.5 $\mu g/kg_{DM}$) and 3.4 ng/mL in biochar (corresponding to 3.4 $\mu g/kg_{DM}$). Averaged LOQ from three experiments using standard addition are depicted in table 5.2 together with the instrumental LOQ.

Most compounds showed similar LOQ in the sewage sludge and the biochar matrix. For PFBA, PFPA and PFHxA higher deviations (SD) were obtained because the biochar matrix interfered with their mass transitions. For PFBS matrix effects caused a high LOQ of 21 $\mu g/kg_{DM}$ in sewage sludge and 17 $\mu g/kg_{DM}$ in biochar.

Table 5.2 Limits of quantification (LOQ) of perfluorinated compounds. Standard addition was applied for quantification. In sewage sludge and biochar the instrumental LOQ in ng/mL equals the corresponding unit $\mu g/kg_{DM}$

	Instrumental LOQ ng/mL n= 7	Sewage sludge LOQ $\mu g/kg_{DM}$ n = 3	Biochar LOQ $\mu g/kg_{DM}$ n = 3
PFBA	2.0	3.6	9.1
PFPA	0.2	8.5	16
PFHxA	0.2	3.4	23
PFHpA	0.2	8.8	6.3
PFOA	0.2	2.3	1.5
PFNA	0.3	3.5	3.4
PFDA	0.3	1.8	2.0
PFBS	0.2	21	17
PFHxS	0.1	6.8	5.3
PFOS	0.2	6.0	9.2

We investigated the recovery rates by spiking the compounds into the sample prior to PLE. Method recovery was checked by quality control (QC) samples (see fig. 5.1), which contained inert sand material instead of sewage sludge or biochar. All 10 investigated PFC show good recovery rates during extraction and clean-up despite the many sample preparation steps. Relative SD remained below 10% for most PFC indicating good reproducibility except for PFHpA (21%), PFHxS (17%) and PFOS (13%).

Recovery rates were also determined in sewage sludge and biochar samples comparing standard addition and the internal standard method, which is shown in fig. 5.2.

Fig. 5.2 A displays reduced recovery rates for some PFC in sewage sludge compared to the QC samples. Nevertheless, for most compounds recovery rates of 70 - 80% were achieved. Only for PFBA considerable losses of 71% were observed with both approaches. Altogether, the approach of standard addition resulted in slightly higher recovery rates (75%) compared to using internal standards (67%). Likewise, a lower SD of 13% using standard addition compared to 17% by use of internal standards was achieved. However, in both approaches the matrix of sewage sludge caused higher SD than observed for the QC samples.

Fig. 5.2 B depicts that the internal standard method resulted in insufficient recovery rates for biochar. This matrix suppressed the internal standard MPFOS, which resulted in enhanced calculated recovery rates up to 1,900% and high SD for the three PFC containing sulfonate groups.

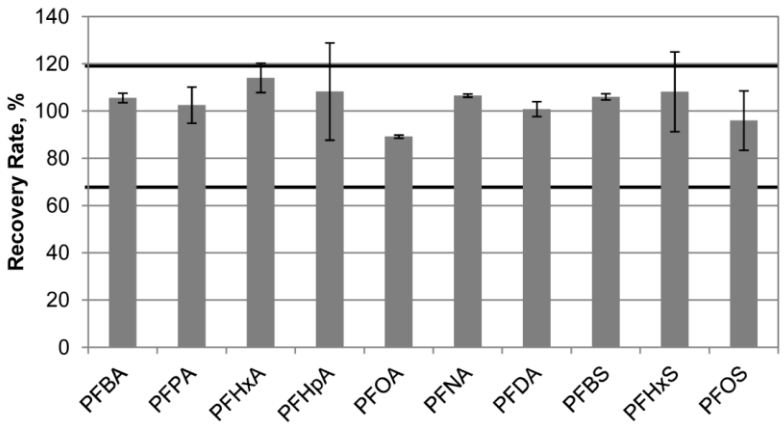

Fig. 5.1 Recovery rates of perfluorinated compounds in quality control samples, n = 5. The bold lines mark the accepted range of recovery rates between 70 and 120%

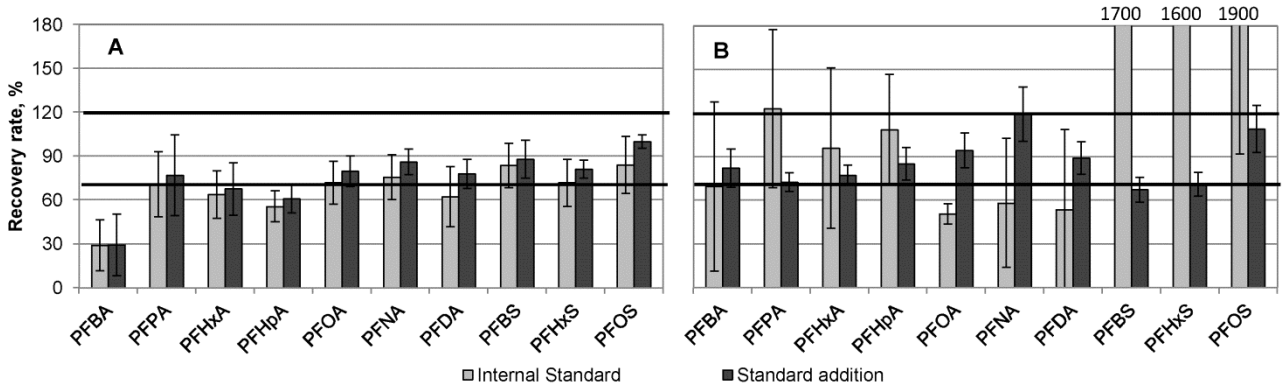

Fig. 5.2 Recovery rates of perfluorinated compounds in sewage sludge (A) and biochar (B) samples determined by the internal standard method (bright grey) and standard addition (dark grey), n = 3. The bold lines mark the accepted range of recovery rates between 70 and 120%

Fig. 5.3 shows the chromatogram for MPFOS in the different matrices. For comparison, PFBS chromatograms are also shown. The MPFOS signal was suppressed in biochar to a significant extent. No suppression was observed in sewage sludge. In contrast, PFBS was slightly suppressed in sewage sludge and considerably in biochar. However, suppression greatly differed from MPFOS. Therefore, correlation of both substances failed and MPFOS could not serve as internal standard for PFBS. Wang et al. and Radjenovic et al. have already reported that internal standards might fail to compensate suppressed signals in complex matrix [16, 17]. Matrix effects differ on each MRM transition, which hinders comparability of analyte and its internal standard. The internal standard method also failed in a previous HTC study investigating pharmaceuticals in sewage sludge and biochar [18].

Recovery rates of the other seven compounds ranged between 51% and 123% (fig 5.2). However, SD was around 50% except for PFOA (6.9%).

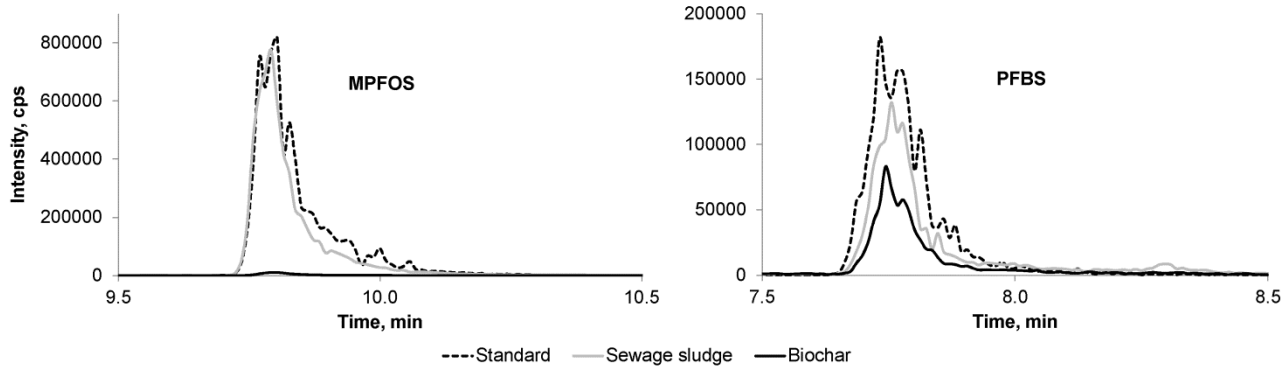

Fig. 5.3 Comparison of signal suppression for MPFOS (c = 200 ng/kg$_{DM}$) in comparison to PFBS (c = 50 µg/kg$_{DM}$)

In contrast, the use of standard addition showed good recovery rates for all PFC ranging from 67% to 119%. The averaged SD of 11% conforms to the matrix of sewage sludge (13%).
Clearly, only with the standard addition approach meaningful results were obtained in contrast to the use of internal standards. Therefore, this should be the method of choice when analyzing PFC in sewage sludge and particular biochar samples.

5.4.2 Inert experiments

Inert experiments were carried out with PFOA and PFOS in sand to check whether the compounds readily degrade during HTC. Fig. 5.4 displays the results comprising HTC temperatures of 180 °C, 210 °C and 240 °C.
The reference test (24 h stirring) resulted in an overall recovery rate for PFOA of 79%, to which mainly the liquid phase contributed (78%) and only a minor fraction of PFOA was attached to the sand (1%). HTC removed half of the PFOA at 180 °C. At higher temperatures (210 °C and 240 °C) full removal (> 98%) was achieved. Considerable removal rates at HTC conditions have also been reported for other compound classes [14, 19].
The PFOS recovery rate after 24 h stirring amounted to 70% in the liquid phase and 7% extracted from the sand. Higher PFOS adsorption compared to PFOA has already been reported for sewage sludge and ascribed to hydrophobic interactions [20].
PFOS remains stable at 180 °C, but partial removal of 40% was observed at 210 °C and 240 °C. Considering its thermal and chemical inertia no degradation was expected for PFOS. However, the elevated pressure in the reactor might promote compound decomposition. The exact mechanism, however, remains unknown. Adsorbed PFOS amounts to 5 - 7% of the starting concentration independent of the applied temperature.

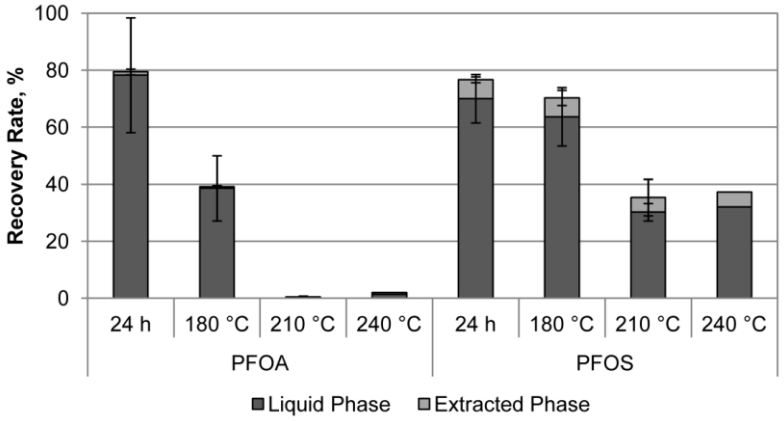

Fig. 5.4 Recovery rates of PFOA and PFOS after hydrothermal carbonization in inert experiments with sand at different temperatures, n = 2 for 24 h stirring, n = 3 at 180 °C and 210 C, n = 1 at 240 °C. The liquid phase is displayed in dark grey and extracted solid phase in bright grey

Overall, results suggest that HTC is appropriate to reduce PFOS and PFOA. Hori et al. also reported partial PFOS degradation in subcritical water (> 250 °C and 20 bar) in the presence of zero-valent iron. However, without addition of iron, they reported less degradation (< 10%) [21]. Other degradation processes like advanced oxidation have been attributed the potential to reduce PFC. Photo-Fenton, for example, degrades PFC releasing fluoride ion, carbon dioxide and shorter chain PFC [5]. With sulfate radicals fluoride ion release occurred preferable at low temperature (80 °C), while higher temperature (150 °C) promoted formation of 1H-perfluoroalkanes [22]. However, the reaction proceeds via a thermodynamically unfavored pathway, which limits the reaction in real systems. Accelerated temperature is supposed to accelerate removal, which has been shown in the presence of sulfate radicals for PFOA, which needed 648 h at 20 °C and 72 h at 40 °C for full removal [23].

5.4.3 Occurrence of PFC in sewage sludge and biochar

PFC removal during HTC was investigated for a real sample using sewage sludge from the WWTP Hollenstedt. Based on method validation, the PFC were quantified using the standard addition approach. Fig. 5.5 shows PFC concentrations in sewage sludge and the corresponding biochar after 4 h at 210 °C.

Eight of ten investigated PFC were detected in the sewage sludge. PFPA and PFBS remained below the LOQ. Concentrations ranged between 3.7 µg/kg_{DM} (PFHxA) and 43 µg/kg_{DM} (PFOS). PFOA and PFOS sum up to 51 µg/kg_{DM}, which is around the level requiring labeling according to the German fertilizer regulation. The sum of all PFC amounts to 104 µg/kg_{DM}, which agrees with levels reported in other European studies [6, 7].

After HTC, only PFBA and PFOS concentrations exceeded the LOQ. The sum of PFOA and PFOS concentrations remained below 50 µg/kg_{DM}. Therefore, the biochar product would not be subject to labeling when applied in agriculture. All detected PFC in biochar summed up to 32 µg/kg_{DM}, HTC reduced the PFC load by two third. However, no reduction could be confirmed for PFPA and PFHxA because the biochar LOQs exceeded their sewage sludge concentration. Nevertheless, the trend of reducing PFC loads by HTC was shown with this real sample. It also confirmed full removal of PFOA and halving of PFOS from inert experiments suggesting that the sewage sludge matrix may not considerably affect the PFC reduction.

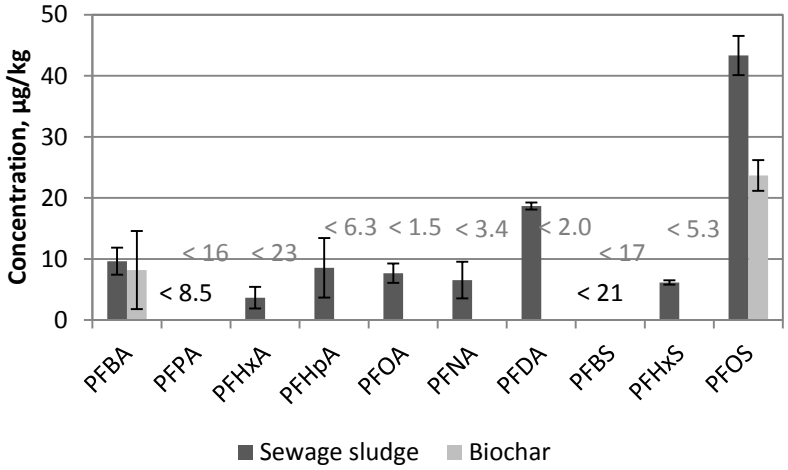

Fig. 5.5 Concentration of perfluorinated compounds in sewage sludge and biochar from hydrothermal carbonization determined by use of standard addition, n = 3

5.5 Conclusion

This study developed and validated an LC-MS/MS method to determine PFC in sewage sludge and HTC biochar. The quantification approaches internal standards and standard addition method showed good recovery rates in sewage sludge samples. In biochar, standard addition was necessary in order to avoid matrix effects. Inert experiments revealed the potential of HTC to reduce PFOA and PFOS load, which was corroborated in reals sewage sludge for all PFC. Following studies should focus on detailed reaction mechanisms and elucidation of transformation products of PFC during HTC.

5.6 Acknowledgement

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6. General conclusion and outlook

6.1 General conclusion

This thesis provides the first detailed insight into the HTC process considering the behavior of micropollutants in a complex medium by use of LC-MS methods.

New LC-MS/MS methods are presented to determine micropollutants in sewage sludge and biochar samples. The complex source and product matrices are visualized and evaluated using matrix effect profiles. Comparing different quantification methods reveals that matrix effects hinder common quantification approaches using external standards as they do not consider any matrix effects. Internal standards also fail because of different matrix effects on different mass traces in the MS. The complex biochar matrix requires analysis by use of standard addition over the whole analytical procedure despite of clean-up procedures, for example for the PFC.

Distinct compounds accumulate in sewage sludge during wastewater treatment, which is proven by the frequent determination of these compounds in sewage sludge. Therewith, they entail the risk to enter the environment, for example by applying the sludge in agriculture. Here, HTC is a promising technology to degrade micropollutants in sewage sludge while producing biochar. Pharmaceutical and PFC loads are considerably reduced, although not fully removed in all cases. Four of initially 11 pharmaceuticals and two of ten PFC are detected in low amounts in biochar. Compared to sewage sludge, only the load of phenazone is slightly increased. Inert HTC experiments with sand replacing the sewage sludge prove the general ability of the process to degrade micropollutants. Especially the potential of the HTC process to remove PFC might entail an interest for the scientific community as few methods can remove these recalcitrant compounds. The higher the temperature the better the removal is in the inert experiments with PFC. Inert experiments with diclofenac show that compound reduction is increased by increasing the HTC runtime.

Quantification methods for sewage sludge and biochar are crucial tools to evaluate the potential of biochar from HTC as a fertilizer or soil amendment. This thesis provides an evaluation tool by use of HPLC-MS/MS. With respect to micropollutants the biochar shows advantages compared to sewage sludge. Besides micropollutant removal, HTC increases the dewaterability of the input material for reactions exceeding one h, which might reduce the waste disposal costs. In fact, lower water content comes along with reduced transport costs. Additionally, lower water content facilitates subsequent incineration, which represents one of the main disposal routes for sewage sludge. Therewith, economic benefits are achieved. Otherwise, the process could replace or add on to conventional drying technologies like centrifugal systems or filter presses. Further advantages are described in other studies like decreased compound mobility in biochar [1] or an increased water holding capacity for sandy solids [2]. Based on these findings, one can conclude, that the residue sewage sludge can be transformed into a reusable biochar material.

Compound removal is followed in detail for diclofenac. HTC reactions, like dehydration and decarboxylation, which have been reported for glucose and lignin in an ideal medium, are shown for diclofenac in sewage sludge as well. This good transferability from glucose and lignin to diclofenac shows that the HTC process undergoes the same reactions despite of the surrounding medium and compounds present. Moreover, this indicates that all substances susceptible for these reactions really react this way. Hence, observing a distinct compound like diclofenac in its complex medium improves our understanding of transformation reactions in the HTC process. Results also show that mass spectrometry is a selective and sensitive tool to follow such processes even in a complex medium like sewage sludge.

6.2 Outlook

Further studies should enhance the understanding of micropollutant reduction during HTC. Behavior of other compound groups should be considered like PAH, PCB and volatile organic compounds. Especially, the increase of PCDD/F requires attention because high loads might restrict the application in agriculture. Preliminary tests have shown that the pH might be a decisive process parameter. Alternatively, Wiedner et al. [3] assume that dioxins only evolve from reactions above a temperature of 250 °C. Therefore, the dioxin load only depends on the initial content of the feedstock. However, they only investigate materials with low dioxin contents like heat straw, poplar wood and olive residues. The experimental plan should compare the load of PCDD/F in biochar after HTC of sewage sludge at different process parameters. Depending on these results, after-treatment methods might be required to guarantee a contaminant-free biochar product. However, the exact design and set up of the after-treatment of biochars has not been considered yet.

Hygienic aspects cause acceptance problems for the use of sewage sludge in agriculture. Therefore, the microbial load of material produced by HTC should be tested. Generally, a temperature of more than 121 °C for a few min is enough to sterilize water. However, effects of the complex HTC reaction medium should be addressed. These results could help to erase public doubts regarding the application on fields.

Besides, further notice of the pollutant load helps to evaluate the product quality. Especially, its capability to withhold the micropollutants in the biochar or its leaching behavior into adjacent water bodies plays a major role. Here, a higher holding capacity of biochar is expected compared to sewage sludge. However, little is known about the uptake of micropollutants in plants after fertilizing with biochar. First studies have reported promising results about the effects on plant growth and thriving. Yu et al. [4], for example, reported reduced plant uptake of pesticides with biochar additions to soil. Schulz et al. [5] concluded that biochar addition increases soil fertility. Contrary, Rillig et al. [6] reported reduced plant growth. Thus, the effect may be influenced by the

type of biochar and the source material. An after-treatment step might help to reduce negative effects after the HTC [7]. Still, identifying the cause of growth inhibition requires more research.

HTC of sewage sludge has already been transferred from laboratory scale to the first industrial scale plants. Building of HTC plants on-site provides a possible strategy because the plants can run efficiently at low size. However, more long-time experience with industrial scale plants is needed.

Besides adding to filed, the HTC biochar can also be incinerated. The heating value is high enough that mono-incineration plants can run with the biochar. An economic consideration of conventional handling, HTC plants on-site including subsequent utilization or bigger central HTC plants, for example next to incineration plants, should be compared regarding building and current costs.

Additionally to the mentioned topics, researchers should continue to enhance process elucidation in detail, for example by observing distinct degradation routes. Therewith, the process understanding for complex matrices could be deepened. Furthermore, one could learn more about the general behavior of pollutants in such processes, which could affect the optimum process parameters. These findings might also be transferred to other thermal or chemical processes.

Other analytical techniques to determine further physical, biological and chemical characteristics could help to deepen the process understanding. For example, gas chromatography could provide a complex picture of the gaseous compounds, for example the volatile organic compounds, produced by HTC. Another aspect is the nutrient distribution in the HTC product, which might provide important information about the fertilizing properties. In fact, either the biochar or the process water contains the nutrient rich fraction. Subsequently, availability of these nutrients in plants should be followed to investigate if the nutrients remain in an available mode for the uptake in plants. Concurrently, utilization options for the nutrient-poor fraction are required. The process water contains a high fraction of dissolved organic carbon [8]. This limits its utilization scope and complicates the further handling. One handling strategy tries to reduce the amount of water by recirculation. However, dissolved organic carbon and other pollutants might accumulate by and by. In another strategy a membrane filtration unit is attached to the process water flow, which cleans up the water efficiently but involves high costs and a highly concentrated waste fraction. Another possible strategy might be to include an advanced oxidation unit, which can reduce organic loads in wastewater streams for example. However, this has not been tested yet.

6.3 References

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7. Appendix

7.1 Chapter 2 - supplementary material (suppl.)

Suppl. 7.1 List of the 12 pharmaceuticals and their corresponding internal standards

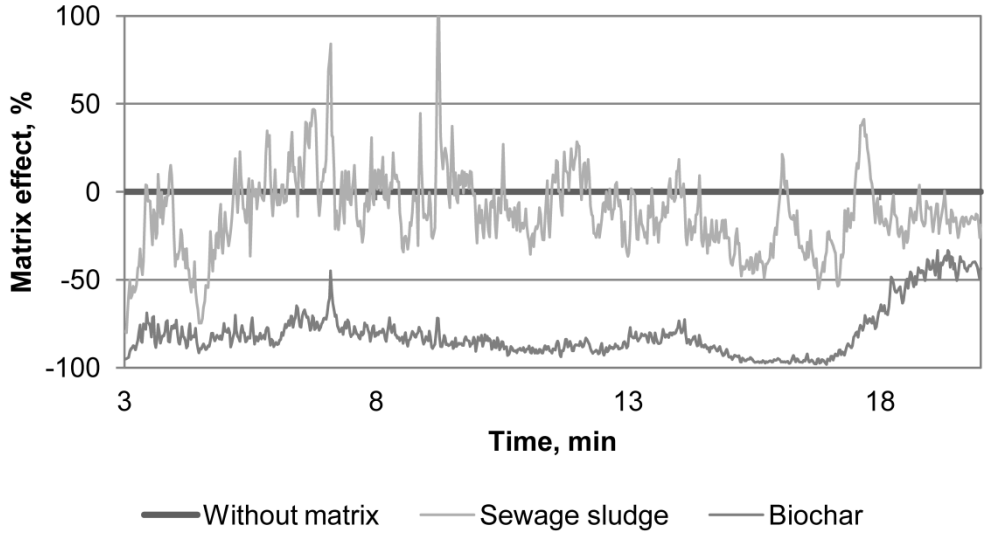
Compound	Internal standard
Diclofenac	Diclofenac-d4
Ibuprofen	Ibuprofen-d3
Phenazone	Cyclophosphamide-d6
Carbamazepine	Carbamazepine-d10
Sulfamethoxazole	Sulfamethoxazole-d4
Bezafibrate	Cyclophosphamide-d6
Fenofibric acid	Cyclophosphamide-d6
Metoprolol	Metoprolol-d7
Propranolol	Metoprolol-d7
Clarithromycin	Erythromycin-C_{13}-d3
Roxithromycin	Erythromycin-C_{13}-d3
Dehydrato-erythromycin	Erythromycin-C_{13}-d3

Suppl. 7.2 Qualitative comparison of clean-up via solid phase extraction (Strata-X, 500 mg, 6 mL, Phenomenex, Aschaffenburg, Germany) and nanofiltration (Amicon Ultra-4 Centrifugal Filter Devices, Millipore, Eschborn, Germany); analyte sensitivities from direct measurement are correlated to sensitivities after solid phase extraction and after nanofiltration in sewage sludge and biochar; samples were prepared with standard addition over the whole procedure; equal sensitivities (80-120%) are shown as "=", improved sensitivities (> 120%) are shown as "+" and sensitivity losses (< 80%) are shown as "-"

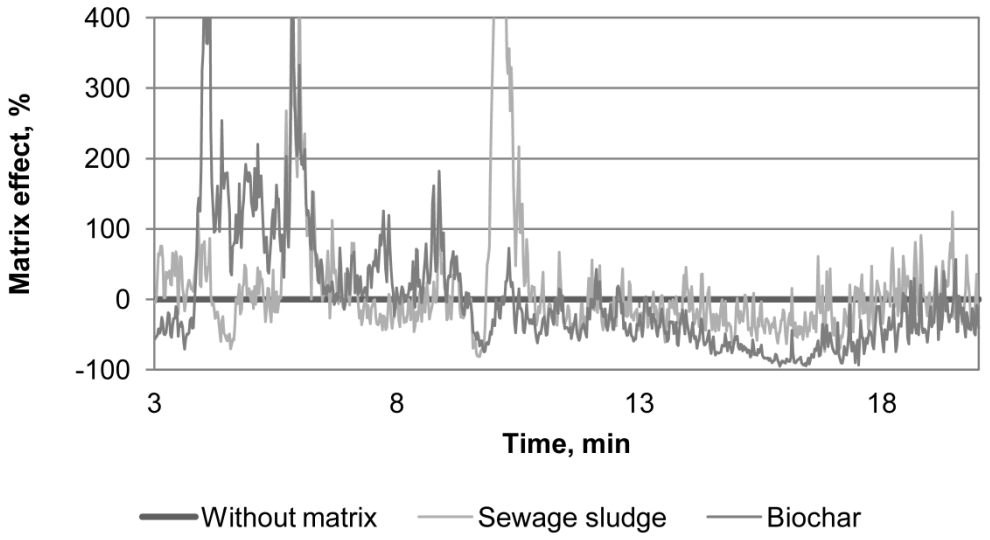
	Sewage sludge		Biochar	
	Solid phase extraction	Nanofiltration	Solid phase extraction	Nanofiltration
Diclofenac	+	+	-	+
Ibuprofen	=	-	-	-
Phenazone	+	+	+	-
Carbamazepine	=	+	+	+
Sulfamethoxazole	-	-	+	+
Bezafibrate	=	+	+	+
Fenofibric acid	=	+	=	+
Metoprolol	+	-	+	-
Propranolol	+	-	+	-
Clarithromycin	-	-	+	+
Roxithromycin	-	-	+	=
Erythromycin	+	-	=	-

Suppl. 7.3 Matrix effect profiles with water as the reference line; the bright grey area indicates signal suppression and enhancement for sewage sludge and the dark grey area reflects signal suppression in biochar measurement

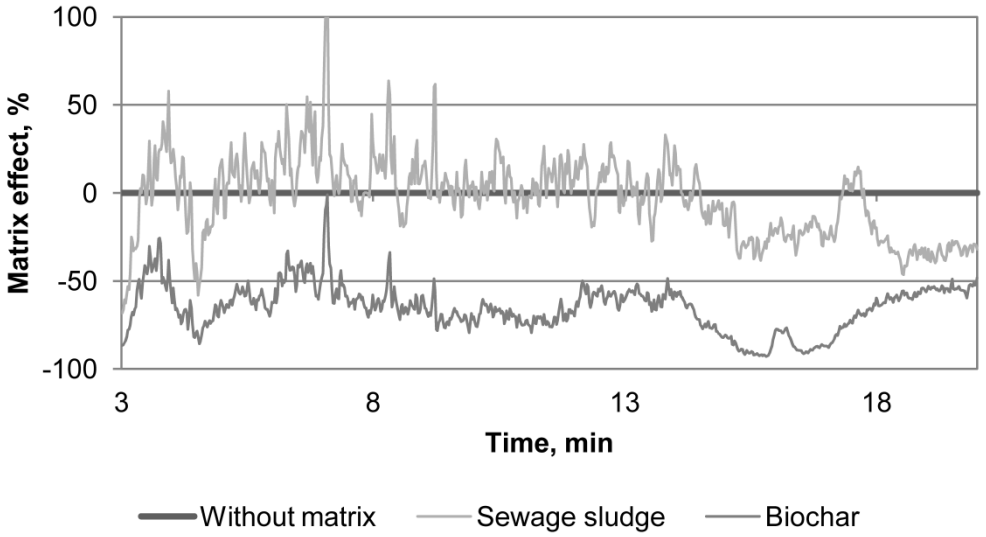
a) Diclofenac (expected retention time: 15.2 min), XIC: *m/z* = 296 → 214



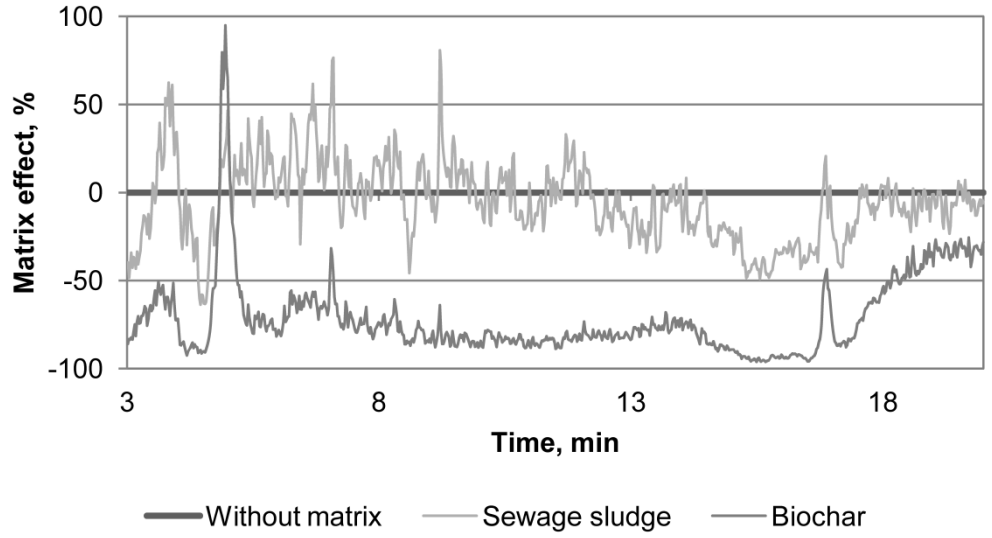
b) Ibuprofen (expected retention time: 15.3 min), XIC: *m/z* = 207 → 161



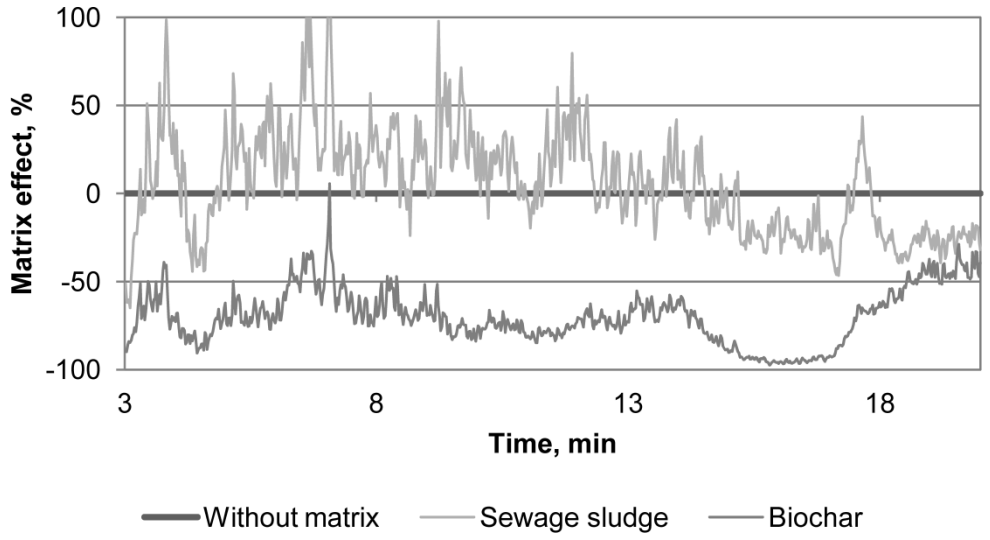
c) Carbamazepine (expected retention time: 12.3 min), XIC: *m/z* = 237 → 194



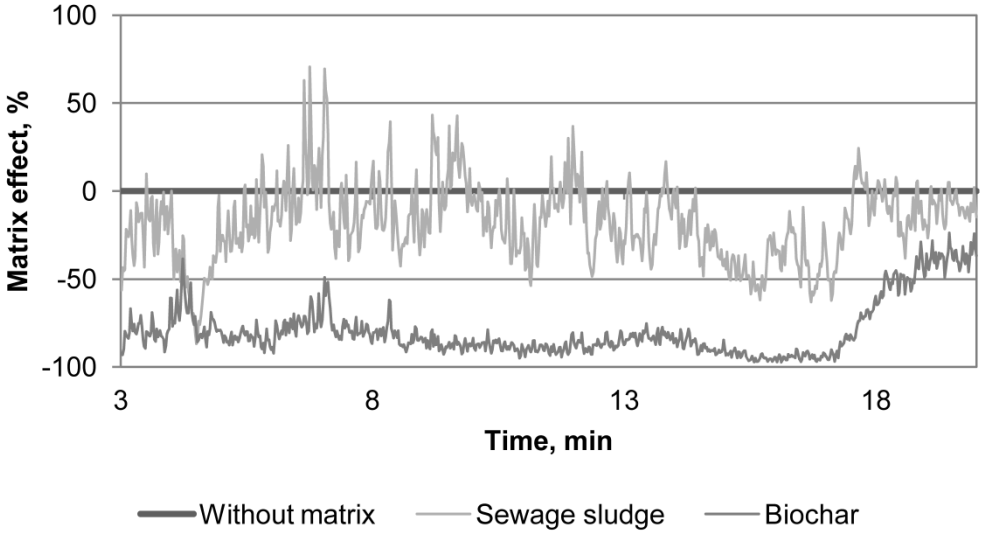
d) Sulfamethoxazole (expected retention time: 6.5 min), XIC: *m/z* = 254 → 156



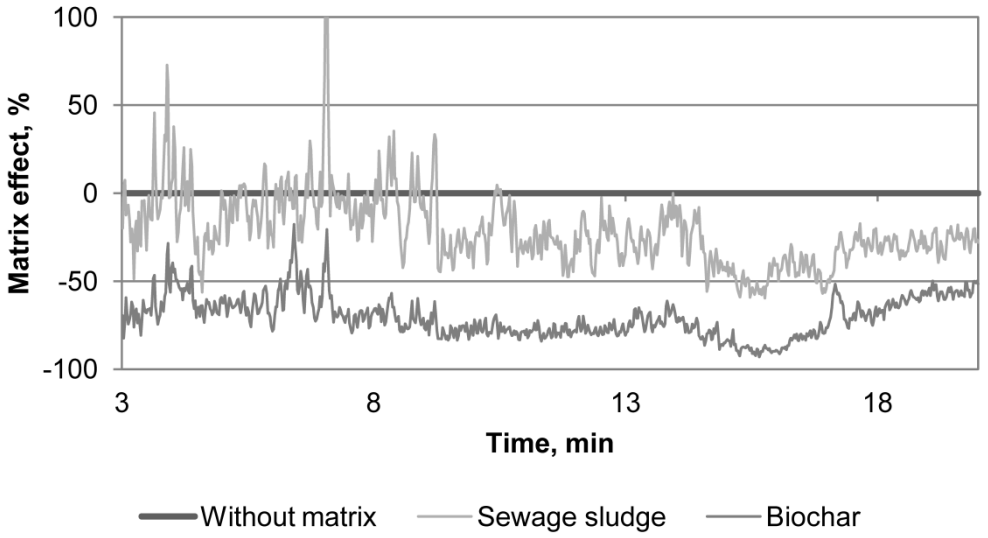
e) Bezafibrate (expected retention time: 14.6 min), XIC: *m/z* = 362 → 139



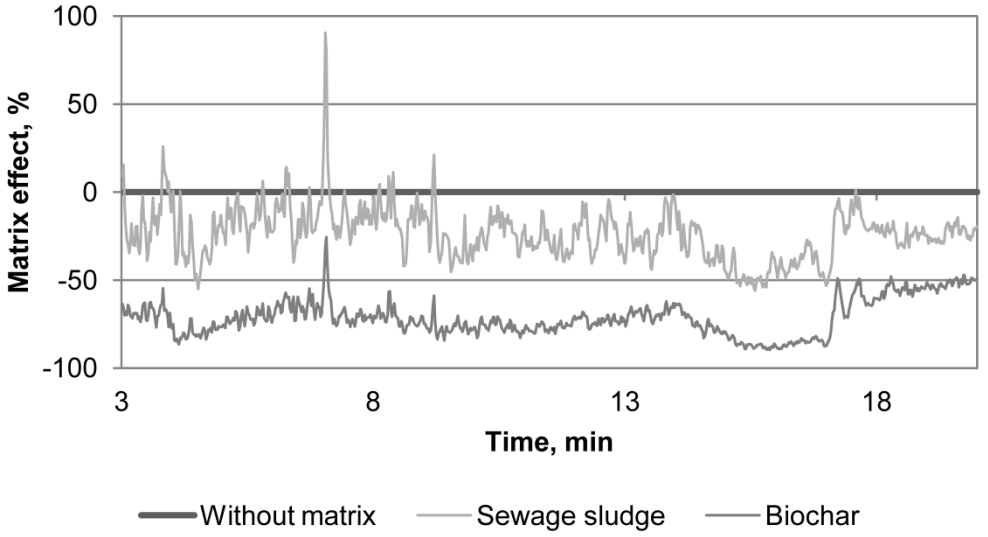
f) Fenofibric acid (expected retention time: 15.2 min), XIC: *m/z* = 319 → 139



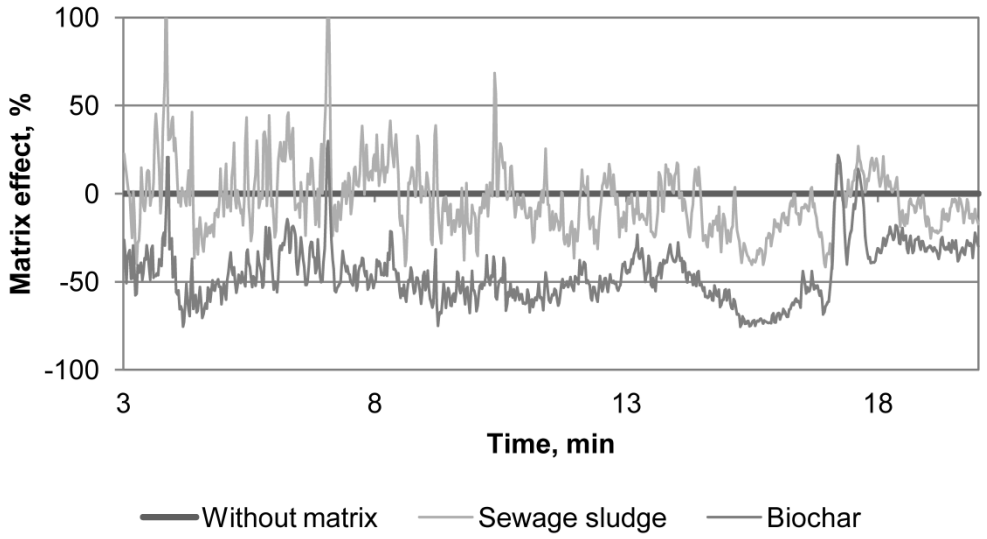
g) Metoprolol (expected retention time: 8.5 min), XIC: *m/z* = 268 → 72



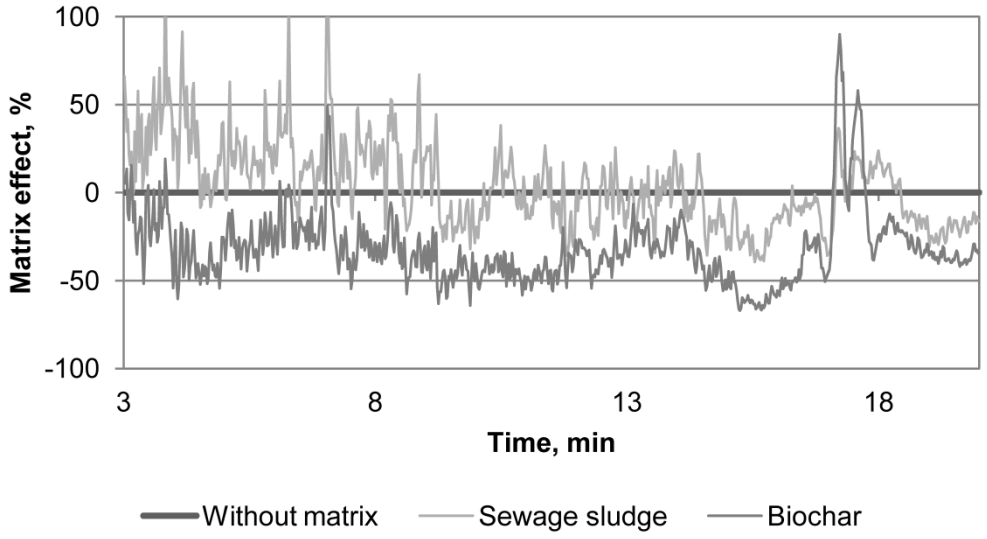
h) Propranolol (expected retention time: 12.0 min), XIC: *m/z* = 260 → 116



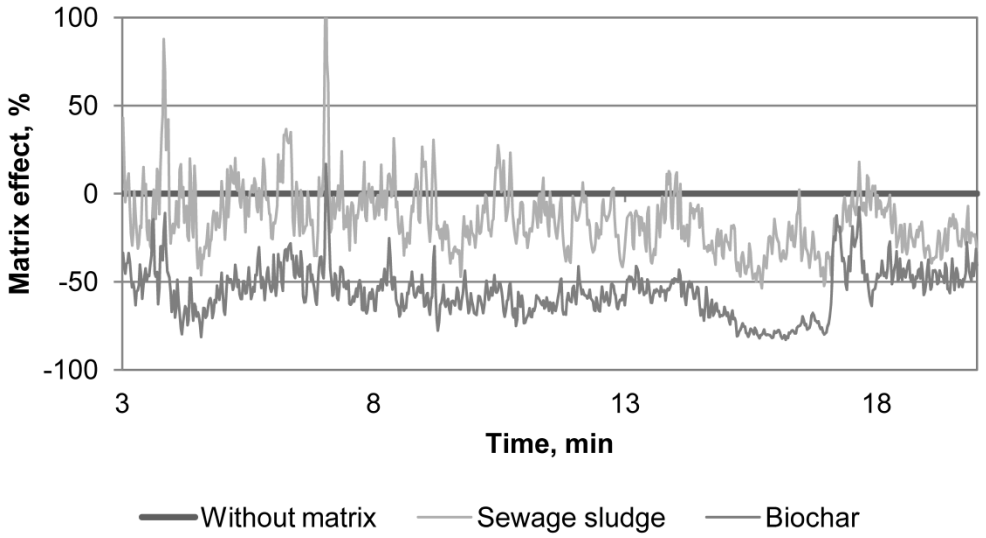
i) Clarithromycin (expected retention time: 15.1 min), XIC: *m/z* = 749 → 83



j) Roxithromycin (expected retention time: 15.0 min), XIC: *m/z* = 838 → 158



k) Erythromycin (expected retention time: 14.6 min), XIC: *m/z* = 716 → 83



Suppl. 7.4 Comparison of standard deviation (SD) and measurement uncertainty (MU) in sewage sludge and biochar, both given in %, in a quality control sample (n = 4) and for the quantification approaches external calibration (n = 3) and internal standards (n = 3) using type A evaluation of standard uncertainty; advanced MU with k = 3.2 for the quality control and k = 4.3 for external calibration and internal standard analysis is based on the t-distribution and defines a 95% confidence interval

			Sewage sludge				Biochar			
	Quality control		External calibration		Internal standard		External calibration		Internal standard	
	SD	MU	SD	MU	SD	MU	SD	MU	SD	MU
Diclofenac	12	20	113	282	9.2	23	102	254	17	42
Ibuprofen	8.6	14	147	366	39	98	295	733	n.d.*	n.d.
Phenazone	6.4	10	43	107	4.5	11	50	124	16	40
Carbamazepine	14	22	35	86	8.5	21	41	101	8.9	22
Sulfamethoxazole	11	18	60	148	9.4	23	51	127	13	33
Bezafibrate	8.5	14	58	145	6.5	16	66	165	26	64
Fenofibric acid	23	37	48	119	11	28	48	120	23	57
Metoprolol	6.0	10	103	256	12	30	80	198	14	35
Propranolol	9.9	16	112	279	7.1	18	78	194	17	41
Clarithromycin	10	16	55	137	25	62	135	334	21	53
Roxithromycin	12	19	71	175	18	44	78	193	6.5	16
Erythromycin	8.0	13	59	147	14	34	33	81	4.9	12

*n.d. = not determined, because less than three samples were evaluable

Suppl. 7.5 Comparison of standard deviation (SD) and measurement uncertainty (MU) in sewage sludge and biochar, both given in %, for the quantification approaches standard addition before LC-MS/MS and standard addition over the whole procedure using type B evaluation of standard uncertainty; factor k for advanced MU is based on the t-distribution for varying degrees of freedom (depending on the number of standards used in the calibration, υ = n − 2) and defines a 95% confidence interval

	Sewage sludge				Biochar			
	Standard addition before LC-MS/MS		Standard addition whole procedure		Standard addition before LC-MS/MS		Standard addition whole procedure	
	SD	MU	SD	MU	SD	MU	SD	MU
Diclofenac	24	29	12	38	38	130	3.0	57
Ibuprofen	n.d.*	14	4.0	47	n.d.	74	n.d.	78
Phenazone	12	23	5.6	27	17	13	4.0	13
Carbamazepine	4.8	16	1.9	36	12	22	1.4	18
Sulfamethoxazole	36	52	27	66	28	37	8.8	26
Bezafibrate	3.5	22	7.7	27	25	43	7.0	19
Fenofibric acid	39	20	11	30	25	65	4.2	18
Metoprolol	35	60	7.2	30	36	22	7.7	17
Propranolol	18	68	9.2	78	30	70	6.9	19
Clarithromycin	28	71	7.0	58	6.2	18	6.9	17
Roxithromycin	32	68	2.7	61	7.2	28	10	18
Erythromycin	20	47	6.5	41	8.0	23	7.7	16

*n.d. = not determined, because less than three samples were evaluable

Suppl. 7.6 Limits of detection and limits of quantification for the twelve investigated pharmaceuticals derived from six samplings of sewage sludge from the treatment plant Rheinhausen and six biochars from different production charges

	Limit of detection in sewage sludge					
	Sampling 1	**Sampling 2**	**Sampling 3**	**Sampling 4**	**Sampling 5**	**Sampling 6**
Diclofenac	7.1	25	3.2	4.5	4.3	6.3
Ibuprofen	20	47	58	91	260	93
Phenazone	6.1	4.9	1.2	4.6	2.1	4.7
Carbamazepine	13	15	1.3	1.1	0.9	1.1
Sulfamethoxazole	0.4	17	3.5	4.5	3.6	4.6
Bezafibrate	5.5	5.1	2.8	2.0	1.4	5.2
Fenofibric acid	35	10	9.5	4.0	1.4	4.0
Metoprolol	17	13	20	15	14	9.1
Propranolol	35	16	3.0	3.1	0.9	7.6
Clarithromycin	34	54	7.7	4.6	1.5	4.2
Roxithromycin	8.3	6.1	10	4.1	8.5	5.2
Erythromycin	33	13	10	2.3	4.6	16

	Limit of quantification in sewage sludge					
	Sampling 1	**Sampling 2**	**Sampling 3**	**Sampling 4**	**Sampling 5**	**Sampling 6**
Diclofenac	24	84	11	15	14	21
Ibuprofen	68	160	190	300	850	310
Phenazone	20	16	4.1	15	7.1	16
Carbamazepine	44	50	4.4	3.8	2.8	3.8
Sulfamethoxazole	1.2	58	12	15	12	15
Bezafibrate	18	17	9.4	6.6	4.7	17
Fenofibric acid	120	34	32	13	4.6	13
Metoprolol	57	45	68	50	47	30
Propranolol	120	52	10	10	2.8	25
Clarithromycin	110	180	26	15	5.1	14
Roxithromycin	28	20	34	14	28	17
Erythromycin	110	44	34	7.7	15	53

	Limit of detection in biochar					
	Sampling 1	**Sampling 2**	**Sampling 3**	**Sampling 4**	**Sampling 5**	**Sampling 6**
Diclofenac	1,300	210	160	110	44	190
Ibuprofen	400	260	960	1,100	2,200	990
Phenazone	17	33	20	24	22	40
Carbamazepine	38	23	9.2	22	6.8	17
Sulfamethoxazole	260	20	92	110	110	120
Bezafibrate	69	44	1.8	11	5.3	8.7
Fenofibric acid	180	56	10	34	15	19
Metoprolol	62	51	47	50	33	53
Propranolol	11	23	6.8	9.0	5.5	4.3
Clarithromycin	31	35	10	16	8.6	18
Roxithromycin	19	20	2.9	7.9	5.7	15
Erythromycin	14	16	6.7	8.2	7.9	23

	Limit of quantification in biochar					
	Sampling 1	**Sampling 2**	**Sampling 3**	**Sampling 4**	**Sampling 5**	**Sampling 6**
Diclofenac	4,300	680	530	360	150	640
Ibuprofen	1,300	850	3,200	3,600	7,300	3,300
Phenazone	57	110	68	80	74	130
Carbamazepine	130	76	31	72	23	56
Sulfamethoxazole	870	68	310	360	360	420
Bezafibrate	230	150	6.1	38	18	29
Fenofibric acid	590	190	35	110	51	63
Metoprolol	210	170	160	170	110	180
Propranolol	36	78	23	30	18	14
Clarithromycin	100	120	33	53	29	60
Roxithromycin	63	67	10	26	19	51
Erythromycin	47	53	22	27	26	77

7.2 Chapter 3 - supplementary material

Suppl. 7.7 Sewage sludge parameters from the wastewater treatment plants Hollenstedt, Rheinhausen and Rahmedetal (values are kindly provided by the wastewater treatment plants)

	Wastewater treatment plant		
Parameter	**Hollenstedt**	**Rheinhausen**	**Rahmedetal**
Dry matter	23%	24%	28%
pH	6.6	7.3	7.0
Organic substance	84.4%	56.6%	46.5%
Mineral substance	15.6%	43.4%	53.5%
Total nitrogen, kg/t	77.8	46.9	39.5
Phosphate (P_2O_5), kg/t	57.5	73.7	65.5

Suppl. 7.8 Limits of quantification (LOQ), indication of best values (n = 8) in sewage sludge and biochar. Matrix effects cause LOQ variation between samples. Recovery rates were determined from samples spiked before pressurized liquid extraction (n = 3)

	Sewage sludge		Biochar	
	LOQ, $\mu g/kg_{DM}$	**Recovery rate, %**	**LOQ, $\mu g/kg_{DM}$**	**Recovery rate, %**
Diclofenac	1.3	89 ± 11	64	116 ± 4
Ibuprofen	28	124 ± 5	22	125*
Phenazone	2.1	103 ± 6	6.5	85 ± 3
Carbamazepine	2.2	118 ± 2	19	104 ± 2
Sulfamethoxazole	3.7	33 ± 9	29	96 ± 8
Bezafibrate	1.9	95 ± 7	23	101 ± 7
Fenofibric acid	4.7	105 ± 12	52	101 ± 4
Metoprolol	3.7	92 ± 7	22	93 ± 7
Propranolol	5.4	98 ± 9	6.4	113 ± 8
Clarithromycin	1.2	108 ± 8	7.6	115 ± 8
Roxithromycin	2.3	96 ± 3	2.3	106 ± 11
Erythromycin	2.2	97 ± 6	5.3	98 ± 8

*n = 1

The pyrazolones have been addressed in additional experiments. Suppl. 7.9 lists limits of quantification and recovery rates of these experiments.

Suppl. 7.9 Limits of quantification (LOQ), indication of best values (n = 4) in sewage sludge and biochar. Matrix effects cause LOQ variation between samples. Recovery rates were determined from samples spiked before pressurized liquid extraction

	Sewage sludge		Biochar	
	LOQ, $\mu g/kg_{DM}$	Recovery rate, %	LOQ, $\mu g/kg_{DM}$	Recovery rate, %
Propyphenazone	0.6	90	13	110
4-aminoantipyrine	17	108	180	88
4-methylaminoantipyrine	4.3	70	11	78

Suppl. 7.10 Chromatographic and mass spectrometric data of the analytes

Diclofenac, ibuprofen, phenazone, carbamazepine, sulfamethoxazole, bezafibrate, fenofibric acid, metoprolol, propranolol, clarithromycin, erythromycin and roxithromycin were analyzed using an LC 20 HPLC (Shimadzu, Duisburg, Germany) connected to a 3200 QTRAP (AB Sciex, Darmstadt, Germany) MS. A Waters Atlantis T3 column (100 x 2.1 mm, 3 µm, Waters GmbH, Eschborn, Germany) was applied for chromatographic separation of 20 µL sample aliquots at 45 °C with water (A) and methanol (B) containing 0.1% formic acid, respectively, in a 20-min gradient. MRM mode served to quantify the analytes with the most intense mass transition, which are listed in Suppl. 7.11. A second MRM transitions was recorded to verify the compound. Erythromycin was measured as dehydrato-erythromycin. Ion source parameters were set to: 11 psi curtain gas, 520 °C ion source temperature, 63 and 68 psi ion source gases, 5,000 V positive electrospray ionization and 4 V collision cell exit potential

Suppl. 7.11 Compound specific quantification parameters during the HPLC-MS/MS measurement

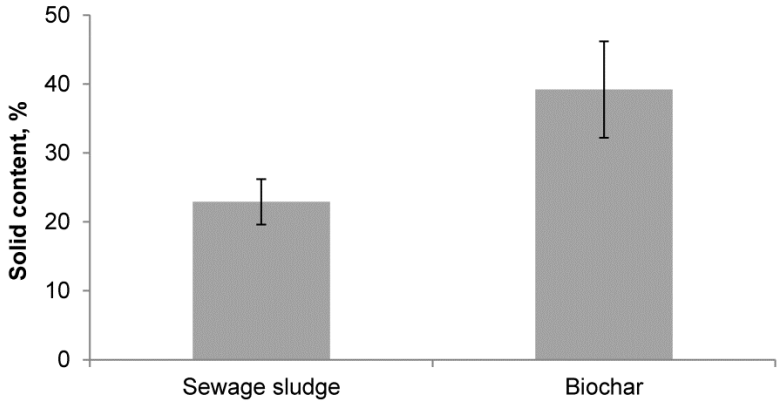
Compound	Quantification transition	Verification transition	Declustering Potential	Entrance Potenial	Collision Cell Entrance Potential	Collision Energy
	m/z	*m/z*	V	V	V	eV
Diclofenac	296 → 214	296 → 250	31	4.0	22	43
Ibuprofen	207 → 161	207 → 119	16	6.0	14	17
Phenazone	189 → 56	189 → 77	51	10	12	45
Carbamazepine	237 → 194	237 → 165	46	5.0	14	27
Sulfamethoxazole	254 → 156	254 → 92	36	5.0	18	21
Bezafibrate	362 → 139	362 → 121	36	4.5	18	33
Fenofibric acid	319 → 139	319 → 233	46	4.0	16	41
Metoprolol	268 → 72	268 → 56	51	5.0	16	33
Propranolol	260 → 116	260 → 56	46	4.0	14	23
Clarithromycin	749 → 83	749 → 158	51	8.5	32	65
Roxithromycin	838 → 158	838 → 83	56	8.0	34	47
Dehydrato-erythromycin	716 → 158	716 → 83	41	6.5	34	41

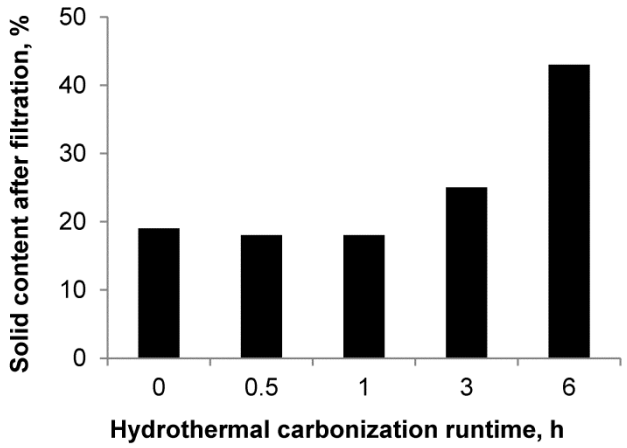
The pyrazolones were separated in a 15-min linear chromatographic run using water + 0.1% formic acid and methanol + 0.1% formic acid at 40 °C with an injection volume of 20 µL. The MS recorded the two most intense mass transitions for each compound in MRM mode with optimized curtain gas (16 psi), ion source temperature (600 °C), ion source gases (70 and 65 psi), positive electrospray ionization (4,800 V) and collision cell exit potential (4 V) settings. Suppl. 7.12 provides detailed compound specific parameters to analyze the pyrazolones

Suppl. 7.12 Compound specific quantification parameters for mass spectrometric measurements of the pyrazolones

Compound	Quantification Transition	Verification Transition	Declustering Potential	Entrance Potenial	Collision Cell Entrance Potential	Collision Energy
	m/z	*m/z*	V	V	V	eV
Phenazone	189 → 56	189 → 77	51	10	12	45
Propyphenazone	231 → 56	231 → 189	56	6.0	14	47
4-aminoantipyrine	204 → 56	204 → 159	41	11.5	10	35
4-methylaminoantipyrine	218 → 56	218 → 97	31	5.0	14	33

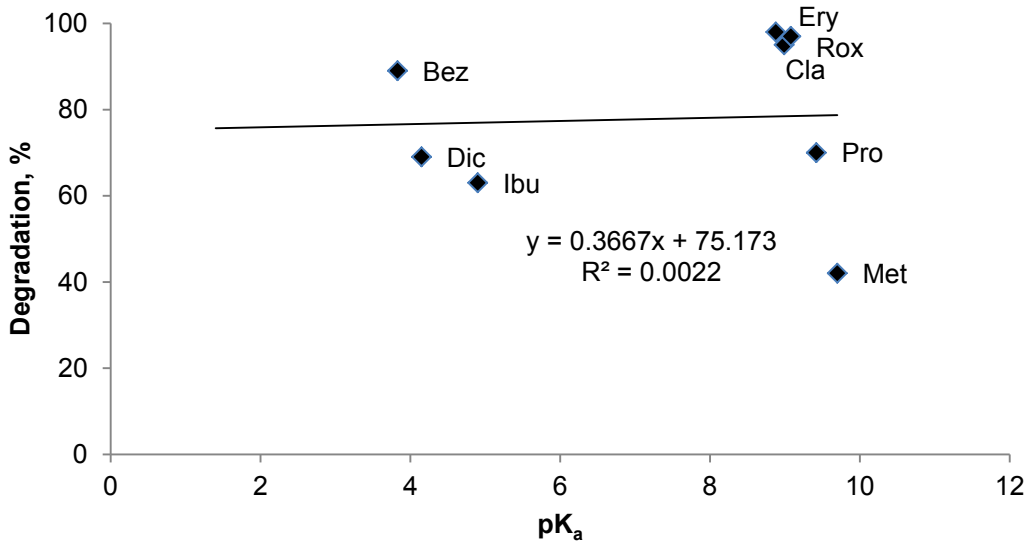
Suppl. 7.13 Dewaterability of sewage sludge (n = 7) and biochar (n = 11) from hydrothermal carbonization of sewage sludge and dewaterability of hydrothermal carbonized sewage sludge at different runtimes



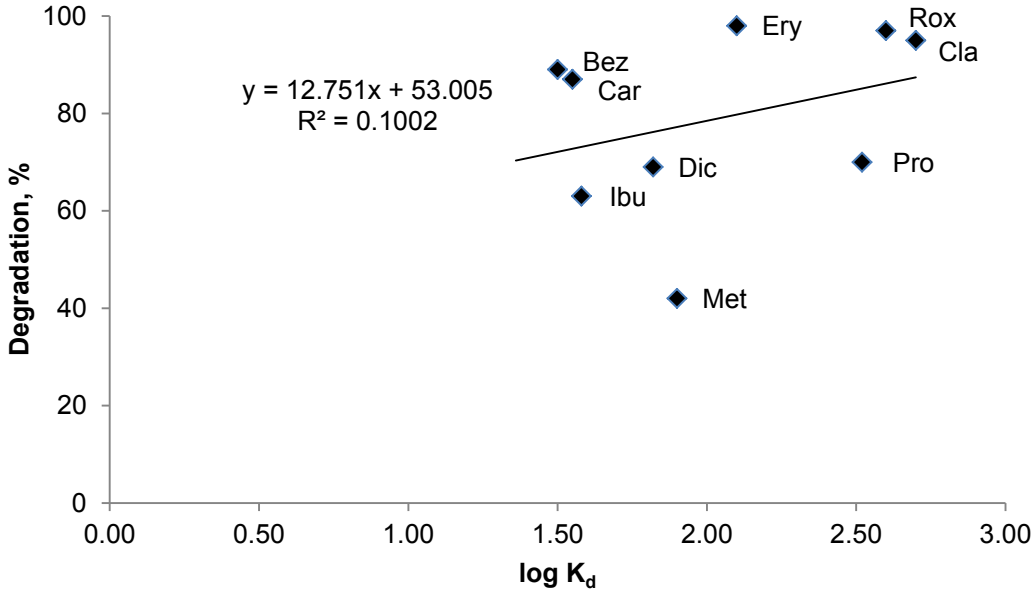


Suppl. 7.14 Pharmaceutical removal during hydrothermal carbonization of sewage sludge: Correlation of removal rates and physico-chemical properties

Compound degradation of eight pharmaceuticals was correlated to their pK_a values (Dic = diclofenac, Ibu = ibuprofen, Bez = bezafibrate, Met = metoprolol, Pro = propranolol, Cla = clarithromycin, Rox = roxithromycin, Ery = erythromycin). The antibiotics clarithromycin, roxithromycin and erythromycin show high degradation rates and high pK_a values. The pK_a of metoprolol is also high; however, degradation rate remains low. Bezafibrate and diclofenac have similar pK_a values, but differ considerably in their degradation rate. Overall, degradation rate during hydrothermal carbonization cannot be correlated to the pK_a value of the pharmaceutical substances



Alternatively, compound degradation of nine pharmaceuticals was correlated to their log K_d value, which was taken from literature data (Dic = diclofenac, Ibu = ibuprofen, Car = carbamazepine, Bez = bezafibrate, Met = metoprolol, Pro = propranolol, Cla = clarithromycin, Rox = roxithromycin, Ery = erythromycin). The trend that high log K_d values go along with high degradation rates is visible. Ibuprofen and diclofenac, both showing moderate log K_d values, were degraded by about 50%. The antibiotics, which have high log K_d, show high degradation rates. However, bezafibrate and carbamazepine were removed by more than 80%, although they have moderate log K_d values. And metoprolol, which has a higher log K_d value than bezafibrate and carbamazepine, was removed to a lower extent. Therefore, a clear correlation cannot be derived


y = 12.751x + 53.005
R² = 0.1002
Ery
Rox
Cla
Bez
Car
Dic
Pro
Ibu
Met
Degradation, %
log K_d
100
80
60
40
20
0
0.00
0.50
1.00
1.50
2.00
2.50
3.00

7.3 List of publications

7.3.1 Publications in peer-reviewed journals

A. Tekle-Röttering, C. von Sonntag, E. Reisz, C. vom Eyser, H. V. Lutze, J. Tuerk, S. Naumov, W. Schmidt, T. C. Schmidt
Ozonation of anilines: Kinetics, stoichiometry, product identification and elucidation of pathways
Water Research, submitted December 2015.

C. vom Eyser, K. Palmu, T. C. Schmidt, J. Tuerk
Fate and behavior of diclofenac during hydrothermal carbonization
Chemosphere, submitted September 2015.

C. vom Eyser, K. Palmu, T. C. Schmidt, J. Tuerk
Pharmaceutical load in sewage sludge and biochar produced by hydrothermal carbonization
Science of the Total Environment, **537** (2015) 180-186.

C. vom Eyser, K. Palmu, R. Otterpohl, T. C. Schmidt, J. Tuerk
Determination of pharmaceuticals in sewage sludge and biochar from hydrothermal carbonization using different quantification approaches and matrix effect studies
Analytical and Bioanalytical Chemistry, **407** (2015) 821-830.

J. Richard, A. Boergers, C. vom Eyser, K. Bester, J. Tuerk
Toxicity of the micropollutants Bisphenol A, Ciprofloxacin, Metoprolol and Sulfamethoxazole in water samples before and after the oxidative treatment.
International Journal of Hygiene and Environmental Health, **217(4-5)** (2014) 506-514.

C. vom Eyser, A. Börgers, J. Richard, E. Dopp, N. Janzen, K. Bester, J. Tuerk
Chemical and toxicological evaluation of transformation products during advanced oxidation processes
Water Science and Technology, **68(9)** (2013) 1976-1983.

7.3.2 Oral presentations

C. vom Eyser, A. Mohren, S. Wiese, C. Portner, J. Türk, T. Teutenberg
Möglichkeiten und Grenzen der Online SPE zur Untersuchung von Arzneimitteln in Wasser
Spark Anwendertreffen, Sprockhövel, Germany
10/12/2015

C. vom Eyser, A. Mohren, S. Wiese, C. Portner, J. Türk, T. Teutenberg
Einsatz eines CTC basierten Online SPE-LCMS/MS Systems zur Bestimmung von Arzneimitteln in Wasser
PAL Anwendertreffen, Sprockhövel, Germany
09/02/2015

C. vom Eyser
A short example of the Online-SPE system in use
Analytical Forum Water Contaminants (AFWC) 2015, Koblenz, Germany
04/28/-04/30/2015

T. Teutenberg, C. vom Eyser, J. Türk
Möglichkeiten und Grenzen der Online Festphasenextraktion gekoppelt mit Flüssigkeitschromatografie und Massenspektrometrie
Mülheimer Wasseranalytisches Seminar, Mülheim an der Ruhr, Germany
09/10/-09/11/2014

C. vom Eyser, C. Portner, J. Türk, T. Teutenberg
Systematische Erfassung von Matrixeffekten bei der Analyse komplexer Umweltproben
LC-MS in der Umweltanalytik 2014, Leipzig, Germany
06/16/-06/18/2014

J. Türk, C. vom Eyser, K. Palmu, R. Otterpohl, T.C. Schmidt
Verhalten von Spurenstoffen bei der HTC von Klärschlamm
Weltleitmesse für Wasser-, Abwasser-, Abfall- und Rohstoffwirtschaft (IFAT), München, Germany
05/05/-05/09/2014

C. vom Eyser, T. Teutenberg, J. Türk
Möglichkeiten der Online Festphasenextraktion zur Aufreinigung und Anreicherung pharmazeutischer Formulierungen
Forschungsvereinigung der Arzneimittel-Hersteller (FAH) Informationsveranstaltung, Bonn, Germany
05/07/2014

C. vom Eyser, C. Portner, T. Teutenberg, J. Tuerk
Experiences with QTRAP in water analysis – Matrix effects
AFWC 2014, Antwerp, Belgium
03/13/2014

C. vom Eyser, K. Palmu, T. C. Schmidt, J. Tuerk
Quantification of pharmaceuticals in complex environmental samples
24. Doktorandenseminar des Arbeitskreises Separation Science, Hohenroda, Germany
01/05/-01/07/2014

C. vom Eyser, A. Salma, A. Börgers, S. Thoröe-Boveleth, J. Tuerk
Elimination of pharmaceuticals and investigation of transformation products during oxidative water treatment
Waters User Meeting, Berlin, Germany
10/09/2013

C. vom Eyser, A. Salma, A. Börgers, S. Thoröe-Boveleth, J. Tuerk
Elimination of pharmaceuticals and investigation of transformation products during oxidative water treatment
2013 MS Technology Days, Stuttgart, Germany
10/08/2013

C. vom Eyser, K. Palmu, T. Haeselhoff, R. Otterpohl, T. C. Schmidt, J. Tuerk
Product quality of hydrochar from sewage sludge in terms of micropollutants
1st International Conference on Terra Preta Sanitation, Hamburg, Germany
08/28/-08/30/2013

C. Portner, L. Gehrmann, A. Börgers, C. vom Eyser, J. Türk
Kombination von instrumenteller und wirkungsbezogener Analytik zur Untersuchung und Bewertung der oxidativen Abwasserbehandlung
Gesellschaft Deutscher Chemiker: Forum Umweltwissenschaftler, Blomberg, Germany
05/27/-05/29/2013

J. Türk, A. Börgers, C. vom Eyser, K. Bester, X. Chen, J. Richard, E. Dopp
Einsatz der instrumentellen und wirkungsbezogenen Analytik zur Bewertung von Transformationsprodukten bei der Abwasserbehandlung
ANAKON 2013, Essen, Germany
03/04/-03/07/2013

C. vom Eyser, K. Palmu, T. Haeselhoff, J. Tuerk, T. C. Schmidt
Hydrothermal carbonization - Fate and behavior of selected micropollutants
2nd Nordic Biochar Seminar, Helsinki, Finland
02/13/-02/16/2013

C. vom Eyser, A. Börgers, J. Richard, E. Dopp, J. Raab, K. Bester, J. Tuerk
Chemical and toxicological evaluation of transformation products during advanced oxidation processes
Advanced Oxidation Processes 6, Goslar, Germany
05/07/-05/09/2012

7.3.3 Poster presentations

C. vom Eyser, T. Werres, J. Schram, S. Giegold, T. Teutenberg
Determination of estrogens in water samples using High Performance Liquid Chromatography - Fluorescence Detection
Novia HPLC-Tage 2015, Bad Soden, Germany
11/17-11/18/2015

T. Teutenberg, C. vom Eyser, A. Mohren, C. Portner, J. Türk
Vergleich von Online-SPE und klassischer SPE bei der Spurenanalytik von Arzneimittelwirkstoffen mittels LC-MS/MS
Langenauer Wasserforum, Langenau, Germany
11/08/-11/10/2015

C. vom Eyser, J. Türk, T. C. Schmidt
Target und Non-Target Analytik von Arzneimitteln in komplexen Umweltproben
Wasser 2015 Schwerin, Germany
05/11/-05/13/2015

C. vom Eyser, T. Teutenberg
HPLC-MS Anreicherung mit integrierter Online-SPE Anreicherung zur Bestimmung von sechs Neonicotinoiden
LC-MS in der Umweltanalytik 2014, Leipzig, Germany
06/16/-06/18/2014

C. Portner, C. Kowalewski, C. vom Eyser
Development and multivariate optimization of a simultaneous extraction method for 10 mycotoxins from wheat and oats
36th Mycotoxin Workshop, Göttingen, Germany
06/16/-06/18/2014

C. vom Eyser, K. Palmu, R. Otterpohl, T. C. Schmidt, J. Tuerk
Occurrence of selected pharmaceuticals in sewage sludge and biochar
Activated Sludge… 100 years and counting, Essen, Germany
06/12/-06/14/2014

C. vom Eyser, C. Portner, S.-D. Freihoff, J. Türk, T. Teutenberg
Systematische Aufklärung von Matrixeffekten bei der Kopplung von Flüssigchromatographie und Massenspektrometrie bei der Analyse komplexer Umweltproben
Wasser 2014, Haltern am See, Germany
05/26/-05/28/2014

T. Teutenberg, C. vom Eyser, S.-D. Freihoff, C. Portner
Systematic elucidation of matrix effects in liquid chromatography hyphenated to mass spectrometry
13th International Symposium on hyphenated Techniques in Chromatography and Technology and 3rd International Symposium on Hyphenated Techniques for sample preparation, Bruges, Belgium
01/28/-01/31/2014

J. Richard, A. Boergers, C. vom Eyser, X. Chen, K. Bester, E. Dopp, J. Tuerk
Chemical and biological evaluation of transformation products during advanced oxidation processes
Micropol & Ecohazard 2013, Zurich, Switzerland
06/17/-06/21/2013

C. Portner, L. Gehrmann, A. Börgers, C. vom Eyser, J. Türk
Kombination von instrumenteller und wirkungsbezogener Analytik zur Untersuchung und Bewertung der oxidativen Abwasserbehandlung
GDCh Forum Umweltwissenschaftler, Blomberg, Germany
05/27/-05/29/2013

C. vom Eyser, T. C. Schmidt, J. Tuerk
Untersuchung von Matrixeffekten bei der Analytik komplexer Umweltproben
ANAKON 2013, Essen, Germany
03/04/-03/07/2013

C. vom Eyser, A. Boergers, J. Richard, E. Dopp, K. Bester, J. Tuerk
Degradation and by-product formation of ofloxacin with ozonation, UV and UV/H2O2-treatment
Ecotechnologies for wastewater treatment, Santiago de Compostela, Spain
06/25/-06/27/2012

C. vom Eyser, K. Palmu, R. Otterpohl, T. C. Schmidt, J. Tuerk
Fate and behavior of pharmaceuticals during Hydrothermal carbonization
Ecotechnologies for wastewater treatment, Santiago de Compostela, Spain
06/25/-06/27/2012

C. vom Eyser, J. Tuerk, A. Boergers, M. Launer, H. Herbst
Full scale application of two ozone injection systems for the removal of micro pollutants at the wastewater treatment plant Duisburg-Vierlinden
Advanced Oxidation Processes 6, Goslar, Germany
05/07/-05/09/2012

C. vom Eyser, A. Boergers, J. Richard, E. Dopp, K. Bester, J. Tuerk
Chemical and biological evaluation of Ofloxacin transformation products
Wasser 2011, Norderney, Germany
05/30/-06/01/2011

7.4 Curriculum vitae

Der Lebenslauf ist in der Online-Version aus Gründen des Datenschutzes nicht enthalten.

7.5 Erklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit mit dem Titel

„Behavior of micropollutants during hydrothermal carbonization of sewage sludge"

selbst verfasst und keine außer den angegebenen Hilfsmitteln und Quellen benutzt habe, und dass die Arbeit in dieser oder ähnlicher Form noch bei keiner anderen Universität eingereicht wurde.

Essen, im Juli 2016

7.6 Danksagung

An dieser Stelle möchte ich all denen danken, die für mich da waren und somit auf Ihre Weise einen wertvollen Beitrag zur Vollendung der vorliegenden Arbeit geleistet haben.

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