

Potential of bioreactors for attenuation of herbicides and nitrate in drainage waters

vorgelegt von

Dipl.-Ing.
Björn Krause Camilo
geb. in Frankfurt an der Oder

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- Dr.-Ing. -

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Promotionsausschuss:
Vorsitzender: Prof. Dr. Martin Kaupenjohann
Betreuer und Gutachter: Prof. Dr. Gerd Wessolek
Gutachter: Prof. Dr. Dr. Wilfried Pestemer
Gutachter: Dr. Dieter Felgentreu

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List of Abbreviations

Anammox	-	Anaerobic ammonium oxidation
ATR	-	Atrazine
CaCl ₂	-	Calcium chloride
DEA	-	Deethyl-atrazine
DNRA	-	Dissimilatory nitrate reduction into ammonium
DOC	-	Dissolved organic carbon
DT ₅₀	-	Half-life, in this study attenuation time of 50% of the substance
GC/MS	-	Gas Chromatography/Mass Spectrometry
GUS	-	Groundwater Ubiquity Score
H ₂ S	-	Hydrogen sulphide
HA	-	Hydroxy-atrazine
HRT	-	Hydraulic residence time
IPU	-	Isoproturon
K _F	-	FREUNDLICH coefficient
K _{OC}	-	Soil organic carbon-water partitioning coefficient
λ [lambda]	-	Attenuation rate
LC/MS	-	Liquid Chromatography/Mass Spectrometry
N ₂	-	Dinitrogen, elementary nitrogen
N ₂ O	-	Nitrous oxide
NH ₃	-	Ammonia
NH ₄ ⁺	-	Ammonium
NO	-	Nitric oxide
NO ₂ ⁻	-	Nitrite
NO ₃ ⁻	-	Nitrate
PO ₄ ³⁻	-	Phosphate
SO ₄ ²⁻	-	Sulphate
TOC	-	Total organic carbon
n.a.	-	not available
e.g.	-	for example
i.e.	-	which means

Abstract

The wash-out of agrochemicals, such as fertilizers and herbicides, via tile drainage into surface waters presents an increasing risk for drinking water production and biodiversity in rivers and lakes. Mitigation systems are important measures to attenuate agricultural emissions at the source and relieve surface waters downstream. Under extreme high flow conditions the effectiveness of such facilities is restricted, due to bypass of untreated waters or short contact times.

The study to the present PhD thesis “Potential of bioreactors for attenuation of herbicides and nitrate in drainage waters” is part of the Aquisafe II project, in which the focus is laid on drainage water decontamination in mitigation systems. The project aims at implementing new effective mitigation systems and improving existing mitigation systems in contamination hot spots. In the PhD study, organic substrates were examined for their potential to attenuate a set of herbicide agents and nitrate (NO_3^-), simultaneously and efficiently, at short hydraulic residence times (HRT) between 0.2 and 2.5 days. The selected herbicide agents, bentazon, atrazine and isoproturon are classified with a high risk for drinking water production.

Based on comprehensive literature studies, the design of bioreactors with organic substrates, such as bark mulch and straw, emerged to be of high potential for decontamination of drainage waters in mitigation systems.

In laboratory scale, the substrates were tested in sorption-, degradation- and column displacement experiments for hydraulic conductivity and attenuation of NO_3^- and the selected herbicide agents at temperatures around 21 °C.

In technical macro scale, simultaneous attenuation of NO_3^- and atrazine and attenuation of NO_3^- and isoproturon were investigated. The potential of the mixture of bark mulch and straw was tested under realistic conditions, in macro scale under the influence of seasonally changing temperatures. The hydraulic residence times ranged between 0.4 and 2.5 days.

The study revealed that the tested organic substrate provides a high and long-term stable hydraulic conductivity to ensure and maintain high flow.

Straw is a readily available organic carbon source, which can support effective and efficient denitrification at short hydraulic residence times. Bark mulch is an effective adsorbent for the herbicide agents. The conditions of the experiment support denitrification as major NO_3^- attenuation process.

The potential of the organic substrate to attenuate the selected herbicide agents is different for each compound and based on different attenuation paths. Non-aerobic conditions in the bioreactor are in general disadvantageous for attenuation of the selected herbicide agents. Bentazon is too persistent and mobile to be considerably attenuated under high flow conditions. Atrazine can be substantially reduced in drainage waters. It appears that atrazine is predominantly attenuated by sorption to the organic substrate and by formation of bound residues. Isoproturon seems to be effectively attenuated by degradation to metabolites under denitrifying (suboxic) condition. Uncertainty remains about the contribution of the metabolite hydroxy-atrazine to bound residues and about the kind of isoproturon metabolites.

Denitrification and herbicide agent attenuation correlate positively with temperature. The NO_3^- attenuation performance of the organic substrate decreases gradually with operating time, since denitrification is based on depletion of the organic carbon source. Atrazine attenuation is less influenced by substrate aging, but nevertheless linked to the presence of organic matter as adsorbent. The organic substrate has to be frequently exchanged, while intervals depend on contaminant loads in the drainage water, temperature and water-saturation of the substrate. When denitrification is limited by NO_3^- input, the organic carbon source is used for competing respiratory processes.

Design and sizing of the mitigation system need to be adapted to present conditions at the desired site to prevent or, at least, to minimize possible negative effects, such as discharge of oxygen-free water, dissolved organic carbon and hydrogen sulphide into receiving waters.

In conclusion, the studied design provides a valuable and effective tool in the set of best management practices in agriculture to attenuate NO_3^- and atrazine as well as isoproturon in drainage waters.

Abstract (German)

Die Auswaschung von Agrochemikalien, wie Dünger und Pflanzenschutzmittel, über Dränagen in Oberflächengewässer, stellt ein wachsendes Risiko für die Trinkwasserbereitstellung und die Biodiversität in Flüssen und Seen dar. Rückhaltezone sind wichtige Einrichtungen, um landwirtschaftliche Emissionen an der Quelle zu reduzieren und nachgeschaltete Gewässer zu entlasten. Bei extrem hohen Ablaufbedingungen ist die Effektivität solcher Maßnahmen jedoch durch den Umlauf nicht behandelten Wassers oder durch kurze Kontaktzeiten eingeschränkt.

Die vorliegende Doktorarbeit „Das Potential von Bioreaktoren für den Rückhalt von Pflanzenschutzmitteln und Nitrat aus Dränagewässern“ fasst einen Teil der Ergebnisse und Erkenntnisse des Aquisafe II Projektes zusammen. Der Fokus des Projektes ist auf die Dekontamination von Dränagewässern in Rückhaltezone gerichtet. Das Ziel ist die Einrichtung von neuen effektiven und die Verbesserung von existierenden Rückhaltezone an Kontaminationsschwerpunkten. In der Studie wurden organische Substrate auf ihr Potential hin untersucht, eine Reihe von Pflanzenschutzmittelwirkstoffen (PSMW) und Nitrat (NO_3^-) gleichzeitig und effizient bei kurzen hydraulischen Aufenthaltszeiten im Bereich von Stunden bis zu wenigen Tagen zurückzuhalten. Die PSMW Bentazon, Atrazin und Isoproturon wurden ausgewählt, weil ihr Einsatz mit einem hohen Risiko für die Trinkwasserbereitstellung verbunden ist.

Auf der Grundlage umfassender Literaturstudien haben sich Bioreaktoren mit organischen Substraten, wie Stroh und Rindenmulch, als besonders geeignet für die Dekontamination von Dränagewässern in Rückhaltezone erwiesen.

Im Labormaßstab bei Raumtemperatur (21°C) wurden die Substrate in Sorptions-, Abbau- und Verlagerungsexperimenten auf hydraulische Durchlässigkeit und den Rückhalt von NO_3^- und der ausgewählten PSMW getestet.

Im technischen Maßstab wurde der gleichzeitige Rückhalt von NO_3^- und Atrazin bzw. NO_3^- und Isoproturon untersucht. Das Potential der Mischung von Rindenmulch und Stroh wurde unter realistischen Bedingungen, in großer Skala bei saisonal bedingten

Temperaturschwankungen getestet. Die hydraulischen Aufenthaltszeiten lagen zwischen 0,4 und 2,5 Tagen.

Die ausgewählten Substrate gewährleisten eine andauernd hohe hydraulische Durchlässigkeit, wodurch hoher Abfluss realisiert und aufrechterhalten werden kann.

Stroh ist eine leicht verfügbare Quelle organischen Kohlenstoffs, welche eine effektive und effiziente Denitrifikation bei geringen hydraulischen Aufenthaltszeiten ermöglicht. Rindenmulch stellt ein gutes Adsorbens für die PSMW dar. Unter den Bedingungen des Experiments wird NO_3^- hauptsächlich durch Denitrifikation zurückgehalten.

Das Potential der organischen Substrate, die ausgewählten PSMW zurückzuhalten, ist sehr unterschiedlich und beruht auf verschiedenen Wirkmechanismen. Sauerstofffreie Bedingungen sind in der Regel von Nachteil für ihren Abbau. Bentazon ist zu persistent und mobil, um bei hohem Abfluss überhaupt zurückgehalten zu werden. Atrazin kann substantiell aus dem Dränagewasser entfernt werden, vermutlich hauptsächlich durch Adsorption und Bildung von nicht extrahierbaren Rückständen im Substrat. Isoproturon scheint unter denitrifizierenden (suboxischen) Bedingungen effektiv durch den Umbau zu Metaboliten zurückgehalten zu werden. Unklarheit besteht über die Beteiligung des Metaboliten Hydroxiatrazin an der Bildung von nicht extrahierbaren Rückständen und über die Art der Metabolite des Isoproturon.

Denitrifikation und Rückhalt der PSMW korrelieren positiv mit der Temperatur. Die Rückhaltewirkung der organischen Substrate nimmt mit der Einsatzzeit graduell ab. Sie beruht im Falle der Denitrifikation auf dem Verzehr des organischen Kohlenstoffes durch mikrobielle Atmungsprozesse. Der Rückhalt der PSMW, vor allem des Atrazins, scheint weniger stark vom Alter des Substrats beeinflusst, ist jedoch an die Präsenz des organischen Materials als Adsorbens geknüpft. Das organische Substrat muss ständig ersetzt werden, wobei die Intervalle besonders von der Kontaminantenbelastung im Dränagewasser, der Temperatur und des Wassersättigungsgrades des Substrates abhängen. Im Fall einer vollständigen Denitrifikation wird die Kohlenstoffquelle für konkurrierende Atmungsprozesse verwendet und die Effizienz der Kohlenstoffausnutzung durch die Denitrifikation verringert sich.

Um mögliche negative Nebeneffekte, wie die Auswaschung organischen Kohlenstoffes, Hydrogensulfats oder sauerstofffreien Wassers in die anschließenden Gewässer zu

verhindern oder zumindest zu verringern, muss die Gestaltung und die Dimensionierung des Systems an die Bedingungen im gewünschten Einsatzgebiet angepasst werden.

Zusammengefasst stellt das untersuchte Design ein wertvolles und effektives Werkzeug der guten landwirtschaftlichen Praxis dar, um NO_3^- und Atrazin sowie NO_3^- und Isoproturon in Dränagewässern zurückzuhalten.

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Study

Chapter 1

Introduction

1.1 Motivation and design of the Aquisafe II Project

The Aquisafe II project is focused on reduction of diffuse pollution in surface waters by decentralised measures to mitigate negative impacts of contaminants on drinking water production. The aim of work package V in the Aquisafe II project is to determine efficient conditions and designs for the attenuation of selected plant protection products in mitigation system. The present PhD thesis has been developed for scientific evaluation of the results of work package V.

The Aquisafe II project was designed and has been conducted by partners in Germany (KompetenzZentrum Wasser Berlin (KWB), Umweltbundesamt (UBA), Institut für Landschaftsökologie und Ressourcenmanagement der Universität Gießen (ILR)), in France (Ste d'Environnement d'Exploitation et de Gestion de Travaux (S.E.E.G.T.), Syndicat Mixte Environnement Goëlo l'Argoat (SMEGA)) and in the United States of America (Center on Earth and Environmental Science, Indiana University - Purdue University of Indianapolis). It has been financed and supervised by Veolia eau, France. The experimental studies of work package V were conducted at the European Center for Aquatic Simulations, which is operated by UBA and located in Berlin, Germany.

Motivation and design of the Aquisafe II project is comprehensively described in the project proposal (Matzinger et al. (2009)) and in Périllon and Matzinger (2010). As introduction into the project, its essence is given in the following paragraphs.

The improvement of waste water treatment, by new effective and efficient methods and techniques, did substantially increase the quality of raw waters in urban regions. Far-reaching elimination of point-source pollution, by collection of waste water and centralized treatment, was the basis for the positive change. Diffuse pollution of surface waters via runoff and erosion, base flow seepage, tile drainage, spray drift and atmospheric deposition after volatilization, and diffuse pollution of ground waters via leaching are still a challenge for water resource management.

In most countries surface water is one of the major sources of public drinking water supply, in some even the only one. In the European Union and in the United States of America, 40 (EEA (2003)) and 63% (Hutson et al. (2004)) of the drinking water originate from above ground sources, respectively (Tedesco et al. (2009)). If surface

water is used for drinking water production, diffuse pollution can increase the costs of purification or even prevent the exploitation of the source.

In the previous Aquisafe I project relevant contaminants (1) and sufficiently effective mitigation systems (2) were identified. Analytical methods (3) were developed and tested to recognize hot spots of pollution.

1. Since intensification of agriculture, the diffuse discharge of nutrients has been predominantly responsible for the pollution of surface waters in rural regions (EEA (2005) and EPA (2002) in Tedesco et al. (2009)). Wash-off and wash-out of excessively applied artificial or natural fertilizers result in high loads of nutrients in surface waters. As effect, eutrophication can lead to misbalance of natural populations. Increase of algae and its toxins, lack of oxygen and bad odour are only some negative impacts, which result in problems for water purification. In the European Union, raw waters with nitrate (NO_3^-) concentrations above the threshold of the drinking water directive are not allowed to be used for drinking water production, even if purification techniques are sufficiently effective.

Herbicides in urban and rural regions are of increasing concern for drinking water production. High threshold-exceeding concentrations bring along increased costs for treatment and increased risks for public health. Admittedly, higher loads of herbicides enter into surface water via point-sources (farmyard runoff, sewage plants, sewer overflows and accidental spills). Jaeken and Debaer (2005) in Reichenberger et al. (2007) revealed that in Europe point-sources contribute 40 to 90% of total herbicide load in surface waters. However, the pathway of herbicides via diffuse sources is significant.

Further substances, such as other plant protection products, hormones, nano particles and pharmaceutical products from industry, households and agriculture, were considered, but not found significant in comparison with nutrients and herbicides, then. Environmental concentrations did not reach the relevant level for drinking water production and/or findings were little or not generally accepted. However, it was recommended to observe the development of their relevance for drinking water production in future.

2. The most effective measure to reduce diffuse pollution in surface waters is,

without doubt, the reduction of applied loads of nutrients and herbicides. However, it is often not the most feasible method. The fact that, before discharge into receiving water, the pollutants are still called auxiliary substances in intensified agriculture visualizes the contrary perspective of stakeholders. A solution could be a temporary compromise, which balances the demands of different sides, before the implementation of more sustainable alternatives is possible. The Aquisafe I project was focused on the treatment of the agricultural discharge in mitigation systems. As a result, the implementation of decentralised and near-natural mitigation systems at hot spots of diffuse pollution was expected to be a valuable measure to attenuate the input of contaminants into surface waters. Mitigation systems have been proven as effective nutrient sinks in practice. This generalization could be specified in experimental studies for a range of specially designed denitrification systems (Schipper et al. (2010)).

3. Numerical based models were tested to narrow down the choice of optimal sites for the implementation of mitigation systems. Also, less complex diagnosis tools were tested for applicability and prediction reliability to identify regional hotspots of diffuse pollution.

Based on the outcome of Aquisafe I, in Aquisafe II the focus has been laid on performance analysis of: (i) selected mitigation system designs with particular consideration of NO_3^- , herbicides and high flow events in practice and in technical macro scale experiments and (ii) selected diagnosis tools for implementation of new mitigation systems and for effectiveness of existing mitigation systems.

Different, field-sized mitigation systems were implemented in diverse locations to evaluate the performance of nutrient attenuation under near-natural conditions, e.g. varying discharges. Experiments in laboratory scale and technical macro scale were conducted to estimate the attenuation potential of bioreactors with organic substrates for selected herbicide agents and NO_3^- under influencing conditions, such as temperature and flow (the present study). Simple Geo Information System- and statistically- based diagnosis tools and improvements of the numerical based model were tested for their capability to: (i) point out hot spots of contamination and (ii) estimate the attenuating effects of mitigation systems on the water shed balance.

In the Aquisafe I project in a comprehensive literature review by Tedesco et al. (2009), a wide range of rural and semi-rural diffuse contaminants were compared for their relevance for drinking water production in terms of:

- presence in surface waters and drinking water in Germany, France and the United States of America and
- technical effort of elimination in raw waters, used for drinking water production.

In the Aquisafe II project in Work package V, the literature review was specified towards the contaminant group of herbicides. For further description of relevance and risk and in preparation of experiments, the herbicide agents of highest concern were revised in detail for characteristics, such as:

- chemical, biological and physical properties,
- toxicity,
- thresholds,
- trends of application,
- presence in ground waters and
- relevance of metabolites.

The herbicide agents atrazine, isoproturon and bentazon were selected, next to NO_3^- , as model substances to be investigated for their attenuation in presence of organic carbon sources.

The selected herbicide agents are important representatives in weed control and commonly used in the United States of America (atrazine), Europe (isoproturon) or both regions (bentazon). They are ranked in top positions for occurrence in surface waters. Isoproturon stands for the phenylurea- and atrazine for the triazine-derivates, both leading groups of herbicide agents worldwide. The herbicide agents are characterized by high persistence at non-aerobic conditions. In terms of mobility, they range from highly sorptive (atrazine) to highly soluble (bentazon). They are often found with legal threshold-exceeding concentrations in surface waters and they are expected to be of high importance for agriculture in future.

Detailed information about the herbicide agents, NO_3^- and the tested organic substrates are presented in chapter: Materials and Methods, Section: Substances and substrates.

Results and discoveries of the Aquisafe projects have been continuously documented and published in several articles, reports and conference papers, e. g. Krause Camilo (2015, in press), Wicke et al. (2014), Krause Camilo et al. (2014), Krause Camilo et al. (2013), Rouault et al. (2012), Holsten et al. (2012), Litz et al. (2011), Périllon et al. (2010), Krause et al. (2010), Matzinger et al. (2010b), Matzinger et al. (2010a) and Jacinthe et al. (2009).

1.2 State-of-the-art of herbicide agent- and nitrate attenuation in mitigation systems, a literature review

A variety of comprehensive reviews on a range of measures for herbicide mitigation and their effectiveness can be found in literature. In Reichenberger et al. (2007), one of the most recent contributions, the efficiencies of available mitigation measures are compiled with respect to different input pathways. For herbicides in drain flow, positive effect was observed only in few mitigation measures, mostly on the application side. The establishment of collection ponds for tile drain outflow, in analogy to constructed wetlands, is suggested as a possible effective mitigation measure and recommended for further investigations.

Indeed, wetlands and constructed wetlands seem to have potential to be effective end-of-pipe solutions for herbicide attenuation in drain flow. Schulz and Peall (2001) analysed studies with strongly sorbing hydrophobic insecticides and suggested that constructed wetlands are very effective in reducing pesticide inputs into surface waters. Moore et al. (2001) in Reichenberger et al. (2007) confirmed these assumptions for the moderately sorbing herbicide atrazine. Comparatively high atrazine inflow concentrations (73 and 147 $\mu\text{g L}^{-1}$) were reduced to less than 20 $\mu\text{g L}^{-1}$ by transfer and transformation processes in months. Wang et al. (1991) in Detenbeck et al. (1996) demonstrated that the carboxylic groups in humic acids of organic sediments (and presumably plant litter) are responsible for binding atrazine and catalysing it via hydrolysis to hydroxy-atrazine. Chung et al. (1996) examined atrazine degradation in non-aerobic wetland sediments and could report an increase of hydroxy-atrazine formation by adding external organic carbon. Runes et al. (2001) found that adaption of microorganisms or bioaugmentation with atrazine degraders increase mineralization, i. e. the herbicide agent is completely broken down. Furthermore, it was shown that plant cover in wetlands promotes survival of atrazine degraders in the absence of atrazine. Far less information on attenuation in wetlands is available for isoproturon and bentazon. Passeur et al. (2011) proved attenuation potential of artificial wetlands for isoproturon. In sediments, high adsorption coefficients ranging from 84 to 372 L kg^{-1} were observed. Bentazon, on the other side, is not attenuated (Braskerud and Haarstad (2003) in Artwet (2010)).

Wetlands are adequate mitigation measures for nutrients. Vymazal (2007) comprehensively described constructed wetlands as very effective NO_3^- sinks, since denitrification is the major attenuation mechanism. A potential drawback is that constructed wetlands can be quite area-consuming.

Schipper et al. (2010) presented an extensive review of mitigation systems for NO_3^- attenuation with focus on the effect of organic carbon addition on denitrification. The addition of organic carbon increases the efficiency of mitigation systems, thus needed areas decrease. Taking into account attenuation rates, found by Périllon and Matzinger et al. (2010), and assuming an average depth of 0.5 m, denitrification in constructed wetlands without carbon addition is in average $0.37 \text{ g-nitrogen m}^{-3} \text{ day}^{-1}$. Denitrification rates in mitigation systems with carbon addition range from 2 to $22 \text{ g-nitrogen m}^{-3} \text{ day}^{-1}$ (Schipper et al. (2010)). The addition of organic carbon as source of energy for suboxic respiration (denitrification) enables effective NO_3^- attenuation, even under high-flow conditions.

It was suggested earlier that organic substrates have also positive effect on herbicide attenuation. In biobeds experiences are gained about the role of organic substrates as basis for microbial activity and co-oxidation of herbicides. Biobeds are relatively new technical systems to prevent herbicide contamination of the environment from point-sources, such as cleaning sites for farming machines and herbicide application devices as well as contaminated discharges from herbicide storage and handling sites. Biobeds are usually filled with layers of different organic materials, such as straw, peat, coco fibres or garden waste compost. The contaminated liquid percolates vertically with relatively high residence time in the order of days to weeks, predominantly under aerobic conditions (Castillo et al. (2008)).

Castillo et al. (2000) observed in relatively high concentrated experiments (10 mg L^{-1}) that degradation of bentazon in bioreactors of straw was related to the enzyme production of the white rot fungus. Information on atrazine degradation in biobeds is not available. Still, comparable substances from the triazine family, such as terbuthylazine and cyanazine, were attenuated successfully (Castillo et al. (2009)). Coppola et al. (2011) observed degradation of isoproturon and bentazon in biobeds filled with lingo-cellulosic substrates. The development of lignin-degrading microorganisms, which have shown to be able to degrade persistent pesticides, was assumed.

However, conditions in biobeds are aerobic and the hydraulic residence time is high. Best performance is proven, when contaminated water is cycled (Felgentreu and Bischoff (2010)). Biobeds are optimal end-of-pipe solutions for mitigation of herbicides, originated from point-sources in agriculture.

Diffuse contamination of surface waters is characterized by occasionally high flow conditions and short contact times. The combination of NO_3^- , the major contaminant in drain flow, and organic substrates results in suboxic conditions. Under suboxic conditions oxygen is depleted and NO_3^- is the major electron acceptor in respiratory processes. These circumstances are disadvantageous for herbicide degradation (Stieber et al. (2007)).

On the other hand, the addition of organic substrates has the potential to support attenuation of herbicides, even under suboxic conditions (Kourtev et al. (2006)). An active microbial community and co-metabolic herbicide degradation can be expected in presence of major nutrients (carbon from substrate, NO_3^- and phosphorus from drain flow) and a high surface for biofilm accumulation. The decomposition of organic substrates for suboxic respiration (denitrification) and anoxic respiration (dissimilatory sulphate reduction) releases enzymes, which enable co-metabolic biodegradation under non-aerobic conditions (Hovarth (1972), Hazen (2010)). Organic substrates are also known for sorption of organic compounds, such as herbicides (Gendrault (2004), De Wilde et al. (2009b)).

In conclusion, mitigation systems, amended with organic substrates, which enable effective NO_3^- attenuation under high flow conditions, could be an effective sink for herbicide agents as well. Unfortunately, the behaviour of herbicide agents, such as atrazine, isoproturon and bentazon, is poorly studied in non-aerobic, drain flow-treating mitigation systems. In particular little information is available on the herbicide agents in non-aerobic bioreactors of natural organic substrates and several questions wait to be answered.

Are bioreactors of organic substrates effective designs for simultaneous NO_3^- and herbicide agent attenuation under non-aerobic, high flow conditions? Which organic substrates support the simultaneous attenuation of NO_3^- and herbicides? How does the performance of bioreactors changes with time, when the concept of attenuation is based

on the decomposition of the substrate? How do environmental conditions, such as temperature and flow, influence the performance of bioreactors of organic substrates? Does the presence of herbicides interfere with NO_3^- attenuation?

1.3 Object of this study

The present study evaluates the potential of effective and simultaneous attenuation of NO_3^- and one selected herbicide agent (atrazine, bentazon or isoproturon) in bioreactors of straw and bark mulch, through experiments in laboratory and technical macro scale. The focus is laid on environmental conditions with relevant impact on the effectiveness of mitigation systems, such as temperature and flow. The applicability and the performance of the reactive substrates bark mulch and straw, as organic carbon sources for denitrification at high flow conditions, were investigated. Further, it was researched, whether the organic substrates bark mulch and straw also have the potential to facilitate herbicide agent attenuation at non-aerobic conditions, which are disadvantageous for degradation of the selected herbicide agents. The outcome of this study may help optimize the design of mitigation systems for simultaneous herbicide and NO_3^- attenuation.

Chapter 2

Materials and Methods

2.1 Substances and substrates

The characterization of the herbicide agents atrazine and bentazon has been produced by Dr. S. Heise, Dr. N. Litz and the author and was partly published earlier in Krause et al. (2010). The characterization of the organic carbon sources has been produced by the author and was partly published earlier in Krause Camilo et al. (2013). The characterization of the herbicide agent isoproturon has been produced by Dr. S. Heise, Dr. N. Litz and the author and is, in parts, included in the project report of Aquisafe 2 (Krause Camilo et al. (2014), unpublished).

2.1.1 Atrazine

Effect and application

Atrazine has been on the market since 1957 (PPDB (2014)). Its Chemical Abstract Service number is 1912-24-9. It is a systemic herbicide agent. Its phytotoxic action is defined by the inhibition of photosynthesis in the contaminated plant. Atrazine is mainly absorbed by the plants via their roots, but also via their leaves and is distributed in the plant via the xylem (Schmidt in Litz et al. (eds.) (2003a)).

Atrazine is used for control of broadleaf and grassy weeds, before and after plant emergence. It is applied on a number of crops, such as corn, sorghum, sugarcane and sweet corn, as well as in Christmas trees and conifer reforestation plantings and on lawns and golf courses. It is also used as a non-selective herbicide agent on non-cropped industrial lands and forests (EXTOXNET (1996), EPA (2003a)). Typical application times for the three most important crops corn, sorghum and sugar cane in temperate climate are April to June, June to August and fall and spring, respectively.

Over a long period atrazine was the most used conventional herbicide agent in agriculture in the United States of America (1987 (71 to 76 million pounds), 1993 (70 to 75 million pounds), 1995 (68 to 73 million pounds), 1997 (75 to 82 million pounds), 1999 and 2001 (74 to 80 million pounds)). Despite stable or slightly increased use, atrazine was displaced from top position by glyphosate (85 to 90 million pounds) in 2001 (Kiely (2004)). Based on a survey between 1990 and 1997, 83, 10 and 3% of total atrazine, used in the United States of America, were applied to corn, sorghum and sugar cane, respectively. Less than 2% were used in forestry and non-agricultural purposes. In

terms of cropped area, 75% of all corn fields, 56% of all sorghum fields, 76% of all sugarcane fields and 49 to 58% of all sweet cornfields were treated with atrazine (EPA (2003a)). The application rate of atrazine varies remarkably between 0.4 and 4.5 kg ha⁻¹ year⁻¹ in dependence on type of use (Burgoa and Wauchope (1995)). The use of atrazine is restricted in Europe. Since 2004, atrazine has been on the European Union list of banned herbicides; only Spain, Portugal, Ireland and the United Kingdom were allowed to use it under restrictions until 2007 (EC (2004)).

Chemical, biological and physical parameters

Atrazine (6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine) has a moderate solubility in water (33 mg L⁻¹ at 22°C and pH 7) (Schmidt in Litz et al. (eds.) (2003a)). It volatilizes barely (K_{HENRY} : $2.99 \cdot 10^{-4}$ Pa m³ mol⁻¹ (25°C)), but can drift via aerosols, due to application methods, such as spraying (Reichenberger et al. (2007)). Atrazine dissociates in water, i.e. changes reversible from non-polar molecule form into polar ion form, at low pH values (pK_A : 1.68 (22°C)). The distribution coefficient between octanol and water is moderate ($\log K_{\text{OW}}$: 2.5 (25°C)) for atrazine in its non-polar form (Schmidt in Litz et al. (eds.) (2003a)). The sorption on soil is moderate (K_{OC} : 39 to 155 mL g⁻¹ (Schmidt in Litz et al. (eds.) (2003a)), 100 mL g⁻¹ (EXTOXNET (1996))) and determined by clay minerals and organic material (Spongberg and Ganliang (2000)). Under aerobic conditions chemical hydrolysis (dechlorination) and degradation by soil microorganisms (dealkylation) account for most of the breakdown of atrazine. In acidic or alkaline environments hydrolysis is rapid and the most important transformation pathway. At neutral pH values hydrolysis slows down and microbiological degradation becomes dominant. Addition of organic material increases the rate of hydrolysis. In soils, atrazine is characterised by a medium persistence against microbiological transformation in the aerobic soil (half-life (DT_{50}): 35 to 50 days (Schmidt in Litz et al. (eds.) (2003a)), 146 days (Gilliom et al. (2006))) and a high persistence in the deeper soil layers with low oxygen content (DT_{50} : 105 to 200 days) (Schmidt in Litz et al. (eds.) (2003a)). Favourable conditions for fast degradation are aerobic conditions and the efficiency of present microorganisms in catabolic processes, not their amount or high temperatures (Schmidt in Litz et al. (eds.) (2003a)). However, works by Ghosh and Philip (2006) indicate that the addition of carbon sources can enhance the degradation of atrazine under denitrifying or even anoxic conditions.

Atrazine is moderately to highly mobile in soils with low clay or organic matter content (EXTOXNET (1996)). According to the leaching potential index, atrazine is highly leachable (GUS value: 3.3 (PPDB (2014)), 3.6 (Gan (2002))) and thus of high potential for groundwater contamination. The Groundwater Ubiquity Score (GUS) is a method to estimate the potential of plant protection products to contaminate groundwater on the basis of their persistence and binding behaviour to solid particles. Migration into deeper soil is also supported by macro pores, which promote preferential flow and therefore shorter contact times, and by adsorption of atrazine to soil colloids and particles, which can be transported by water flow (Schmidt in Litz et al. (eds.) (2003a)). In Germany atrazine is classified as highly hazardous for water, due to its toxicity for water organisms, mammals and humans, its persistence and its distribution behaviour in environmental compartments (BMJ (1999)). According to the Directive 91/414/EEC, deethyl-atrazine and deisopropyl-atrazine are relevant metabolites (PPDB (2014), i.e. (i) the metabolites have comparable intrinsic properties to the parent chemical in terms of its biological target activity, (ii) the metabolites have toxicological properties that are considered severe and (iii) the metabolites pose a higher or comparable risk to organisms than the parent substance (EC (2003)).

Occurrence in waters

Gilliom et al. (2006) found atrazine, during a stream survey in the United States of America between 1992 and 2001, in 90.2% of all examined surface waters (rank 1 of the most frequently detected conventional plant protection products). In 42.9% concentrations exceeded $0.1 \mu\text{g L}^{-1}$ (rank 1). In a more recent study (2004 to 2005) in the United States of America, Kingsbury et al. (2008) found atrazine in 82% of all surface water samples with maximum concentration of $5 \mu\text{g L}^{-1}$ (rank 1). In particularly exposed streams (White river, Indiana) temporarily very high concentrations in the order of 10 to $50 \mu\text{g L}^{-1}$ can be observed. These high concentrations ($10 \mu\text{g L}^{-1}$) with a seasonal pattern are linked to non-restricted use, and can reach exceptionally even higher values (Tedesco et al. (2009)).

In Germany atrazine is banned since 1991 (Schmidt in Litz et al. (eds.) (2003a)), in France the use of atrazine is forbidden since 2003. Nevertheless atrazine and its metabolite deethyl-atrazine are still detected in surface waters in Germany (rank 2 and 7 (Sturm et al. (2007))) and France (rank 4 and 3 (IFEN (2006) in Tedesco et al. (2009))),

due to its persistence in the environment and its legal application (or later ban) in neighbouring countries (e.g. Switzerland).

In an UBA study between 1990 and 2006, atrazine and its main metabolite deethyl-atrazine led the list of the herbicide agents, which were detected most frequently above $0.1 \mu\text{g L}^{-1}$ in German shallow ground waters. However, the tendency of their occurrence is decreasing from 2.2% of 12353 sampling sites between 1996 and 2000 to 1.78% of 4496 sampling sites in 2006 (Sturm et al. (2007)). In a survey all over the United States of America from 1992 to 2001, atrazine was detected in approximately 13% of all searched ground water bodies under agricultural land with concentrations more than or equal to $0.1 \mu\text{g L}^{-1}$ (Gilliom (2006)).

Kingsbury et al. (2008) examined finished drinking water of 9 water works in the United States of America and found atrazine in 87 % of all samples with maximum concentration of $3.4 \mu\text{g L}^{-1}$ (rank 2).

2.1.2 Bentazon

Effect and application

The herbicide agent bentazon has been on the market since 1972 (PPDB (2014)). Its Chemical Abstract Service number is 25057-89-0. It is applied after plant emergence and used for selective control of broadleaf weeds and sedges in sweet corn, beans, rice, corn, peanuts, mint and others. In Germany bentazon is most frequently used for cereals next to corn (Sturm et al. (2007)). According to the French ministry of agriculture and fishery (2010), the herbicide agent is allowed to be used for a wide range of cultured plants, such as sweet corn, sorghum, rice, corn, soya, peas and different kinds of cereals. Bentazon is a contact herbicide agent, i.e. it affects only the parts of the plant, to which it is applied. It interferes with the ability of certain plants to use sunlight for photosynthesis (EXTOXNET (1996)).

In the United States of America bentazon is applied in comparably low quantities, predominantly for specific plants. In particular it is used on 5 and 23% of all fresh and processing sweet corn fields, respectively (EPA (2003b)). The most recent figures of use are: 1987 (6 to 9 million pounds, rank 15), 1993 (4 to 7 million pounds, rank 18), 1995 (4 to 8 million pounds, rank 23) and 1997 (6 to 8 million pounds, rank 21). From

1999 to 2001 it was no longer listed in the ranking of the 25 most important conventional plant protection products, because of the increased use of other products and a slightly decreased use of bentazon (Kiely (2004)). Bentazon is used all over Europe, except in Malta (PPDB (2014)). In Brandenburg (a German state) the sales figures were stable from 1998 to 2003 (around 30 metric tons per year). In 1999 bentazon was the 5th most used herbicide agent in Brandenburg. In 2001 and 2003 it was in the 6th position (LUA Brandenburg (2003)). Sturm et al. (2007) report that 250 to 1000 metric tons of bentazon were sold in Germany in 2005 and that bentazon is usually applied at a rate of 0.75 kg ha⁻¹ year⁻¹ (corn) and 1 kg ha⁻¹ year⁻¹ (cereals).

Chemical, biological and physical parameters

Bentazon (3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide) is highly soluble in water (570 mg L⁻¹ at pH 7 (20°C)) and barely volatilizes (K_{HENRY} : $7.2 \cdot 10^{-5}$ Pa m³ mol⁻¹ (25°C)). Still, a drift of the substance to non-target areas via aerosols, due to application methods, such as spraying has to be anticipated (Reichenberger et al. (2007)). The distribution coefficient between octanol and water is low ($\log K_{\text{OW}}$: -0.46 at pH 7 (20°C)). The dissociation constant ($\text{p}K_{\text{A}}$) of bentazon in water is 3.28. The sorption on soil is low (K_{OC} : 34 mL g⁻¹ (EXTOXNET (1996)), 13 to 176 mL g⁻¹ (Sturm et al. 2007)). Boivin et al. (2005) found a negative correlation between bentazon adsorption to soil and natural soil pH values (5.3 to 8.2). He assumed that higher desorption rates of bentazon, compared to other herbicide agents such as atrazine, could be connected to weaker interactions of the molecule to soil components. On the contrary, Knauber et al. (2000) reported on formation of bound residues of bentazon in humic substances depending on microbial activity, which was stimulated by the presence of oxygen. However, the immobilization of bentazon in the absence of oxygen was also significant. De Wilde et al. (2009b) tested the sorption of bentazon, among other herbicide agents, on various materials with high organic carbon content, such as straw, peat mixture, cocos chips, garden waste compost and willow chopping. They found that bentazon was best adsorbed to peat mixture and coco chips and to a medium extent to straw (K_{OC} : 6 L kg⁻¹).

Bentazon is characterised by a low persistence against microbiological transformation in aerobic soil (half-life (DT_{50}): 13 days (PPDB (2014)), 35 days (Gilliom (2006))). Field experiments resulted in similar half-life values (DT_{50} : 14 days, range 4 to 21 days

(PPDB 2014)). The mineralization rate of bentazon is quite low and given with 6 to 9% in 90 days (EC (2000)). Knauber et al. (2000) observed that under non-aerobic conditions, the mineralization activity was less than 5% of the activity under aerobic conditions. Microbiological breakdown in water is lower than in soil (DT_{50} : 161 days (Sturm et al. (2007)), >200 days (Gilliom et al. (2006))). Moreover, bentazon appears to be stable to hydrolysis. However, it has a comparably low half-life in surface water, because it is readily broken down by sunlight (photolytic half-life: < 24 hours (EXTOXNET (1996)), 122 hours at pH 5 and 63 to 93 hours at pH 7 (Sturm et al. (2007))). Bentazon has the potential to contaminate surface water via run-off from treated crops, because of its high mobility. This characteristic of bentazon also suggests a strong potential for groundwater contamination. However, its breakdown by photolysis above ground and its rapid degradation by soil bacteria and fungi in unsaturated soil are expected to prevent significant contamination of groundwater (EXTOXNET (1996)). According to the leaching potential index, bentazon is a marginal leacher (GUS value: 2.55 (PPDB (2014)), 2.62, (Papa et. al. (2004))) or a leacher (GUS value: 3.22 (Gan (2002))). The Groundwater Ubiquity Score (GUS) is a method to estimate the potential of plant protection products to contaminate groundwater on the basis of their persistence and binding behaviour to solid particles. In Germany bentazon is classified as hazardous for water, due to its toxicity for water organisms, mammals and humans, its persistence and its distribution behaviour in environmental compartments (BMJ (1999)). According to the Directive 91/414/EEC, bentazon has no relevant metabolites (PPDB (2014)).

Occurrence in waters

In a stream survey in the United States of America between 1992 and 2001 by Gilliom et al. (2006), bentazon was listed on rank 11 of the most frequently detected conventional plant protection products. It was found in 16.3% of all examined surface waters. In the same study bentazon was listed on rank 6 of the most frequently detected conventional plant protection products with concentrations above $0.1 \mu\text{g L}^{-1}$. It exceeded the threshold in 7.6% of all examined surface waters. These high rankings cannot be explained by bentazon application (see above), but by its high mobility. In a more recent study (2004 to 2005) in the United States of America, Kingsbury et al. (2008) found bentazon in 18% of all examined surface waters with a maximum

concentration of $0.2 \mu\text{g L}^{-1}$ (rank 21). The different ranking is the result of the inclusion of a great number of metabolites, which were not measured by Gilliom et al. (2006). If the additionally measured substances were neglected, bentazon would be on rank 8 in the study by Kingsbury et al. (2008). Bentazon was listed in the 9th place in the ranking based on detection frequency in German surface waters (Sturm et al. (2007)). In more than 10% of German surface water monitoring stations, concentrations were above $0.1 \mu\text{g L}^{-1}$ (Sturm et al. (2007)). In France bentazon was in the 11th place in the ranking based on detection frequency in surface waters (IFEN (2009) in Tedesco et al. (2009)).

In the survey by Gilliom et al. (2006), which covered the entire United States of America, bentazon was detected at concentrations higher than and equal to $0.1 \mu\text{g L}^{-1}$ in approximately 2.5% of all examined groundwater bodies under agricultural land (rank 8). According to a UBA study, bentazon climbed between 1990 and 2000 from the 9th to the 4th position in the list of the most detected herbicide agents above $0.1 \mu\text{g L}^{-1}$ in German shallow ground waters. In 2006 bentazon was displaced from 4th to 5th position. Its occurrence decreased from 0.82% of 8578 sampling sites between 1996 and 2000 to 0.77 % of 3769 sampling sites in 2006 (Sturm et al. (2007)). In 1995 bentazon was in the 6th position of the French list of herbicide agents, which exceeded the threshold of $0.1 \mu\text{g L}^{-1}$ in ground water sampling sites (0.09% of 8524) (Isenbeck-Schröter et al. (1998)).

Kingsbury et al. (2008) examined finished drinking water of 9 water works in the United States of America and found bentazon in 16% of all samples with maximum concentration of $0.2 \mu\text{g L}^{-1}$ (rank 17 or rank 7 without metabolites). Bentazon is frequently detected in finished drinking waters in Germany and France (Tedesco et al. (2009)).

2.1.3 Isoproturon

Effect and application

Isoproturon has been on the market since 1974 (PPDB (2014)). Its Chemical Abstract Service number is 34123-59-6. It is a selective systemic herbicide agent. Its phytotoxic action is defined by the inhibition of photosynthesis in the contaminated plant. Isoproturon is used selectively in resistant crops (wheat, barley, rye) for weed control. It

is mainly absorbed by the plants via their roots and distributed in the plant via the xylem (Schmidt in Litz et al. (eds.) (2003b)).

Isoproturon is used all over Europe, except Denmark, Finland, Estonia, Lithuania, Cyprus, United Kingdom and Malta (PPDB (2014)). In Brandenburg (a German state) the sales figures were stable from 1998 to 2003 (around 170 metric tons per year). Until 2001 isoproturon was the most frequently used herbicide agent in Brandenburg. Since 2003 Glyphosate has replaced it as the top sold plant protection product (LUA Brandenburg (2003)). In 2005 in Germany more than 1000 metric tons of isoproturon were sold. Isoproturon is usually applied in a rate of 1.5 kg ha⁻¹ (Sturm et al. (2007)). The importance of isoproturon as herbicide agent in France is decreasing, since isoproturon has been listed by the French Ministry of Agriculture and Fishery as a dangerous substance, which results in restrictions of use (INERIS (2007)). Similarly, a decline in use is expected in Germany, following its classification as priority substance in the European water framework directive. At the moment isoproturon is still one of the most relevant herbicide agents on the market and in waters in Germany (Ackermann (2009), TZW (2013)). Isoproturon has no relevance in the herbicide market of the United States of America.

Chemical, biological and physical parameters

Isoproturon (*N,N*-dimethyl-*N'*-[4-(1-methylethyl)phenyl] urea) is of moderate solubility in water (65 mg L⁻¹ (22°C) (Schmidt in Litz et al. (eds.) (2003b)), 70 mg L⁻¹ (pH independent) (Sturm et al. (2007))) and volatilization is unlikely (K_{HENRY} : 1.33 · 10⁻⁵ Pa m³ mol⁻¹ (Schmidt in Litz et al. (eds.) (2003b))). Still, a drift of the substance to non-target areas via aerosols, due to application methods, such as spraying, has to be anticipated (Reichenberger et. al. (2007)). The distribution coefficient between octanol and water (logK_{OW}) is 2.48 at pH 7 (22°C). Isoproturon does not dissociate in water (Schmidt in Litz et al. (eds.) (2003b)). The sorption of isoproturon on soil is moderate (K_{OC}: 125 mL g⁻¹ (Schmidt in Litz et al. (eds.) (2003b)), 36 to 241 mL g⁻¹ (in average 122 mL g⁻¹) (Sturm et al. (2007))) and determined by clay minerals and organic material. Migration into deeper soil is supported by macropores and particular binding (Schmidt in Litz et al. (eds.) (2003b)). Isoproturon is characterized by low persistence against microbiological transformation in the aerobic top soil (half-life (DT₅₀): 4 to 45 days (Schmidt in Litz et al. (eds.) (2003b)), 12 to 33 days (Sturm et al. (2007))) and a

high persistence in the deeper soil layers (DT_{50} : 208 days (15°C) (Schmidt in Litz et al. (eds.) (2003b))). Favourable for fast degradation are aerobic conditions, high microbiological biomass, high catabolic properties of the microorganisms and high temperatures. The mineralization rate of isoproturon is relatively low and given with 10 to 22% after 100 days (EC (2002)). The microbiological degradation in water is slow (DT_{50} : 20 to 223 days (Sturm et al. (2007))). Hydrolysis is not expected to be a significant attenuation pathway in the environment (DT_{50} : 1210 to 1560 days at pH values from 5 to 7 (Sturm et al. (2007))). Photolysis is more influential (DT_{50} : 4.5 to 88 days (Sturm et al. (2007))). Assuming good agricultural practice, the risk of an enrichment of isoproturon and its metabolites in the soil in form of bound residues is low (Schmidt in Litz et al. (eds.) (2003b))). According to the leaching potential index, isoproturon is a marginal leacher (GUS value: 2.00 (PPDB (2014))). The Groundwater Ubiquity Score (GUS) is a method to estimate the potential of plant protection products to contaminate groundwater on the basis of their persistence and binding behaviour to solid particles. In Germany isoproturon is classified as highly hazardous for water, due to its toxicity for water organisms, mammals and humans, its persistence and its distribution behaviour in environmental compartments (BMJ (1999)). According to the Directive 91/414/EEC, desmethyl-isoproturon is a relevant metabolite (PPDB (2014)), i.e. (i) the metabolite has comparable intrinsic properties to the parent chemical in terms of its biological target activity, and (ii) the metabolite has toxicological properties that are considered severe, and (iii) the metabolite poses a higher or comparable risk to organisms than the parent substance (EC (2003)).

Occurrence in waters

In the study of Sturm et al. (2007), isoproturon was listed on the 3rd place in the ranking, based on detection frequency in German surface waters. In more than 25% of German surface water monitoring stations, concentrations were above $0.1 \mu\text{g L}^{-1}$. In France isoproturon was on the 6th place in the ranking based on detection frequency in surface waters in 2009. The number of French monitoring stations with bad quality, because of isoproturon ($> 2 \mu\text{g L}^{-1}$), was 20 (IFEN (2009) in Tedesco et al. (2009)). Isoproturon is frequently detected in finished drinking water in Germany and France (Tedesco et al. (2009)).

2.1.4 Nitrate

Nitrogen as macro nutrient is, next to carbon and phosphor, a major compound in biomass. In oceans plant growth is first limited by nitrogen. Plants ingest nitrogen in form of dinitrogen (N_2), ammonium (NH_4^+) and NO_3^- and incorporate it as NH_4^+ in amino acids. Since the patent for the synthesis of ammonia (NH_3) from dihydrogen (H_2) and N_2 (Haber-Bosch) in 1910 and the subsequent production of mineral nitrogen fertilizer at industrial scale, anthropogenic emission of nitrogen has escalated, especially in agriculture. Thanks to the new artificial fertilizer, agricultural yields could be increased erratically around the world. As a result the human population grew exponentially. Today 30 to 50% of agricultural yields can be affiliated to mineral fertilizers. Half of the world's population is fed with help of artificial nitrogen fertilizers (UBA (2011)).

On the downside around 420.000 metric tons of nitrogen are lost annually in Germany's agriculture by diffuse transfer to ground- and surface waters. Mostly in form of NO_3^- , it accounts for 25 % of the nitrogen that is applied via artificial fertilizers. Around 120.000 metric tons of nitrogen are emitted via drain flow into surface waters. The loss of nitrogen fertilizer in food production in form of reactive species causes a range of negative effects in receiving environmental compartments. Intensification of the greenhouse effect, depletion of the ozone layer, decrease of biodiversity by eutrophication in surface waters and in forests and harm to plant-, animal- and human health are only some examples (UBA (2011)).

In 2010 the quality in 26.5% of German ground water resources was classified as chemically insufficient, because of NO_3^- . Many waterworks had to stop drinking water production or were forced to exploit deeper aquifers (UBA (2011)). Between 2008 and 2011 average NO_3^- concentrations were higher than 50 mg L^{-1} in 16% of reported groundwater sampling points in the European Union (in Germany: 52%, in France: 13%). In surface waters of the European Union, average NO_3^- concentrations exceeded 50 mg L^{-1} in 61% of the sampling points (in Germany: 38%, in France: 48%) (EC (2013)). In 2010 NO_3^- concentrations were below 11.1 mg L^{-1} in only 15% of German surface water sampling points for water quality monitoring (BMU and BMELV (2012)).

In the National rivers and stream assessment from 2008 to 2009, surface waters in the United States of America were compared with sites that were least disturbed by human activities. In terms of water quality, affected by NO_3^- concentrations, only 17% of the rivers and streams were rated fair and 28% were rated poor (EPA (2013)).

Tactics to reduce NO_3^- migration from cropland to surface waters can be listed into three categories. The management of nutrient application, e.g. the use of fertilizer with respect to actual nutrient content in soil, is not sufficient. Vegetation diversification, e.g. intermediate crops, and the management of tile drainage water, e.g. controlled drainage, use of wetlands and bioreactors, have to be considered in parallel.

In the Aquisafe II project the effect of decentralised mitigation systems on natural attenuation of nitrogen in drain flow has been investigated.

The most important forms of nitrogen in drain flow are NH_4^+ , NO_3^- , nitrite (NO_2^-) and dissolved organic nitrogen (DON). Cuadra and Vidon (2011) stated that NO_3^- represents in general more than 80% of nitrogen flux during storm events. In gaseous form also N_2 , nitrous oxide (N_2O), nitric oxide (NO) and NH_3 may be present. In dependence on environmental conditions, the different nitrogen species are involved in transformation processes, which Vymazal (2007) described as more or less relevant for nitrogen attenuation in wetlands. Ammonification (DON into NH_3), nitrification (NH_4^+ into NO_3^-), assimilation (NH_4^+ and NO_3^- into organic nitrogen), ammonium adsorption and dissimilatory NO_3^- reduction into NH_4^+ (DNRA) do not remove nitrogen but transform it into other species. Losses of nitrogen via ammonia volatilization are insignificant below the pH of 7.5 and not serious below the pH of 8. Some fractions of organic nitrogen, incorporated in biomass, may become unavailable for nutrient cycling, due to peat formation and burial. Anaerobic ammonium oxidation (anammox) describes the conversion of NO_2^- and NH_4^+ into N_2 . In Crowe (2012) anammox was responsible for a third of N_2 formation in estuary sediments. Denitrification accounted for two thirds of N_2 formation. Denitrification is a biochemical process in which NO_3^- is converted to N_2 via the intermediates NO_2^- , NO and N_2O . NO_3^- is used as an electron acceptor by facultative heterotrophic bacteria for suboxic respiration. The optimum pH value lies between 6 and 8. Denitrification is positively correlated with temperature. It is slow below a temperature of 5°C with release of high NO and N_2O and increases to a

maximum at temperatures between 60 and 75°C with N₂ as the major end product. Denitrifying conditions establish easily in presence of available organic carbon and NO₃⁻. Therefore, denitrification is expected to be the major NO₃⁻ attenuation process in drain flow- treating bioreactors of organic substrate. Recycling of N₂ by fixation as organic nitrogen is expected to be marginal in a nitrogen rich environment (Vymazal 2007).

2.1.5 Organic carbon sources (pine bark, wheat straw)

The addition of organic substrates to mitigation systems enhances denitrification (Schipper et al. 2010), even at low hydraulic residence times (Robertson et al. 2000). The organic carbon serves as energy sources for suboxic respiration. A wide range of organic carbon sources with different particle sizes has been successively tested for effective support of denitrification, e.g. wood chips, sawdust (Schipper et al. (2010)), bark mulch (Rolf (2002), Witz (2005)), wheat straw (Gibert et al. (2008), Soares and Abeliovich (1998)) and corn cobs (Xu et al. (2009)).

The organic substrates for experimental bioreactors in the Aquisafe II project were chosen on the basis of a comprehensive literature review. The final candidates bark mulch and straw were selected with respect to a set of criteria that included wide availability, cost effectiveness and reported efficiency. Bark mulch is known for its high potential to adsorb organic contaminants (Leitch et al. (2006), Vasquez et al. (2007), Grey et al. (1996), Gendrault (2005), Li et al. (2010), Ratola et al. (2003)). Brás et al. (1999) declared that the lignin in bark mulch would be responsible for adsorption of organo-chlorine pesticides. Bark mulch also provides a long-term source of organic carbon and a stable and highly permeable structure. Therefore, it can be characterized as substrate of high longevity.

Straw is a readily accessible organic carbon source and expected to ensure high biological activity and efficient denitrification at short hydraulic residence times.

The combination of bark mulch and straw has high potential to support effective denitrification and effective herbicide agent attenuation by adsorption and by co-metabolic degradation. In Table 2-1 the selected substrates are characterized in detail, regarding shape and composition.

Table 2-1: Composition of straw and bark mulch

Parameter	Unit	Straw	Bark mulch
Shape	-	Halm cut	Chips and fibre
Specific surface ^a	cm ² cm ⁻³	> 500	350 – 450
Pore volume ^a	volume - %	82	54
Water content (air dry)	mass - %	8	13
Combustion loss	dry mass- %	94.4	94.7
Carbon content	dry mass- %	46 ^b	51 ^b
Carbon/Nitrogen ^c	-	50...100	100...400 (wood)
Cellulose content ^c	dry mass- %	27 to 33 (50 ^d)	48 (15 to 25 ^e)
Hemicellulose content ^c	dry mass- %	21 to 26 (25 ^d)	17 (15 to 20 ^e)
Lignin content ^c	dry mass- %	18 to 21 (20 ^d)	38 (10 to 20 ^e)

^a (Rolf (2002), p. 46)

^b (Kaltschmitt et al. (2009), p.343)

^c (Kretzschmar (2010), p. 58)

^d (Maier et al. (2009), p. 295)

^e (König (2007), p. 7-9)

2.2 Laboratory scale experiments

The description of the laboratory scale experiments has been produced by the author and was partly published earlier in Krause Camilo et al. (2013).

The overall objective of the experiments is to test reasonable organic substrates for their potential to support simultaneous and efficient attenuation of nitrate (NO_3^-) and selected herbicide agents in drainage waters to propose sustainable mitigation system designs for high flow conditions.

In laboratory scale, single processes (adsorption, degradation) were investigated to assess their contribution to contaminant attenuation. Influencing factors, such as type of substrate, substrate aging, hydraulic residence time, availability of organic carbon and interference of herbicide agents with NO_3^- attenuation, were studied for their effect on contaminant attenuation in real adapted systems.

The drainage water was simulated by dosing of NO_3^- and herbicide agents into pond water, which contains a natural matrix. The pond water is further characterized by low nutrient concentrations (NO_3^- : $< 1 \text{ mg L}^{-1}$, phosphate (PO_4^{3-}): $< 0.1 \text{ mg L}^{-1}$, sulphate (SO_4^{2-}): $180 \text{ to } 260 \text{ mg L}^{-1}$, ammonium (NH_4^+): $< 0.1 \text{ mg L}^{-1}$, nitrite (NO_2^-): $< 0.1 \text{ mg L}^{-1}$, dissolved organic carbon (DOC): $2 \text{ to } 4 \text{ mg L}^{-1}$) and stable physico-chemical parameters (pH value: 6.5 to 7.5, dissolved oxygen: $5 \text{ to } 10 \text{ mg L}^{-1}$, redox potential (Eh): $300 \text{ to } 350 \text{ mV}$, temperature: $21 \pm 1 \text{ }^\circ\text{C}$, electrical conductivity: $850 \text{ to } 950 \text{ } \mu\text{S cm}^{-1}$). In the following text the pond water is referred to as process water.

In literature a wide range of herbicide agent concentrations in drainage waters of agriculturally used areas can be found. Reported minimum and maximum concentrations are $0.004 \text{ } \mu\text{g L}^{-1}$ (Kronvang et al. (2003)) and $120 \text{ } \mu\text{g L}^{-1}$ (Warnemuende et al. (2007)) for atrazine, $0.002 \text{ } \mu\text{g L}^{-1}$ (Kronvang et al. (2003)) and $100 \text{ } \mu\text{g L}^{-1}$ (Larson and Jarvis (1999)) for bentazon and 0.02 (Accinelli et al. (2003)) and $465 \text{ } \mu\text{g L}^{-1}$ (Beulke et al. (2001)) for isoproturon, respectively. Concentrations around averages (atrazine: $20 \text{ to } 30 \text{ } \mu\text{g L}^{-1}$, bentazon: $50 \text{ } \mu\text{g L}^{-1}$, isoproturon $20 \text{ to } 25 \text{ } \mu\text{g L}^{-1}$) were chosen in the laboratory experiments. Herbicide agents were applied as technically purified substances without additives.

The nutrient concentrations (NO_3^- : 100 mg L⁻¹, PO_4^{3-} : 5 mg L⁻¹) were chosen, according to common values in soil solution below agriculturally used areas in temperate humid climate zones (Kretzschmar et al. (2010)).

2.2.1 Sorption experiments

Adsorption coefficients of the herbicide agents were evaluated with batch experiments following the OECD guideline for the testing of chemicals N° 106: “Adsorption - Desorption Using a Batch Equilibrium Method” (OECD, (2000)). Air dried substrate and process water, enriched with 0.01 mol L⁻¹ CaCl₂, were mixed in 250 mL centrifugal vessels. The vessels were sealed with glass stoppers and shaken overhead to wet the poorly hygroscopic organic substrate and to equilibrate the transfer processes between substrate and solution. After 16 hours the herbicide agents were spiked. Background- (no herbicide agent) and blind- (no substrate) attempts were realized for control. In the adsorption experiment the vessels were agitated for 24 hours in the overhead shaker and subsequently centrifuged with 7200 rpm. The supernatant was extracted and analysed for herbicide agents.

In a pre-study four different substrates, bark mulch (particle size (ps): 0 to 40 mm and 10 to 40 mm), straw (ps: 20 to 30 mm) and sand (ps: 2 mm), were tested for their adsorption potential. With matrix-solution ratios of 30 g/120 mL, 10 g/100 mL and 100 g/100 mL for bark mulch, straw and sand, respectively, the distribution of the herbicide agents atrazine (initial concentration: 30 µg L⁻¹) and bentazon (initial concentration: 50 µg L⁻¹) on matrix and in solution was measured.

In the main sorption experiment a mixture of bark mulch (10 to 40 mm) and straw with a mass ratio of 2.5:1 (volume ratio: 1:1) was investigated for adsorption coefficients of atrazine, bentazon and isoproturon. The matrix-solution ratio was 17.5 g/100 mL. Three different initial herbicide agent concentrations were realized in logarithmical spacing between 10 µg L⁻¹ and 10 mg L⁻¹. With 3 parallel attempts per herbicide agent and concentration an adsorption isotherm was computed. The lower limit for herbicide agent concentrations was selected with respect to actual values in drainage waters. Immediately after the sorption experiment the extracted supernatant was substituted by 0.01 mol L⁻¹ CaCl₂ enriched process water for estimation of the desorption behaviour. After 24 hours in the overhead shaker the new supernatant was extracted. The

desorption step was conducted twice. The evacuated solutions of the adsorption step and the two desorption steps were analysed for herbicide agents.

2.2.2 Degradation experiments

Batch experiments were set up to study the degradation of the herbicide agents under oxygen-absent conditions, which establish in presence of available organic carbon. Glass vessels with a volume of 1.1 L were filled with 1 L process water, which was enriched with 30 $\mu\text{g-atrazine L}^{-1}$, 30 $\mu\text{g-isoproturon L}^{-1}$ or 50 $\mu\text{g-bentazon L}^{-1}$ and 42 g organic substrate (30 g bark mulch (10 to 40 mm) and 12 g straw (< 60 mm)). The vessels were covered with aluminium foil and sealed with glass stoppers. Per herbicide 2 to 3 parallel attempts as well as background- (no herbicide agent) and blind- (no substrate) attempts were realized for control. Attenuation rates and half-lives of the herbicide agents under suboxic to anoxic conditions were investigated over 3 month-period with exponentially increasing sampling intervals (after 1, 2, 4, 7, 12, 29, 56, 92 days). The solution and the substrate were analysed for herbicide agents to distinguish between degradation and adsorption.

2.2.3 Column displacement experiments

The attenuation of herbicide agents in mitigation systems with organic substrates can be assessed from observations in degradation and adsorption experiments. Column displacement experiments enable to study the overall attenuation potential directly and more accurately in potential bioreactors under more authentic condition.

Temporary high discharges during rainy periods and snow melt and permanently high nutrient concentrations are characteristic for drainage waters. The effectiveness of a mitigation measure under these conditions is decisive for its successful implementation.

Columns with organic substrates (pure and mixed) were set up to study the potential of bioreactors to attenuate the selected herbicide agents under denitrifying conditions and the potential of the herbicide agents to interfere with NO_3^- attenuation. Low hydraulic residence times (HRT) in the range of hours were established and constantly high nutrient concentrations (100 $\text{mg-NO}_3^- \text{ L}^{-1}$ and 5 $\text{mg-PO}_4^{3-} \text{ L}^{-1}$) were continuously dosed to simulate a realistic environment.

The columns were run in parallels for each substrate: pure straw, pure bark mulch, a mixture of straw and bark mulch (volume ratio: 1:1) and a mixture of straw and sand (volume ratio: 1:1). Glass cylinders (width: 9.5 cm, height: 22 cm) were filled to 75 % of their height, step by step to create a homogeneous distribution of the substrates. The end caps were made from PVC and included inlet at the bottom and outlet and ports for probes at the top. The columns were fed contra gravity with a peristaltic pump that was adjustable for different discharges to set different HRT. All connections and the tubing were made from silicon. The feed solution was stocked in 10 L glass vessels. Columns and vessels were completely covered with aluminium foil to prevent photolytic reactions (Figure 2-1).

Atrazine and **bentazon** were studied in pure straw, pure bark mulch, a mixture of bark mulch and straw. HRT was set to approximately 0.2 days (2.88 L day^{-1}). The columns were run in a start phase with nutrients only to observe wash-out of organic carbon and denitrification performance. After 4, 5 and 11 months, atrazine (20 to $25 \mu\text{g L}^{-1}$) and bentazon ($50 \mu\text{g L}^{-1}$) were added each in one parallel column of pure straw, the mixture of bark mulch and straw and pure bark mulch, respectively. Constant herbicide agent concentrations were dosed continuously. The dosing of bentazon was stopped after 3 months in straw and in the mixture and after one month in bark mulch. For observation of the effect of flow rate changes on NO_3^- and herbicide agent attenuation, HRT was adjusted for short-term periods (2 to 3 weeks) to approximately 0.4 days (1.44 L day^{-1}) and 2.0 days (0.29 L day^{-1}).

After one year of operation the parallels with bentazon contact were stopped. The atrazine parallels were continued for additional 0.5 years at HRT of 0.2 days and finally for 0.5 years at HRT of 0.4 days to observe the attenuation potential of the substrates and the effect of HRT more precisely.

Short before the end of the experiment the atrazine parallel of the columns with the mixture of straw and bark mulch was let run dry (unsaturated, but wet) for 3 weeks to observe the effect of a dry period on herbicide agent attenuation. After that phase and at the end of the main experiments the atrazine parallels of the mixed and the pure bark mulch column were washed for 4 and 3 weeks, respectively, with pure process water to observe leaching of attenuated herbicide agents.

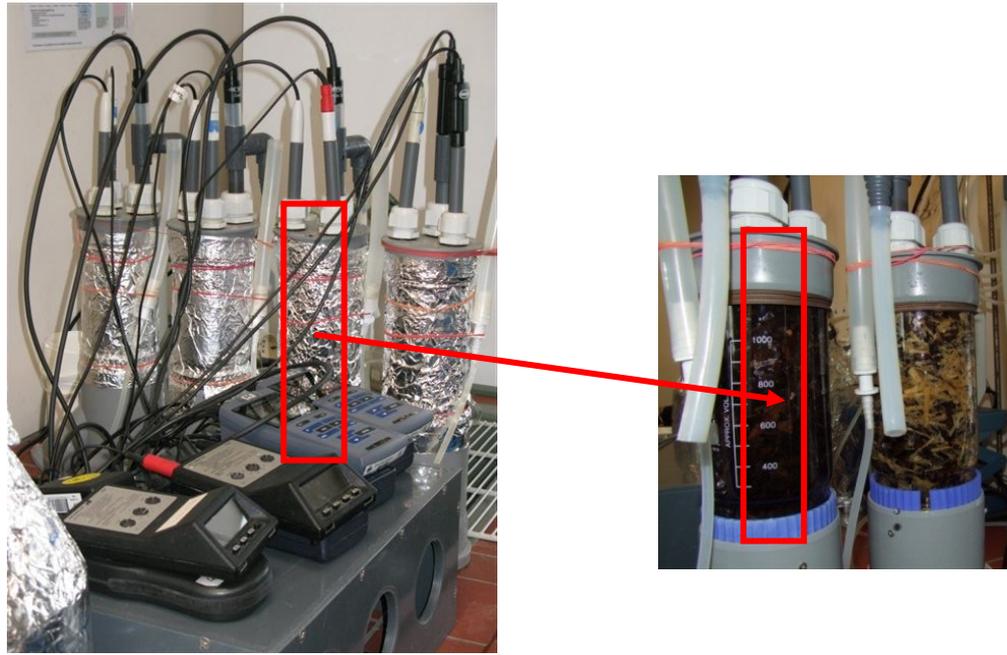


Figure 2-1: Setup of laboratory scale columns.

Column displacement experiments with **isoproturon** (20 to $25 \mu\text{g L}^{-1}$) were carried out after first experiences with atrazine and bentazon in laboratory and technical macro scale. For 1.5 years isoproturon attenuation was studied in the mixture of bark mulch and straw (volume ratio: 1:1), which contributed best to simultaneous NO_3^- and herbicide agent attenuation at low HRT. Continuous and constant dosing of nutrients

(100 mg-NO₃⁻ L⁻¹ and 5 mg-PO₄³⁻ L⁻¹) and isoproturon (20 to 25 µg L⁻¹) was established from the start. In a parallel control column, only nutrients were added to investigate the effect of the herbicide agent on NO₃⁻ attenuation. The HRT in the columns was set to around 0.4 days to compare the results in laboratory scale and technical macro scale. Accordingly, the effect of a lower flow rate was tested at HRT of 2.0 days, but only on short-term. At the end of the laboratory experiment, leaching of isoproturon was evaluated by washing the organic substrate with pure process water and observing the release of attenuated substance fractions.

The solution in the inflow and in the outflow of the columns was sampled on a one week basis and analysed for herbicide agents, metabolites of atrazine (hydroxy-atrazine and deethyl-atrazine) and nutrients. Substrate samples of the straw-bark mulch column with atrazine contact were taken before and after the dry phase and at the end of the experiment and analysed for herbicide agent content. The physico-chemical parameters: temperature, content of oxygen, pH and redox potential were frequently measured to monitor the experimental environment. Hydraulic characteristics of the substrates, such as effective porosity, were identified in tracer tests with sodium chloride.

In five-month duration an extra column displacement experiment without addition of herbicide agents was conducted to study the participation of the organic carbon source in competing respiratory reactions and wash-out. The efficiency of the substrate as organic carbon source for denitrification was evaluated. Lost carbon was estimated stoichiometrically from reduction of major electron acceptors (oxygen, NO₃⁻ and SO₄²⁻) and from wash-out of organic carbon. The results were compared with the actual overall carbon loss in the organic substrate. The experiment was conducted in duplicates in terms of set up and NO₃⁻ load. Inflow concentrations and flow rates were adjusted temporarily to investigate the influence of NO₃⁻ input and HRT on attenuation of NO₃⁻ loads (Table 2-2).

Table 2-2: Time schedule of adjustments in straw-bark mulch columns (hydraulic residence time, nitrate concentration).

Time schedule	1 month	2.5 months	1.5 months
	Hydraulic residence time, nitrate concentration		
Column a	0.2 days, 100 mg L ⁻¹	0.4 days, 200 mg L ⁻¹	0.4 days, 100 mg L ⁻¹
Column b	0.2 days, 100 mg L ⁻¹	0.2 days, 100 mg L ⁻¹	0.2 days, 50 mg L ⁻¹

2.3 Technical macro scale experiments

The description of the technical macro scale experiment has been produced by the author and is, in parts, accepted to be published in the Ecological Engineering – The Journal of Ecosystem Restoration (Krause Camilo (2015)).

There are many potential design configurations possible in laboratory experiments. However, conditions are artificial and design has a strong influence on experimental outcome. Laboratory experiments alone are not suitable for reproducing complex natural processes, but designed well, they give a good first approximation of single effects. They are an important tool to narrow down the variability of possible design solutions and help to prepare macro scale experiments with low risk of failure. Controlled technical macro scale experiments provide more realistic information than laboratory experiments, because near natural conditions can be simulated. The variability of influencing factors can still be limited, e.g. by parallel attempts, to distinguish between single processes and environmental effects.

The technical macro scale experiment of the present study aimed at simulating the operation of designed mitigation systems (bioreactors) under realistic but controlled conditions. The response of NO_3^- and herbicide agent attenuation processes to seasonally changing temperatures was investigated at medium to high flow conditions.

The outcome of the laboratory experiments was considered in the setup of the technical macro scale experiments.

In laboratory scale hydraulic residence time (HRT) had high influence on the ratio of attenuated and dosed contaminants. In technical macro scale, HRTs were adjusted in parallels to around 0.4 and 2.5 days. The range was chosen to lower the risk of overlay of temperature- and HRT- effects, especially at low temperatures and at low HRT and to verify the results of short-term HRT experiments in laboratory under more realistic long-term conditions.

In laboratory scale, simultaneous NO_3^- and atrazine- or isoproturon attenuation was more effective in the mixture of bark mulch and straw than in the pure substrates. The bioreactors in technical macro scale were implemented by filling parallel concrete

channels (length: 8 m, width: 2 m, height: 0.9 m) to a height of 0.8 m with a mixture of straw (550 kg) and bark mulch (1050 kg). The substrates were alternately added in several layers, stepwise mixed and compressed by foot to prevent preferential flow (Figure Appendix-2 and Figure Appendix-3). The bioreactors and the mixing cells were covered with a plastic sheeting to prevent influence of precipitation and deposition. A minimum water level of 0.4 m was ensured by a weir in the outflow cell. The layer of dry organic substrate between the water level and the plastic sheeting was reserved to replace depleted substrate below and to buffer thermal radiation from the cover (Figure 2-2).

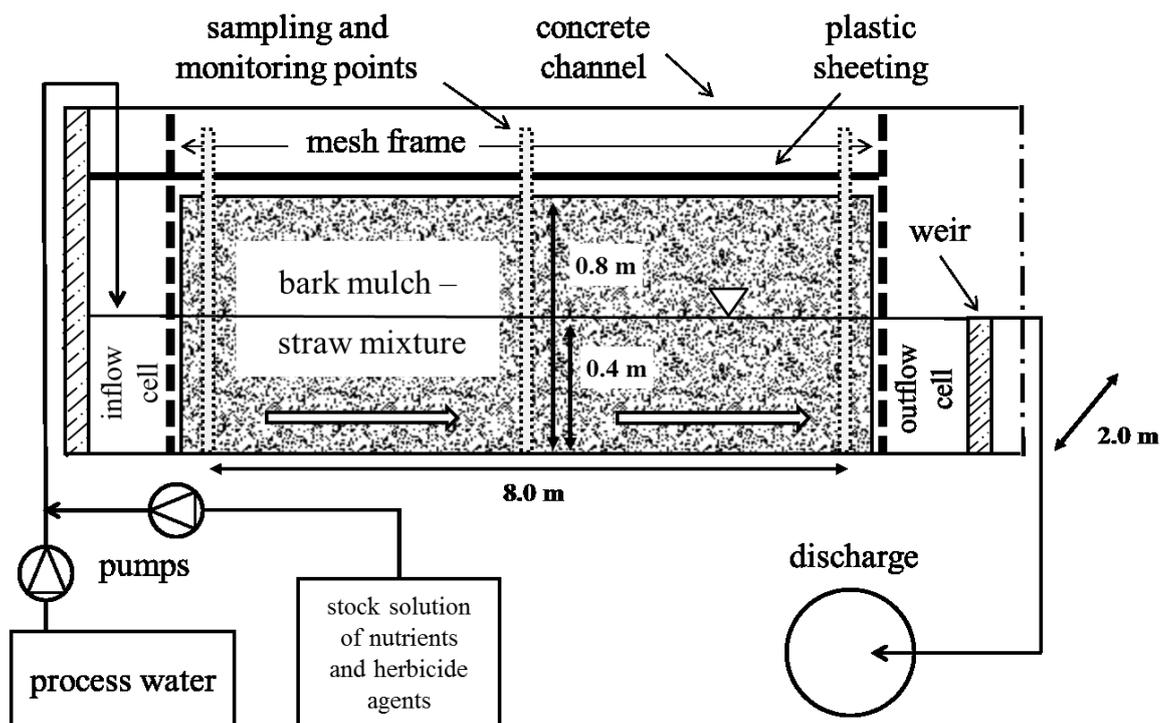


Figure 2-2: Side view (longitudinal section) of bioreactor in technical macro scale (sketch untrue to scale).

The inflow water was of the same origin and equally spiked with nutrient concentrations as in the laboratory column displacement experiments. Inflow concentrations of atrazine and isoproturon were slightly higher (20 to $30 \mu\text{g L}^{-1}$). Bentazon was not applied in the technical macro scale experiments. In laboratory scale experiments the herbicide agent was too mobile and persistent to be attenuated under denitrifying conditions at short HRT.

Since systems were equally sized, HRTs between 0.4 and 2.5 days were established by different flow rates (about 500 L h⁻¹ and 100 L h⁻¹, respectively), which resulted in lower input loads at higher HRT.

Two bioreactors (number 1 and 2) were run with atrazine at HRTs of about 0.4 and 2.5 days, respectively. An identically constructed third bioreactor (number 3) with HRT of about 2.0 days and without atrazine addition was prepared as a blind to be able to compare NO₃⁻ attenuation in presence and in absence of the herbicide agent. The control bioreactor was run for one year, while the two bioreactors with atrazine were run for 1.5 years.

Two further bioreactors (number 4 and 5) were prepared in the same manner as the bioreactors 1 and 2, but to investigate isoproturon attenuation. HRTs were set to around 0.5 and 2.0 days, respectively. The duration of the experiment was one year. In Table 2-3 the experimental plan of technical macro scale experiments is presented.

Herbicide agents, metabolites of atrazine (hydroxy-atrazine and deethyl-atrazine) and nutrients were analysed and physico-chemical parameters were measured analogue to the laboratory column displacement experiments. Hydraulic characteristics of the substrates, such as effective porosity, were identified in tracer tests with sodium chloride.

Table 2-3: Time schedule of technical macro scale experiments in 5 straw-bark mulch bioreactors.

Time schedule		May 2010	Jun 2010	Jul 2010	Aug 2010	Sep 2010	Oct 2010	Nov 2010	Dec 2010	Jan 2011	Feb 2011	Mar 2011	Apr 2011	May 2011	Jun 2011	Jul 2011	Aug 2011	Sep 2011	Oct 2011	Nov 2011	Dec 2011	Jan 2012	Feb 2012	Mar 2012	Apr 2012	May 2012	Jun 2012	Jul 2012													
Bioreactors	1	No additives	A	B		P_{sat}	B											P_{dry}	B																						
	2	-	A	B		P_{sat}	B											P_{dry}	B																						
	3	-	A				P_{sat}	A																																	
	4																		C			P_{sat}																			
	5																			C			P_{sat}																		
<p>Additives in process water: A = $NO_3^- + PO_4^{3-}$, B = $NO_3^- + PO_4^{3-} + ATR$, C = $NO_3^- + PO_4^{3-} + IPU$ ATR = atrazine, IPU = isoproturon, NO_3^- = nitrate, PO_4^{3-} = phosphate</p>																																									
= Hydraulic residence time (HRT): ~ 0.5 days														= HRT: ~ 2.0 days														P_{sat} = paused (saturated) due to frost P_{dry} = 1 st month: dry phase, 2 nd month: flooding phase with pure process water (no additives)													

2.4 Chemical analysis

The description of the chemical analysis has been produced by Dr. S. Heise and the author and was partly published earlier in Krause Camilo et al. (2013).

Atrazine was removed from the liquid sample by solid phase extraction (Bohuss et al. (2005)) and analysed by gas chromatography in combination with mass spectrometry (GC/MS) (TRACE GC/Polaris-Q MS) without derivatization, following the EPA method 525.2 (Eichelberger (1995)). Adsorbed atrazine was extracted by methanol (Del Valle et al. (1994)). For analysis methanol was removed again and the extract was diluted in water and treated as the liquid samples.

Hydroxy-atrazine (HA) and **deethyl-atrazine (DEA)** in the liquid sample were analysed by liquid chromatography in combination with mass spectrometry (LC/MS) (Agilent 1290 Infinity (column: Atlantis T3, 5 μ m, 2.1x150 mm)/AB Sciex Qtrap 5500) with following transitions for quantification: 198/156 (HA) and 188/146 (DEA) (Jeannot et al. (2000)). Entrance potentials were 10 V for both substances, declustering potentials were 126 V (HA) and 66 V (DEA), collision energies were 29 V (HA) and 25 V (DEA) and collision cell exit potentials were 16 V (HA) and 4 V (DEA). The mobile phase composition consisted of (A) water with 0.1% formic acid and (B) methanol with 0.1% formic acid. In the beginning the partition of A was 70%. During 6 minutes it was gradually reduced to 40 %. After being kept constant for 3 minutes it was gradually increased during one minute to the starting value of 70% and kept on this level for 5 more minutes (flow rate: 0.25 ml minute⁻¹). Before analysis of hydroxy-atrazine and deethyl-atrazine by LC/MS the liquid samples were filtered (0.45 μ m).

Bentazon was removed from the liquid sample by solid phase extraction (Vink et al. (1996)) and methylated in a derivatization step (Gaynor et al. (1981)) before analysis by GC/MS (TRACE GC/Polaris-Q MS), following the procedure described in the method DIN EN ISO 15913 (2003) and in the EPA method 515.2 (Munch (1995)). Adsorbed bentazon was extracted by methanol. Prior to analysis methanol was removed again and the extract was diluted in water and treated as the liquid sample (Thorstensen and Lode (2001)).

Isoproturon was removed from the liquid sample by solid phase extraction (Bohuss et al. (2005)) and analysed by GC/MS (TRACE GC/Polaris-Q MS) without derivatization following the EPA method 525.2 (Eichelberger (1995)). Adsorbed isoproturon was extracted by methanol (Del Valle et al. (1994)). For analysis methanol was removed again, the extract was diluted in water and isoproturon was subsequently analysed by LC/MS (Agilent 1290 Infinity (column: Atlantis T3, 5 μ m, 2.1x150 mm)/AB Sciex Qtrap 5500) with following transitions for quantification: 207/72 and 207/165 (Jeannot et al. (2000)). Entrance potential was 10 V, declustering potential was 91 V, collision energy was 23 V and 19 V, respectively, and collision cell exit potential was 8 V. The mobile phase composition consisted of (A) water with 0.1% formic acid and (B) methanol with 0.1% formic acid. In the beginning the partition of A was 70%. During 6 minutes it was gradually reduced to 40%. After being kept constant for 3 minutes it was gradually increased during one minute to the starting value of 70% and kept on this level for 5 more minutes (flow rate: 0.25 ml minute⁻¹). Before analysis of isoproturon by LC/MS the liquid sample was filtered (0.45 μ m).

Deviation of measurements in GC/MS, due to preparation, was considered in comparison with internal standards, which were added to each batch of samples before preparation (terbuthylazine for atrazine and bentazon; chlortoluron for isoproturon). In LC/MS no preparation of liquid samples was necessary.

To quantify the recovery of the substances in the organic substrate, the standards were first added to dry straw and bark mulch and extracted after volatilization of the solvent ethyl acetate after two days.

The inorganic ions NO₃⁻, SO₄²⁻ and NO₂⁻ were measured with ion chromatography (Dionex LC 20 with ultraviolet- and electrical conductivity- detection) following DIN EN ISO 10304-1 (2009). PO₄³⁻ and NH₄⁺ were analysed photometrically (Jenaoptik Typ Specord 205) following DIN EN ISO 6878 (2004) and DIN 38406-5 (1983), respectively. Dissolved organic carbon (DOC) and total organic carbon (TOC) were measured with the Elementar Vario TOC cube after combustion as carbon dioxide (ASTM D 7573 (2009), DIN 38409-46 (2010), DIN EN 1484 (1997)).

All measurements were conducted in duplicates. In Table 2-4 quantification limits and recovery rates in different media are presented.

Table 2-4: Quantification limits and recovery rates.

Method	Substance	Media	Quantification limit	Recovery
<u>Gas Chromatography/</u> <u>Mass Spectrometry</u>	Bentazon	Process water	2.18 $\mu\text{g L}^{-1}$	76 \pm 5%
		Straw	0.2 $\mu\text{g g}^{-1}$	80 \pm 9%
		Bark mulch	1.0 $\mu\text{g g}^{-1}$	
	Atrazine	Process water	0.17 $\mu\text{g L}^{-1}$	86 \pm 1%
		Straw	0.3 $\mu\text{g g}^{-1}$	80 \pm 8%
		Bark mulch	1.1 $\mu\text{g g}^{-1}$	
Isoproturon	Process water	0.17 $\mu\text{g L}^{-1}$	121 \pm 2%	
<u>Liquid Chromatography/</u> <u>Mass Spectrometry</u>	Isoproturon	Straw - bark mulch	0.2 ng g^{-1}	104 \pm 15%
	Deethyl-atrazine	Process water	0.14 $\mu\text{g L}^{-1}$	-
	Hydroxy-atrazine	Process water	0.05 $\mu\text{g L}^{-1}$	-
<u>Ion Chromatography</u>	Nitrate	Process water	1 mg L^{-1}	-
	Sulphate	Process water	5 to 10 mg L^{-1}	-
	Nitrite	Process water	0.1 mg L^{-1}	-
Photometry	Phosphate	Process water	0.1 mg L^{-1}	-
	Ammonium	Process water	0.1 mg L^{-1}	-
<u>Non-Purgeable Organic Carbon</u>	Dissolved and total organic carbon	Process water	0.1 mg L^{-1}	-

2.5 Data analysis

The summary of the data analysis has been produced by the author and was partly published earlier in Krause Camilo et al. (2013).

Sorption experiment

The adsorption of atrazine, bentazon and isoproturon is described with the FREUNDLICH adsorption isotherm (OECD 106 (2000)):

$$C_s^{ads}(eq) = K_F^{ads} \times C_{aq}^{ads}(eq)^{1/n} \quad \text{Equation 1}$$

C_s [$\mu\text{g g}^{-1}$] is the partition of the substance adsorbed to the matrix and C_{aq} [$\mu\text{g L}^{-1}$] is the partition of the substance dissolved in solution after establishment of equilibrium. K_F [$\mu\text{g}^{1-1/n} \text{L}^{1/n} \text{g}^{-1}$] is the FREUNDLICH coefficient and $1/n$ [-] is the FREUNDLICH exponent.

Degradation experiment

Assuming a first order decay, the degradation rate (λ_B [day^{-1}]) in a batch reactor can be calculated with C_0 [$\mu\text{g L}^{-1}$] as the initial concentration, C_t [$\mu\text{g L}^{-1}$] as the concentration at time (t), C_{eq} [$\mu\text{g L}^{-1}$] as the concentration at equilibrium and t_{lag} [days] as duration of the lag-phase at the start without changes in concentration:

$$\begin{aligned} t < t_{lag} : C_t &= C_0 \\ t \geq t_{lag} : C_t &= (C_0 - C_{eq}) \times e^{-\lambda_B(t-t_{lag})} + C_{eq} \end{aligned} \quad \text{Equation 2}$$

Consequently, the half-life value in the batch reactor ($DT_{50,B}$ [days]) is:

$$DT_{50,B} = -\frac{\ln(0.5)}{\lambda_B} + t_{lag} \quad \text{Equation 3}$$

Column displacement and bioreactor experiment (attenuation)

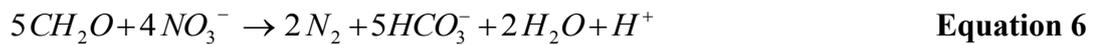
The attenuated loads (P) of oxygen, sulphate, nitrogen from NO_3^- and NO_2^- , atrazine and isoproturon were calculated as the difference between cumulative masses of the

single substance at the inflow and outflow of the column or bioreactor per reactor volume (V_R) and per sampling interval (t). The cumulated masses of the single substance were calculated by multiplying discharged volume (V) and average concentration at the inflow or outflow (c) for each sampling interval.

$$P = \frac{(c_{inflow} \times V_{inflow}) - (c_{outflow} \times V_{outflow})}{V_R \times t} \quad \text{Equation 4}$$

Loads of washed out organic carbon were calculated in analogy to loads of attenuated substances. Absolute attenuation and wash-out are described by the attenuated and washed-out loads. Relative attenuation is the ratio of attenuated load and load in the inflow [%].

Carbon, utilized for oxygen reduction, denitrification and dissimilatory sulphate reduction was stoichiometrically estimated based on the following equations:



Thus, 1 mol, 5 mol and 2 mol of organic carbon are oxidized to reduce 1 mol of oxygen to H_2O , 4 mol of NO_3^- to dinitrogen (N_2) and 1 mol of SO_4^{2-} to hydrogen sulphide (H_2S), respectively (Gibert et al. 2008).

Assuming complete exchange of pore water in the column or bioreactor during flow the degradation or attenuation rate (λ_F [day^{-1}]) can be derived on the basis of the inflow concentration (C_{in} [$\mu g L^{-1}$]), the concentration in the outflow once equilibrium has adjusted (C_{eq} [$\mu g L^{-1}$]) and the hydraulic residence time (HRT [days]) (Maniak (2010), p. 600):

$$\lambda_F = \frac{C_{in} - C_{eq}}{HRT \times C_{eq}} \quad \text{Equation 8}$$

The time needed to reduce half of the inflow concentration in the column or bioreactor ($DT_{50,F}$ [days]) is:

$$DT_{50,F} = -\frac{\ln(0.5)}{\lambda_F}$$

Equation 9

For presentation and statistical evaluation of the results the spreadsheet programs Origin from OriginLab and Excel from Microsoft were used.

Chapter 3

Results and discussion

3.1 Laboratory scale experiments

Results and discussions of the laboratory experiment have been produced by the author and were partly published earlier in Krause Camilo et al. (2013).

3.1.1 Sorption of herbicide agents

In batch experiments with atrazine and bentazon with initial concentrations of $30 \mu\text{g L}^{-1}$ and $50 \mu\text{g L}^{-1}$, respectively, the suitability of different substrates to sorb herbicide agents was tested (Figure 3-1).

In general the herbicide agents were sorbed rather on bark mulch than on straw and merely on sand. However, the matrix-solution mass ratio in the attempt with bark mulch (0.4) was higher than in the attempt with straw (0.1), which could have favoured sorption on bark mulch. Interestingly, little difference was observed between bark mulch with and without fine particles (0 to 10 mm).

Independently on the substrate, more atrazine than bentazon was sorbed. Higher affinity of bentazon to remain in solution was expected, due to a 10-fold higher solubility in water.

Bentazon concentrations in solution at equilibrium were higher in the straw attempt and the sand attempt than in the control without substrate addition. This contradiction reflects, together with high standard deviations, the difficulties with bentazon detection, which was strongly influenced by the composition of the solution.

It can be confirmed that organic matter in substrates has a positive impact on sorption of organic compounds.

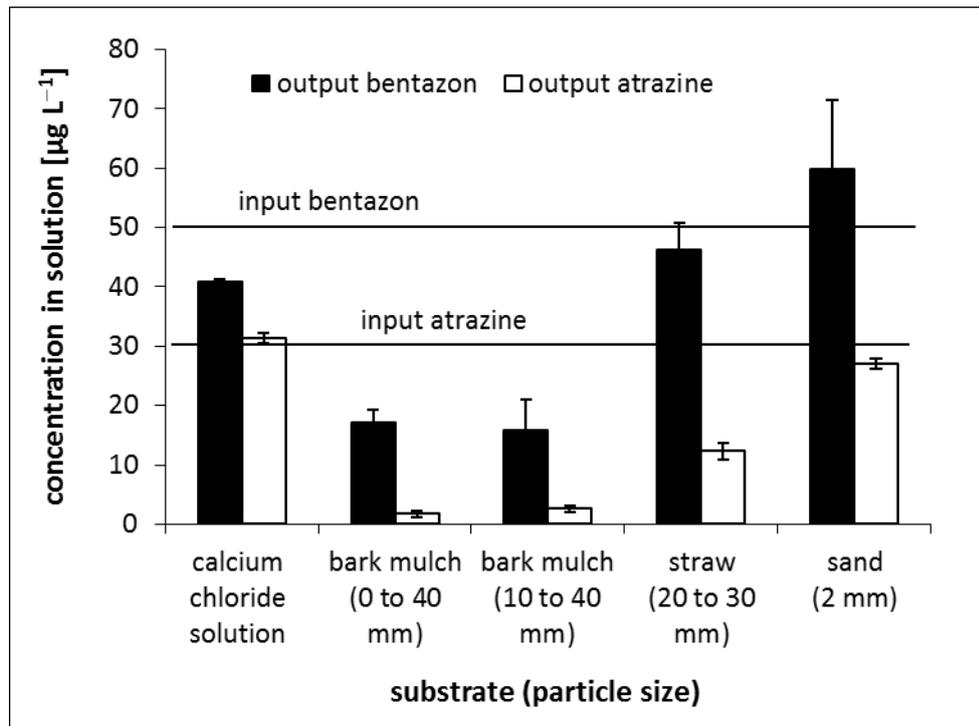


Figure 3-1: Herbicide agent concentration in different substrates after adsorption experiment with initial concentrations of $30 \mu\text{g L}^{-1}$ (atrazine) and $50 \mu\text{g L}^{-1}$ (bentazon).

Since the combination of bark mulch and straw promised best results in simultaneous herbicide agent and NO_3^- attenuation, more comprehensive sorption experiments were conducted with a mixture of the substrates bark mulch and straw with a volumetric ratio of 1:1. After 24 hours in the overhead shaker 82 to 88%, 82 to 83% and 35 to 56% of atrazine, isoproturon and bentazon, respectively, were adsorbed to the substrate. According to OECD guideline 106 (2000), adsorption isotherms for atrazine, bentazon and isoproturon were determined with initial concentrations in the range between $10 \mu\text{g L}^{-1}$ and 10mg L^{-1} . The isotherms were modelled after the concept of FREUNDLICH. It presumes that the ratio of adsorbed and dissolved substance fractions at equilibrium develops degressively with increasing input concentrations (Equation 1). In Figure 3-2 the FREUNDLICH adsorption isotherms are depicted with FREUNDLICH coefficients [$K_F [\mu\text{g}^{1-1/n} \text{L}^{1/n} \text{g}^{-1}]$] and FREUNDLICH exponents [$1/n [-]$].

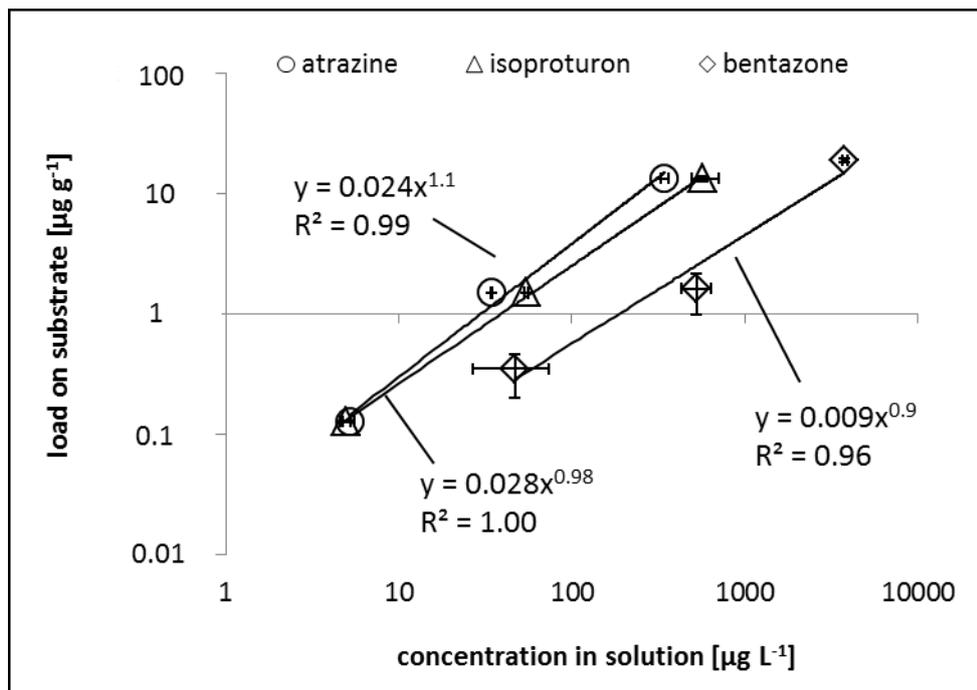


Figure 3-2: FREUNDLICH adsorption isotherms of atrazine, bentazon and isoproturon in mixture of straw and bark mulch with initial concentrations between 10 µg L⁻¹ and 10 mg L⁻¹. Error indicators present minimal and maximal deviation from average values.

With respect to FREUNDLICH exponents between 0.9 and 1.1, the distribution of the herbicide agents between substrate and solution can be assumed as quasi-linear for initial concentrations in the range between 10 µg L⁻¹ and 10 mg L⁻¹. Under that premise, linear distribution of atrazine, bentazon and isoproturon in a mixture of bark mulch and straw can be deduced. The linear distribution coefficient (K_d) is the ratio of adsorbed and dissolved fraction at the equilibrium of distribution. In Table 3-1 the estimated K_d values are compared with K_d values of studies, which tested sorption of the same herbicide agents to organic substrates.

Table 3-1: Linear distribution coefficients (K_d) of atrazine, bentazon and isoproturon in different substrates.

Substance	Initial concentration	Substrate (content of organic carbon)	Distribution coefficient (K_d)	Reference
	mg L ⁻¹	%	L kg ⁻¹	Author/-s (year)
Atrazine	1 to 20	Bark (40)	155	Gendrault (2004)
	0.5 to 8	Wood (46.5)	66	Ilhan (2010)
	0.03, 0.30, 2.70	Straw-bark mulch (46 to 51)	39	This study
	0.003 to 0.8	Bark (40)	23	Gendrault (2004)
	n.a.	Straw (n.a.)	15	Abdelhafid et al. (2000)
	0.25 to 10	Soil (0.62 to 3.5)	1.3 to 6.3	Boivin (2005)
	0.2 to 3	Soil (0.18 to 1.63)	1.7 to 2.6	Sponberg and Ganliang (2000)
Isoproturon	0.03, 0.32, 3.30	Straw-bark mulch (46 to 51)	28	This study
	1 to 5000	Willow chopping (43)	8.65	De Wilde et al. (2009b)
	1 to 5000	Straw (42)	2.05	De Wilde et al. (2009b)
	1 to 5000	Sandy loam soil (0.9)	1.25	De Wilde et al. (2009b)
Bentazon	0.11, 0.81, 7.10	Straw-bark mulch (46 to 51)	5	This study
	1 to 5000	Willow chopping (43)	0.85	De Wilde et al. (2009b)
	1 to 5000	Straw (42)	0.75	De Wilde et al. (2009b)
	1 to 5000	Sandy loam soil (0.9)	0.7	De Wilde et al. (2009b)

The comparison with other studies shows that the observed adsorption coefficients are in the range of values that were measured under similar conditions in similar substrates.

Higher content of organic matter in substrates result in higher K_d values for the tested herbicide agents. Pure organic substrates are more effective than soils in terms of absolute adsorption. On the other hand, soils adsorb more efficiently, which is indicated by higher K_{OC} values, i.e. distribution coefficients (K_d) normalized to total organic carbon content.

It can be assumed that atrazine and isoproturon sorb better to organic substrates than bentazon. Also the bonds of isoproturon and atrazine to the substrate are stronger as indicated by desorption studies. After two desorption steps, the main fractions of initial atrazine (51 to 75%) and isoproturon (52 to 60%) were still adsorbed, while only 9 to 23% of initial bentazon were bound to the matrix of the substrate.

De Wilde et al. (2009b) observed that the presence of adjuvants in formulated herbicides had no significant influence on the sorption of isoproturon and bentazon.

3.1.2 Degradation of herbicide agents

Atrazine, isoproturon and bentazon are organic compounds, which degrade predominantly in presence of oxygen. Organic substrates are expected to provide a microbiologically diversified and active environment, which potentially improves degradation of the herbicide agents in absence of oxygen, e.g. by co-metabolism. In batch experiments of 3 months duration, the degradation of the herbicide agents in presence of organic substrates under suboxic to anoxic conditions was investigated.

Herbicide agents on the substrate and in solution were analysed to distinguish between sorption and degradation. Unfortunately, it is indicated that the extraction method for sorbed herbicide agents was insufficient. It is not ensured that the sorbed herbicide agents, especially aged fractions, were entirely regained. In the following the term attenuation is used for the combination of degradation and sorption.

In the blind attempt low herbicide agent reduction in solution was observed over time, although redox conditions in the process water remained aerobic ($E_h > 300$ mV). The lack of microorganisms, substrate and nutrients is expected to be responsible. Bentazon concentrations in the blind attempt were reduced by 30% in the first week and remained stable until the end. Concentrations of atrazine and isoproturon in the blind attempt decreased more gradually during the entire experiment and to a lower extent by 12 and 10%, respectively.

In presence of organic substrate, redox conditions turned nonaerobic after few days. Bentazon concentrations did not change. Atrazine and isoproturon on the other hand were substantially removed from solution (Figure 3-3 and Figure 3-4).

Hydrolysis and biotic degradation as well as adsorption are expected to be the main attenuation processes for atrazine and isoproturon in organic substrates. Photodegradation was impeded by covered experimental vessels. Volatilization is negligible, due to low vapour pressure and sorption to the organic material (Correia et al. (2007), Ghosh et al. (2001)).

Atrazine

In the first 4 days of the experiment, increasing adsorption is responsible for decrease of atrazine in the liquid phase. After this lag phase, a substantial and comparatively fast reduction of recoverable atrazine (from solution and substrate) was detected over time (Figure 3-3). According to first-order kinetics (Equations 2 and 3) the attenuation rate (λ_B) of atrazine, which combines sorption and degradation, yielded 0.14 day^{-1} and the time for attenuation of 50% of atrazine ($DT_{50,B}$) was 7.9 days.

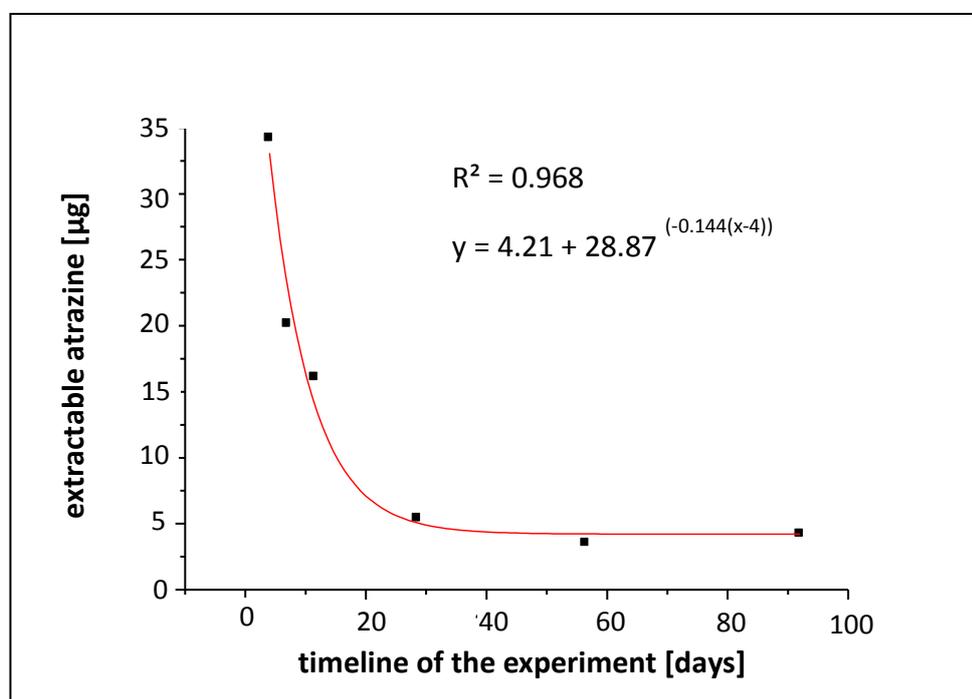


Figure 3-3: Development of total extractable atrazine content in solution and on substrate (mixture of straw and bark mulch) over time under suboxic to anoxic conditions in the degradation experiment. Attenuation of atrazine starts after lag-phase of 4 days

A review by Ghosh and Philip (2006) listed several reports that prove, biodegradation of atrazine is quite possible under suboxic to anoxic conditions. The findings of their study that addition of organic materials enhances facultative, non-aerobic degradation of atrazine are in agreement with Chung et al. (1996). The latter found degradation of

atrazine in non-aerobic wetland sediments by co-metabolic processes, using various carbon sources, such as methanol, sodium acetate, acetic acid and glucose, to provide assimilative organic carbon.

The results of the metabolite analysis of the present study did not support biotic degradation in solution as major reduction process. Deethyl-atrazine, the most drinking water-relevant metabolite of atrazine, was not detected above $0.1 \mu\text{g L}^{-1}$. This may be explained by its preferred formation under aerobic conditions. Under the experiments conditions, hydroxy-atrazine was expected to be the major metabolite. It is predominantly formed under denitrifying conditions (Crawford et al. (1998)) and in non-aerobic sediments with carbon addition (Chung et al. (1996)). As a matter of fact, it was found in solution with increasing concentrations over time up to $1.0 \mu\text{g L}^{-1}$. However, hydroxy-atrazine formation could be accounted for only 3 to 4% of atrazine that was reduced in solution.

The gap between added and recovered atrazine in solution has to be explained by the formation of bound residues (non-extractable atrazine) in the organic substrate. Drori et al. (2005) suggested that the sorption of atrazine in soils occurs in a first fast step to readily available sites and in a second much slower step by interaction with the soil organic matter, which hinders atrazine desorption ability significantly. Lesan and Bhandari (2004) observed that long contact times between atrazine and organic soil decreased water extractability and enhanced the formation of bound residues.

Still, biotic degradation, as important part of atrazine attenuation, has not to be inhibited. Gendrault et al. (2005) found that degradation of atrazine in bark extract by isolated *Pseudomonas sp. strain adp*, a facultative denitrifier, was admittedly reduced from 75 to 50% by irreversible sorption on fine bark particles ($<2 \text{ mm}$), but not stopped. Houot et al. (1996) observed that straw increased the formation of bound residues in soil, but found likewise that partial degradation into hydroxy-atrazine occurred, probably due to enzymatic activity or acidity of the humic components, present in straw. Even direct biotic degradation of atrazine on the matrix may be possible (Abdelfahid et al. (2000)). Trigo et al. (2010) found that atrazine is bio-accessible in organoclay complexes. Park et al. (2003) observed mineralization of soil-sorbed atrazine and proclaimed that high atrazine concentration on the matrix and the presence of microorganisms onto soil particles seem to contribute to bioavailability of atrazine.

Jablonowski et al. (2008) showed that long-term aged bound residues of atrazine are still partly accessible to the degrading microorganism *Pseudomonas sp. strain adp.* Herzberg et al. (2004) observed high degradation of atrazine, which was adsorbed to granulated activated carbon [70 mg-atrazine L⁻¹ day⁻¹] by isolated denitrifier *Pseudomonas sp. strain adp.*, using the herbicide agent as N source.

Nevertheless, it seems more likely that adsorption and subsequent chemical degradation are predominantly responsible for attenuation of atrazine by partial transformation of the parent compound into its metabolite hydroxy-atrazine (Ma and Selim (1996), De Andrea et al. (1997)). Chemical degradation (hydrolysis) of atrazine is soil-surface catalysed, i.e. adsorption favours decomposition to hydroxy-atrazine (Armstrong et al. (1967)).

The organic substrate qualifies as source of important binding sites. Under non-aerobic conditions, as established in the experiment, the formation of humic substances is expected, e.g. by lignin modification. The important role of organic matter (humins, humic and fulvic acids) in soils for atrazine attenuation and formation of bound residues is emphasised in several studies, e.g. Loiseau et al. (2000), Lesan and Bhandari (2004). Munier-Lamy et al. (2002) concluded that before mineralization of the organic carbon, availability (extractability) of atrazine is lowered, due to advanced sorption to the decomposing substrate. De Andrea et al. (1997) observed that the majority of atrazine is bound as hydroxy-atrazine, which shows a more intense binding behaviour than its parent compound (Bouwer et al. (1990), Lerch et al. (1998)). Capriel et al. (1985) detected 9 years after atrazine application still 50% as bound residues in humic matter, mainly hydroxy-atrazine and hydroxy-atrazine metabolites, but also around 13 % of initial atrazine and confirmed the stability of bound residues. With advancing age of bindings, it becomes more uncertain to what extend bound residues of the parent compound and bound residues of metabolites are responsible for attenuation of the herbicide agent.

Gamble (2008) found that atrazine bound residue formation can be described by first-order kinetics. Ma and Selim (1996) concluded that overall atrazine attenuation is pseudo first-order. Ilhan et al. (2011) calculated an attenuation rate in wood chips of 0.8 day⁻¹, not considering the extractable adsorbed atrazine. Taking into account only the loss of atrazine from solution, as done by Ilhan et al. (2011), the half-life in bark mulch

and straw (present study) and in wood chips (Ilhan et al. 2011) are about the same (approximately one day). Gendrault (2004) observed an attenuation rate in bark mulch of 1.4 day^{-1} . The rate is 10-fold higher than in the present study, but it was achieved in grinded bark mulch with a larger specific surface and more accessible binding sites. Gendrault (2004) declared sorption responsible for atrazine attenuation.

Isoproturon

In the beginning, reduction of isoproturon in solution was not correlated with increase of the sorbed fraction. Other than in the atrazine attempt, no lag phase of overall attenuation was observed in the isoproturon degradation experiment (Figure 3-4). Isoproturon is characterized by an attenuation rate (λ_B) of 0.2 day^{-1} and by an attenuation time of 50% ($DT_{50,B}$) of 3.2 days, according to Equation 2 and 3. Unexpectedly, little isoproturon was found on the substrate throughout the experiment, which is controversial to sorption experiments, where substantial reduction in solution was observed. Tests with the herbicide agent and the straw-bark mulch mixture, which resulted in a recovery of $104 \pm 15\%$, were insufficient to reproduce conditions in the batch experiment on short-term and on long-term. Participation of degradation and adsorption or even formation of bound residues on attenuation remains uncertain under the conditions of the experiment.

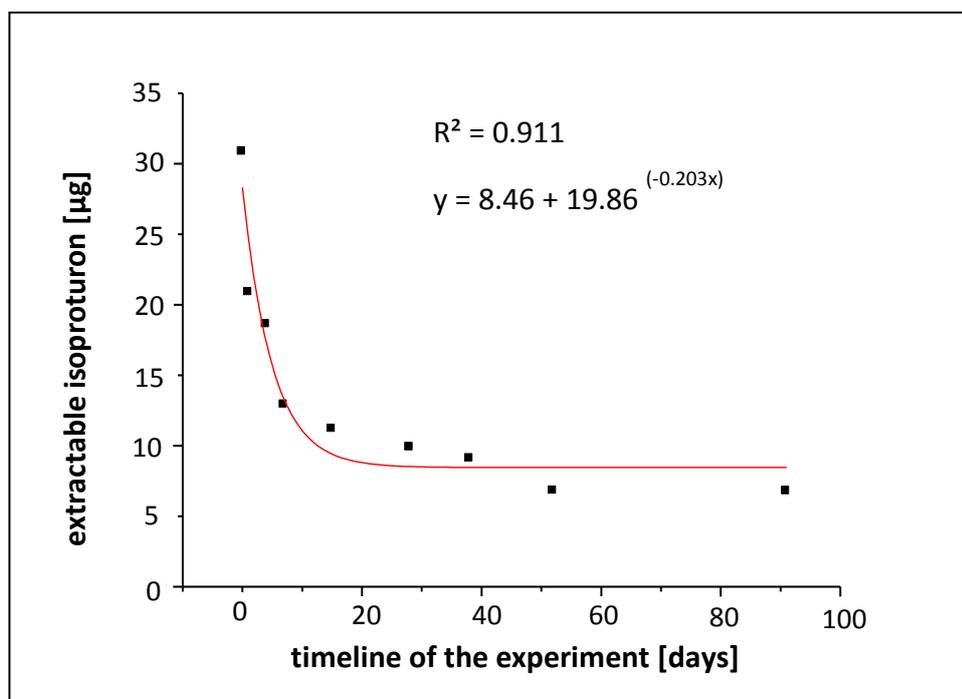


Figure 3-4: Development of total extractable isoproturon content in solution and on substrate (mixture of straw and bark mulch) over time under suboxic to anoxic conditions in the degradation experiment.

Little information can be found about isoproturon biodegradation in absence of oxygen in literature. The potential biodegradation of phenylurea-herbicides under suboxic to anoxic conditions is largely unknown (Sørensen et al. (2003)). Larsen et al. (2001) found no non-aerobic mineralization of isoproturon in the presence of NO_3^- in microcosm experiments with sandy aquifer sediment. In a subsequent study no mineralization of isoproturon in different aquifer sediments under denitrifying, sulfate-reducing or methanogenic conditions was observed (Larsen and Aamand (2001)). However, partial degradation of isoproturon is possible, since detection of metabolites was not included in these studies.

More likely is a predominant role of adsorption and formation of bound residues in the substrate. In a comprehensive review about phenylurea-herbicides in general and isoproturon in detail, Hussain (2010) stated that adsorption of isoproturon is in the same order of magnitude as adsorption of atrazine. Further, isoproturon adsorption is rapid, immediately after its application, and slows down with the course of time (Hussain (2010)). Isoproturon attenuation correlates positively with the content of moisture (Hussain (2010)), which suggests that adsorption could be favoured under saturated conditions.

Ertli (2005) found that, if the insoluble organic content of the soils is high, the immobilization of isoproturon is more likely. Accordingly, De Wilde et al. (2009b) showed that substrates of organic carbon, such as peat mix, straw and willow chopping, have a higher sorption capacity for isoproturon than sandy loam soils. Tian et al. (2010) in Hussain (2010) demonstrated that the addition of charcoal significantly increased the adsorption of isoproturon on soils. Charcoal amendment also enhanced the hysteresis of isoproturon desorption from the soil. Hussain (2010) resumed that relatively weak bindings on soil strengthen proportionally with increase of organic carbon content.

The formation of bound residues is suspected to be more potent than that of atrazine. Gevaio et al. (2001) found that after a 100-day incubation of soils, treated with atrazine and isoproturon, non-extracted C₁₄-labeled residues were 18 and 70%, respectively, despite exhaustive extractions with methanol and dichloromethane. Some metabolites of isoproturon show even higher adsorption affinity than the parent compound. Johannesen et al. (2003) indicated that partial degradation of isoproturon to 4-isopropyl-aniline may lead to reduced mineralization of the herbicide agent, due to sorption of the aniline moiety. In their study, the FREUNDLICH coefficient of 4-isopropyl-aniline was 5-fold higher than the one of isoproturon.

However, the organic substrate has the potential to support, next to formation of bound residues, also co-metabolism and direct biodegradation to metabolites as attenuation mechanisms of isoproturon in a microbial active and diversified environment.

Summarizing the results of the degradation experiment and experiences from other studies, adsorption and formation of bound residues seem to be predominantly responsible for substantial atrazine and isoproturon attenuation in organic substrates. However, the contribution of metabolites to formation of bound residues is unknown. Metabolites can have an even more distinct binding tendency to the matrix than the parent compound (Bouwer et al. (1990), Ma and Selim (1996), Johannesen et al. (2003)). Also, it remains an uncertainty about the content of unknown metabolites of atrazine and isoproturon in solution.

3.1.3 Attenuation of herbicide agents and nitrate in laboratory columns

3.1.3.1 Hydraulic characteristics of organic substrates

Hydraulic parameters of the substrates in the three duplicate column pairs, filled with pure straw, pure bark mulch and a mixture of straw and bark mulch, are summarized in Table 3-2. The tests indicate extremely high saturated hydraulic conductivity in all substrates. Effective porosity, which describes the porosity that contributes to flow, was higher than in sand and gravel. After one year of operation, effective porosity was decreased in average to 75% of starting values.

Table 3-2: Hydraulic parameters of laboratory columns filled with organic substrate

Parameter	Unit	Straw-bark mulch		Bark mulch		Straw	
		1	2	3	4	5	6
Column name	-	1	2	3	4	5	6
Saturated hydraulic conductivity (K_s value) ^a	$\times 10^{-1}$ cm s^{-1}	1.4 \pm 0.1		1.6 \pm 0.1		1.10 \pm 0.05	
Bulk density ^a	g cm^{-3}	0.19		0.27		0.11	
Porosity ^a	%	59.5		48.5		82.5	
Effective porosity (at start/after one year)	%	39/30	51/37	46/32	48/39	37/31	56/37

^a Single values are the average for the column duplicates.

The fourth substrate combination, a mixture of straw (<60 mm) and sand (2 mm) with a volume ratio of 1:1, was not further considered after start phase, because of rapid substantial decrease of porosity with the result of reduced NO_3^- attenuation performance.

3.1.3.2 Attenuation potential of organic substrates

3.1.3.2.1 Bentazon

After fast breakthrough of the herbicide agent in all three substrates, no notable attenuation of bentazon was observed under the conditions of the column displacement experiment (redox potential: 150 mV (suboxic), hydraulic residence time (HRT): 0.2

days, water temperature: $21 \pm 1^\circ\text{C}$). De Wilde (2009a) made similar observations in column displacement experiments with different mixes of organic materials.

The straw-bark mulch column is shown exemplarily in Figure 3-5. The short-term increase of HRT to 0.4 days had no significant impact on attenuation. The oscillation of outflow concentrations of bentazon was directly linked to strongly varying inflow concentrations. This behaviour was observed on day 56 and day 127 of bentazon dosing by simultaneous sampling of inflow and outflow (Figure 3-5).

Degradation and adsorption experiments confirm high mobility and low attenuation of bentazon in the organic substrates. These characteristics are related to the low adsorptive behaviour of the herbicide agent, its high solubility in water and its high persistence under non-aerobic conditions (Krause et al. 2010).

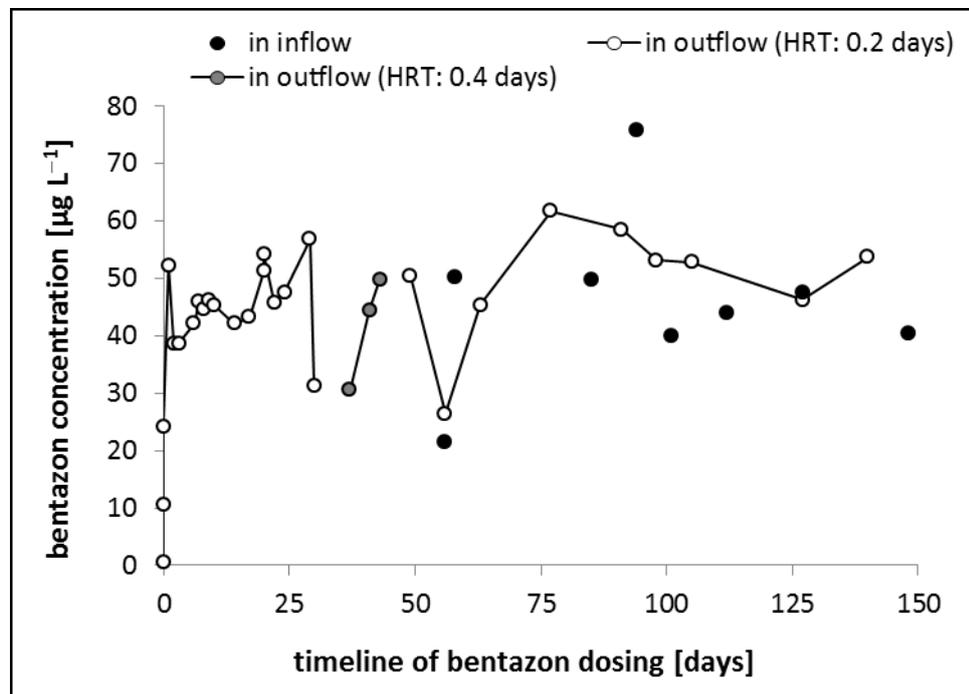


Figure 3-5: Inflow- and outflow concentrations of bentazon at hydraulic residence times (HRT) of 0.2 and 0.4 days in the straw-bark mulch column.

3.1.3.2.2 Atrazine

Atrazine was substantially removed from solution after passage through the straw-bark mulch column at HRT of 0.2 days. Outflow concentrations were lower than inflow concentrations during the whole dosing phase (Figure 3-6).

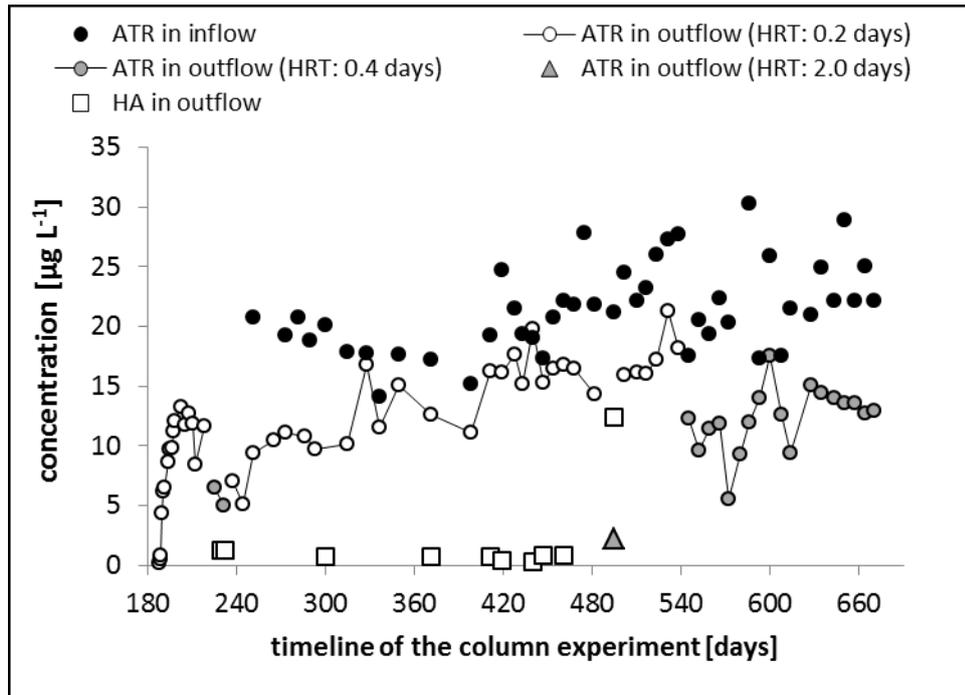


Figure 3-6: Inflow- and outflow concentrations of atrazine (ATR) and outflow concentrations of hydroxy-atrazine (HA) at hydraulic residence times (HRT) between 0.2 and 2.0 days in the straw-bark mulch column.

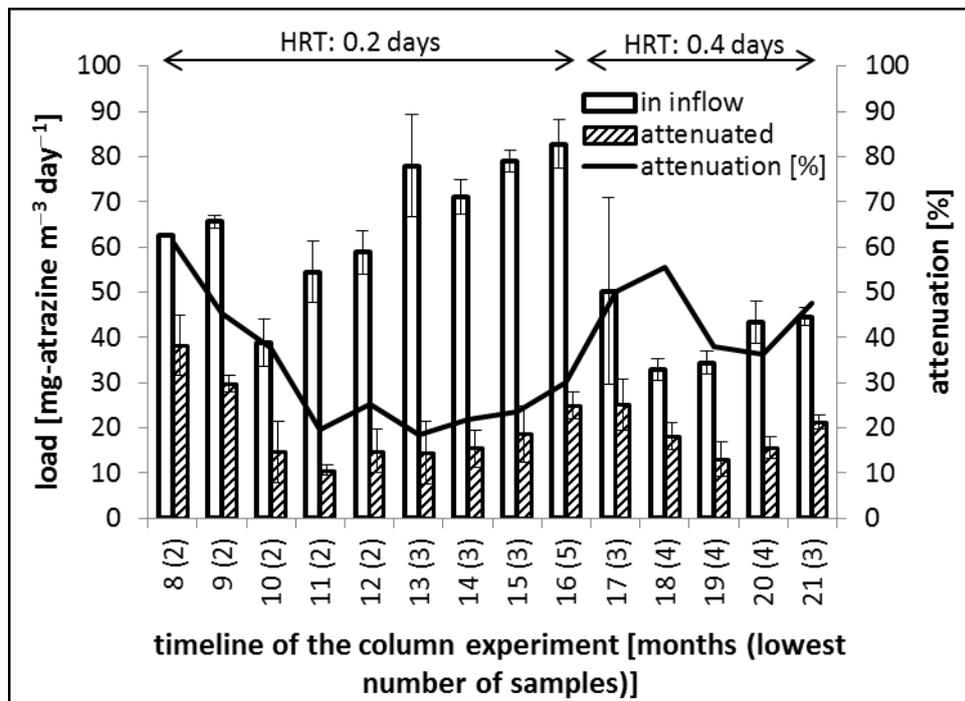


Figure 3-7: Absolute and relative atrazine attenuation in the straw-bark mulch column at hydraulic residence times (HRT) of 0.2 and 0.4 days, with exception of short-term phases in the range of few days at divergent hydraulic residence times.

Initial values of relative attenuation (60%) decreased within 4 months to a level of approximately 25%, at which they remained for 0.5 years, before they increased again to about 45%, due to increase of HRT from 0.2 to 0.4 days (Figure 3-7).

The results of the batch experiments to investigate adsorption (Figure 3-2) and degradation (Figure 3-3) reflect the observations in the column. Atrazine is fast and substantially removed from solution. According to equations 8, attenuation rates (λ_F) in the column can be estimated. At HRT of 0.2 days, λ_F was about 5 day⁻¹ in the beginning. It decreased to values between one and 2 day⁻¹ until HRT was increased. At HRT of 0.4 days, λ_F stabilized between one and 2 day⁻¹ until the end of the experiment. Higher attenuation rates in the column displacement, in comparison to batch experiments, were caused by permanent herbicide agent input.

Increased HRT at same inflow concentrations resulted in increased relative attenuation of atrazine in the mixture of straw and bark mulch. The absolute attenuation of atrazine, which was calculated with Equation 4, did not change much at increasing HRT (Figure 3-7). But, according to Lesan and Bhandari (2004), longer herbicide agent-soil contact times result in reduced water extractability and enhance bound residue formation. Also, the contribution of degradation to atrazine removal from solution is expected to increase with increasing HRT, as indicated by outflow concentrations of the metabolite hydroxy-atrazine (Figure 3-6).

Although degradation can be proven by metabolite analysis, its contribution to atrazine attenuation in solution is of minor relevance (< 15%) at HRT of 0.2 to 0.4 days. Hydroxy-atrazine seems to be the major metabolite. The findings of deethyl-atrazine analysis were in all columns below 0.2 $\mu\text{g L}^{-1}$. At low HRT, adsorption seems to be the major attenuation process.

In Figure 3-8 values of atrazine attenuation in pure bark mulch are presented. Atrazine attenuation is more effective than in the straw-bark mulch mixture, due to higher content of bark mulch in the column. In Figure Appendix-1, pure bark mulch and pure straw are compared with regard to atrazine attenuation potential. It remains uncertain, whether higher bulk density or higher binding affinity of atrazine to bark mulch is responsible for the difference. However, bark mulch qualifies as more adequate adsorbent for atrazine than straw, due to higher resistance against decomposition.

At increased HRT (2.0 days), hydroxy-atrazine formation in the straw-bark mulch mixture resulted in over 50% atrazine attenuation (Figure 3-6), while metabolite concentrations in bark mulch were barely increased. The microbiologically more active environment of straw could have favoured co-metabolic atrazine degradation. But, the significance of single measurements is low and the observation has to be verified in the technical macro scale experiments. Hydroxy-atrazine loads in the outflow were about the same at all HRT.

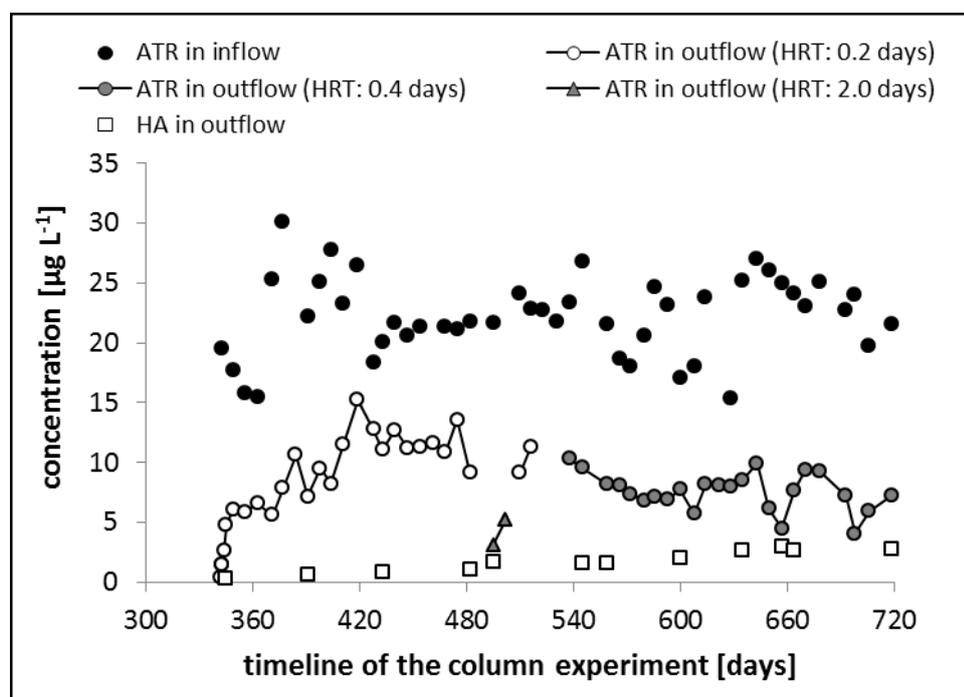


Figure 3-8: Inflow- and outflow concentrations of atrazine (ATR) and outflow concentrations of hydroxy-atrazine (HA) at hydraulic residence times (HRT) between 0.2 and 2.0 days in the bark mulch column.

Effective adsorption leads to high attenuation, but also to high persistence against microbial degradation of the adsorbed fraction, because access of microorganisms is restricted. Even so, direct microbial degradation of atrazine on the matrix may be possible (see 3.1.2. Degradation of herbicide agents/Atrazine).

However, adsorption experiments, literature studies (e.g. Gendault (2004)) and low metabolite concentrations indicate predominantly adsorption to bark mulch and formation of bound residues. The important role of organic matter (humins, humic and fulvic acids) in soils for atrazine attenuation and formation of bound residues is emphasised in several studies, e.g. Loiseau et al. (2000), Lesan and Bhandari (2004).

Under non-aerobic conditions, as established in the experiment, the formation of humic substances is expected, e.g. by lignin modification.

Positively in the scope of sustainable attenuation of atrazine is that, according to De Andrea et al. (1997), the main part of the bound residues is expected to be hydroxy-atrazine, which is predominantly formed under denitrifying conditions (Crawford et al. (1998)) and in non-aerobic sediments with carbon addition (Chung et al. (1996)) by chemical degradation (Ma and Selim (1996)). The hydrolysis of atrazine is soil-surface catalysed, i.e. adsorption favours degradation to hydroxy-atrazine (Armstrong et al. (1967)). The metabolite shows a more intense binding behaviour than atrazine (Bouwer et al. (1990), Lerch et al. (1998)). Hydroxy-atrazine is less phytotoxic than the mother compound (Wang et al. (1991)), in case it is released by desorption from the matrix or by decomposition and dissolution of the organic substrate.

In addition to the effects of different substrates and HRT, the impact of unsaturated phases on atrazine attenuation and the leaching potential of atrazine were studied in the straw-bark mulch column. The results support the theory of bound residue formation.

Analysis of atrazine that was extractable with methanol and water from the matrix before and after the unsaturated phase (3 weeks) revealed a decrease of 75%. But, the extractable fractions of adsorbed atrazine were marginal (<1%) in comparison to the fraction that was attenuated during the experiment and not recoverable in form of hydroxy- or deethyl- atrazine.

Leaching studies after the unsaturated phase and at the end of the experiment demonstrated that, when the reactor was washed with pure process water, the atrazine concentrations in the outflow decreased regressively within 2 to 3 weeks to 5% of former inflow concentrations. The washed out fraction of atrazine was marginal in comparison to the attenuated fraction during the experiment.

Atrazine can be successfully attenuated under high flow and suboxic conditions by adsorption to the studied organic substrates and formation of bound residues. However, the fate of atrazine in the substrates and with that the risk of its revolving into the environment remains uncertain, since the expected comprehensive transformation to hydroxy-atrazine is indicated, but cannot be proven in this study.

3.1.3.2.3 Isoproturon

At the onset, relative attenuation of isoproturon in the column displacement experiment was less effective than relative atrazine attenuation, although contact times were twice as high (compare Figure 3-6 and Figure 3-9). The findings are in contrast to sorption experiments, where similar sorption in the substrate was observed.

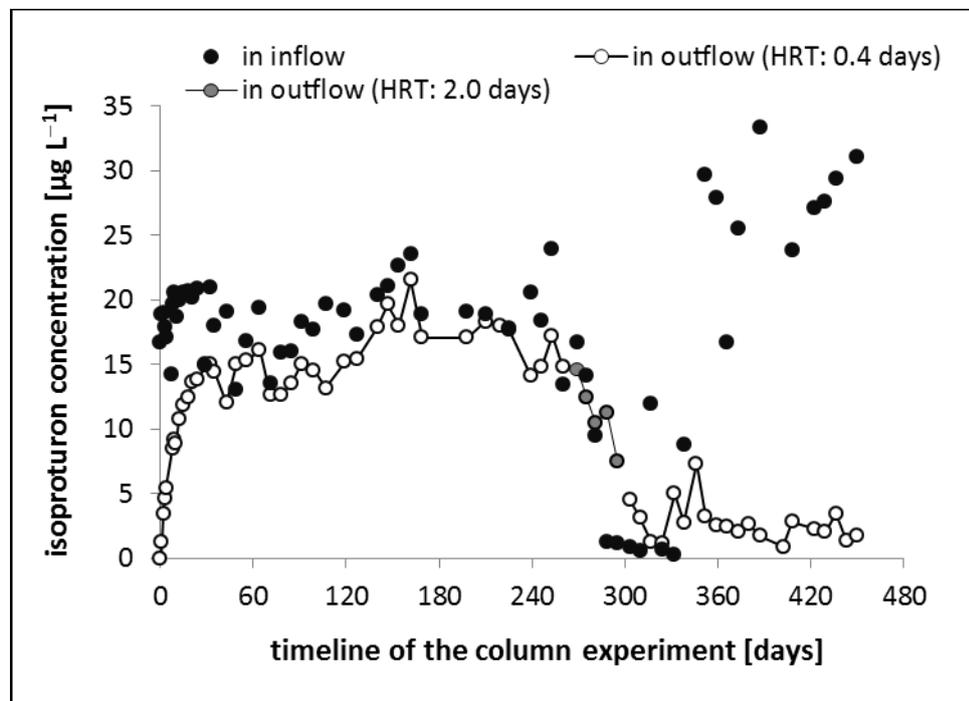


Figure 3-9: Inflow- and outflow concentrations of isoproturon at hydraulic residence times (HRT) of 0.4 and 2.0 days in the straw-bark mulch column.

Initial values of relative isoproturon attenuation (50%) decreased rapidly within one month to approximately 20% (Figure 3-10). Corresponding attenuation rates (λ_F) were 2.5 day^{-1} and 0.6 day^{-1} , respectively (Equation 8). Higher attenuation rates in the column displacement- than in the degradation experiment were caused by continuous herbicide input.

The increase of HRT to 2.0 days in the 10th month did not result in increase of relative attenuation of the herbicide agent in the substrate, as observed in the atrazine column. But, it caused substantial isoproturon attenuation in the dosing vessel, probably by biotic degradation. Interestingly, the mechanism of the attenuation process in the substrate seemed to be modified after change back to standard flow (HRT: 0.4 days) in the 11th month. After the low-flow phase the attenuation rate of isoproturon increased drastically to 90% ($\lambda_F: 22 \text{ day}^{-1}$) and remained stable at this level (Figure 3-10).

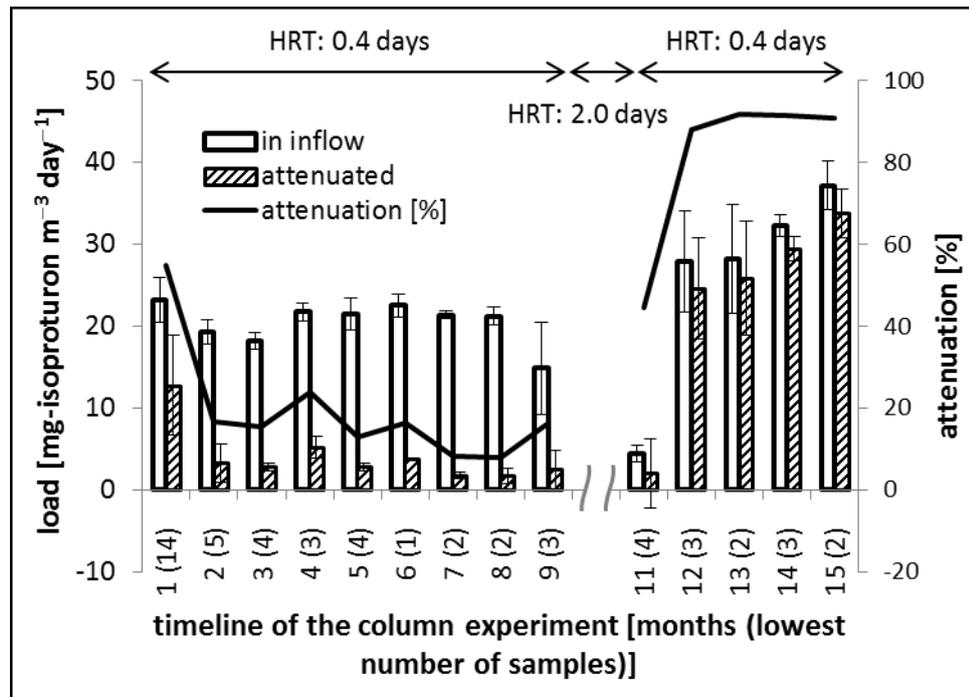


Figure 3-10: Absolute and relative isoproturon attenuation in the straw-bark mulch column at hydraulic residence times (HRT) of 0.4 and 2.0 days, with exception of short-term phases in the range of few days at divergent hydraulic residence times.

The phenomenon was not observed, yet. It has to be investigated in future studies with the help of metabolite- or isotope analysis. The altered conditions could have resulted in the creation of new binding sites for isoproturon or, more likely, in improved effectiveness of direct metabolisms or co-metabolisms in the suboxic environment. Chemical degradation of isoproturon in aqueous solution under moderate temperature and within a pH range of 4 to 10 is of minor importance in most agricultural soils (Sørensen et al. (2003)).

3.1.3.2.4 Nitrate

Denitrification is expected to be predominantly accountable for NO_3^- attenuation in the organic substrates. In studies under comparable conditions, it was shown that competitive NO_3^- transforming processes, such as immobilization into biomass or dissimilatory NO_3^- reduction to NH_4^+ (DNRA), are negligible in relation to reduction of NO_3^- to N_2O and N_2 (Greenan et al. (2006), Gibert et al. (2008)). Schipper et al. (2010) cited NO_3^- attenuation studies with wood substrates in laboratory and field, in which no considerable DNRA was observed, but remained concerned about more labile carbonaceous substrates. In the present study with bark mulch and straw, NH_4^+ concentrations in the column outflow were in general lower than 0.5 mg L^{-1} , including

NH_4^+ , potentially formed by degradation of organic substrate (ammonification). In the following text the terms NO_3^- attenuation and denitrification are used synonymously. Relative denitrification describes the ratio of nitrogen from attenuated NO_3^- and NO_2^- and nitrogen from NO_3^- and NO_2^- in the inflow [%]. Absolute denitrification describes the attenuated loads of nitrogen from NO_3^- and NO_2^- [$\text{g-nitrogen m}^{-3} \text{ day}^{-1}$]. The attenuation of NO_2^- and NO_3^- is subsumed as NO_3^- attenuation or denitrification. NO_2^- in the inflow originated from beginning NO_3^- transformation in the stock solution and NO_2^- in the outflow was the result of incomplete transformation of NO_3^- into gaseous nitrogen species. In the present study NO_3^- attenuation, i.e. denitrification, is considered as transformation of dissolved inorganic nitrogen species in drainage water into gaseous nitrogen species.

Effectiveness of organic substrates and substrate aging

Relative and absolute denitrification in the different organic substrate columns are shown in Figure 3-11 to Figure 3-14. Substrate aging becomes visible by gradually decreasing NO_3^- attenuation. Absolute denitrification performance declined strongly in the beginning, but became steadier to the end of the experiment. These observations are confirmed by Robertson et al. (2010), who reported that wood substrates lost about 50% of their reactivity during their first year of operation. After that, denitrification rates were stable for a considerable number of years.

In the present study, relative denitrification decreased over time from 30 to 18% in pure bark mulch, from 85 to 26% in the mixture of straw and bark mulch and from 95 to 27% in pure straw. The increase of hydraulic residence time (HRT) to 0.4 days at same inflow concentrations resulted in the increase of relative denitrification (Figure 3-11).

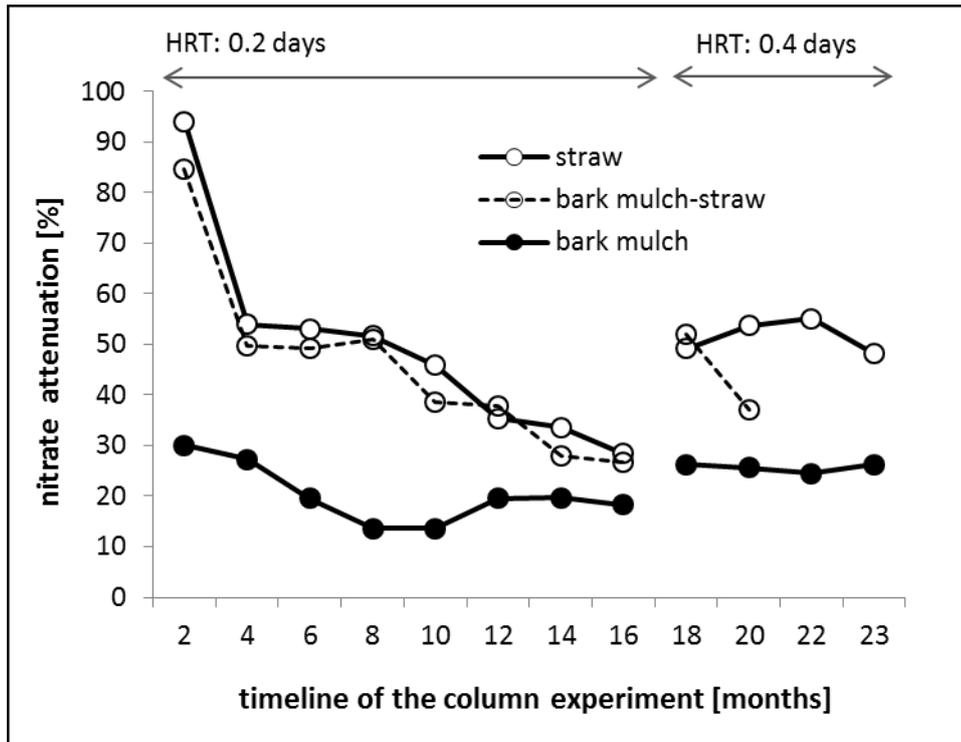


Figure 3-11: Relative denitrification in different substrates at hydraulic residence times (HRT) of 0.2 and 0.4 days.

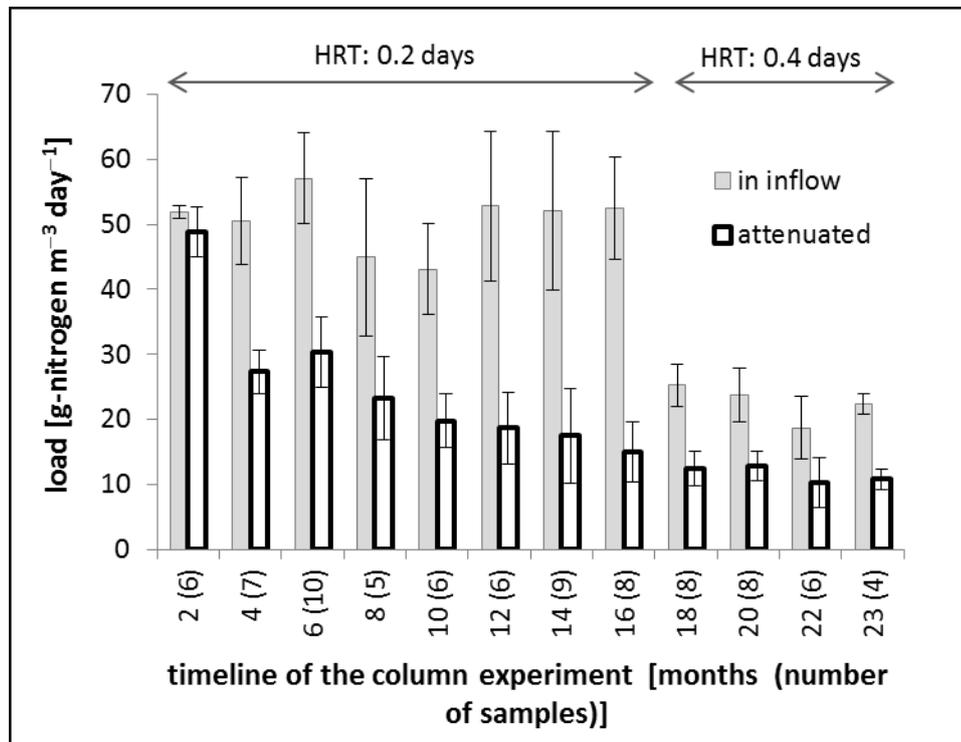


Figure 3-12: Absolute denitrification in straw at hydraulic residence times (HRT) of 0.2 and 0.4 days. The values are average loads of two-month period. The variance of the values is depicted as double standard deviation.

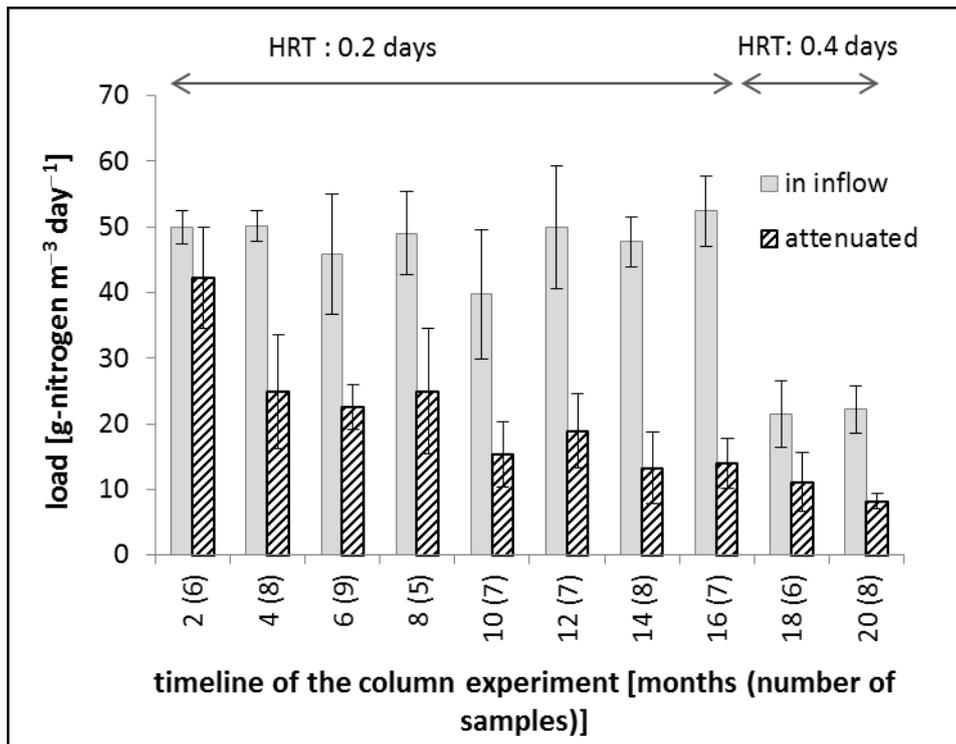


Figure 3-13: Absolute denitrification in a straw-bark mulch mixture at hydraulic residence times (HRT) of 0.2 and 0.4 days. The values are average loads of two-month period. The variance of the values is depicted as double standard deviation.

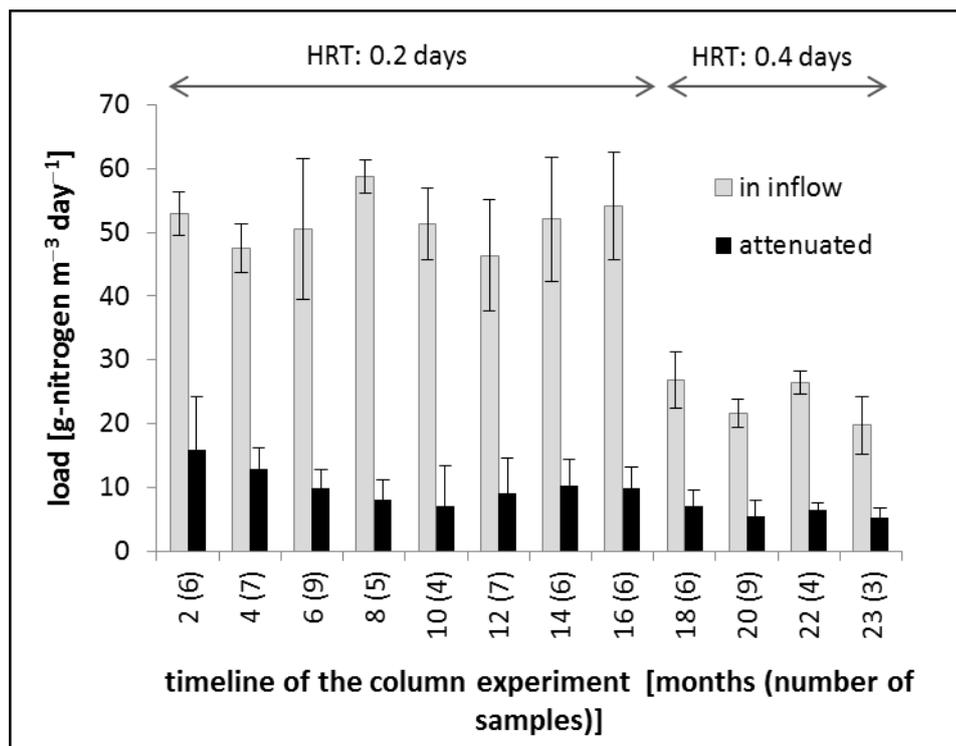


Figure 3-14: Absolute denitrification in bark mulch at hydraulic residence times (HRT) of 0.2 and 0.4 days. The values are average loads of two-month period. The variance of the values is depicted as double standard deviation.

Aslan and Türkman (2005) and Soares and Abeliovich (1998) observed equally high start rates of denitrification (40 and 50 g-nitrogen m⁻³ day⁻¹, respectively). They experimented with laboratory straw columns with inflow concentrations of 100 mg-NO₃⁻ L⁻¹ at room temperatures between 25 and 30°C and at HRT between 0.1 and 0.2 days. In their studies denitrification was kept stable over time by substrate addition as soon as rates started to drop.

In bark mulch the establishment of stable denitrification was slightly delayed in comparison to straw, probably due to low start pH value of 4.5, which increased to 6.5 within one month. Cameron et al. (2010) reported similar experiences with softwood. Trois et al. (2010) explained that pine bark delays the acclimatization time and inhibits the biological denitrification by release of large amounts of phenolic compounds and hydroxylated benzene rings. However, the negative effect of washed out acidic compounds seems to be limited to the start phase.

Laboratory scale results at room temperature (21±1°C) revealed that straw and bark mulch enable effective denitrification at short hydraulic residence times (0.2 days).

With respect to results of related studies (Table Appendix 1), which proved the positive effect of organic substrates on denitrification in mitigation systems, straw and bark mulch show high potential as effective sources of organic carbon for suboxic respiration. Denitrification rates of 5 to 10 g-nitrogen m⁻³ day⁻¹ after 1.5 years (Figure 3-12 to Figure 3-14) are in the range of average denitrification rates between 2 and 22 g-nitrogen m⁻³ day⁻¹, which were observed in wood-filled bioreactors at temperatures between 2 and 22 °C (Schipper et al. 2010).

A drawback of straw as organic carbon source is high leaching of dissolved organic carbon (DOC) at the beginning. The surplus of immediately available organic carbon is provided by enzymatic decomposition of the substrate after death of the plant (senescence). In the first two months nearly the same amount of organic carbon was washed out as was used for denitrification. Start concentrations of DOC in the column outflow were around 250 mg L⁻¹, which decreased gradually to 10 mg L⁻¹ during the first 1.5 months. Bark mulch is a more resistant organic carbon source. Observed denitrification was 2 to 3 times lower than in straw throughout the experiment (Figure 3-11 to Figure 3-14) and the wash-out of DOC in the beginning was lower.

Both substrates are characterized by unstable denitrification performance, especially during the first year, due to progressive decomposition of the substrate and increase of the ratio of resistant and readily available organic carbon species.

The combination of both substrates seems to reduce negative effects of individual characteristics, such as wash-out of DOC and delay of denitrification, while denitrification is nearly as high as in straw (Figure 3-11 to Figure 3-14). The comparison of denitrification in the straw-, the bark mulch- and the mixed column shows that denitrification on the basis of bark mulch as carbon source was higher (+15 %) in the straw-bark mulch column than in the pure bark mulch column. The availability of organic carbon from bark mulch seems to be enhanced, because overall microbial activity is increased, due to access of readily available organic carbon from straw. The more resistant carbon species, such as lignin, can be degraded by co-metabolic reactions (Kögel-Knabener (2010) p.61). According to estimations by Schipper et al. (2010), the high denitrification rates indicate that DOC from straw and bark mulch is predominantly used for denitrification. Since NO_3^- is no limiting factor, with exception of the start phase, and SO_4^{2-} concentrations are barely reduced, dissimilatory sulphate reduction seems to be an insignificant competing organic carbon consumer, at least at HRT lower than 0.4 days.

Effect of hydraulic residence time (HRT)

The HRT is defined as ratio of the volume of the reactor and the flow rate. It can be modified by changing the volume of the reactor, the flow rate or both (under the premise the ratio of both values is changing). In our attempt the HRT was increased by lowering the flow rate. To avoid disrupting the primary experiment, the effect of changing HRT was examined in embedded short-term experiments. For two to three weeks, HRT had been increased via changes in flow to the 2-fold and to the 10-fold value to learn more about the behaviour and the potential of the organic carbon sources. The changes in HRT were adjusted in the parallels with atrazine after the 100th day of the column displacement experiment. At this time, wash-out of readily available organic carbon had decreased and denitrification performance depended on carbon supply by current decomposition of the organic substrate. In Figure 3-15 relative denitrification at HRT of 0.17 days (23 weeks, at least 19 samples), at HRT of 0.34 days (3 weeks, at least 4 samples) and at HRT of 1.7 days (3 weeks, one sample) were compared between

the 100th and the 300th day of the experiment. The resulting uncertainty is shown in the standard deviation. Despite different durations and sample numbers of the three experiments, a trend can be observed. DOC concentrations in the outflow increased, the redox potentials decreased and relative denitrification increased with increasing HRT.

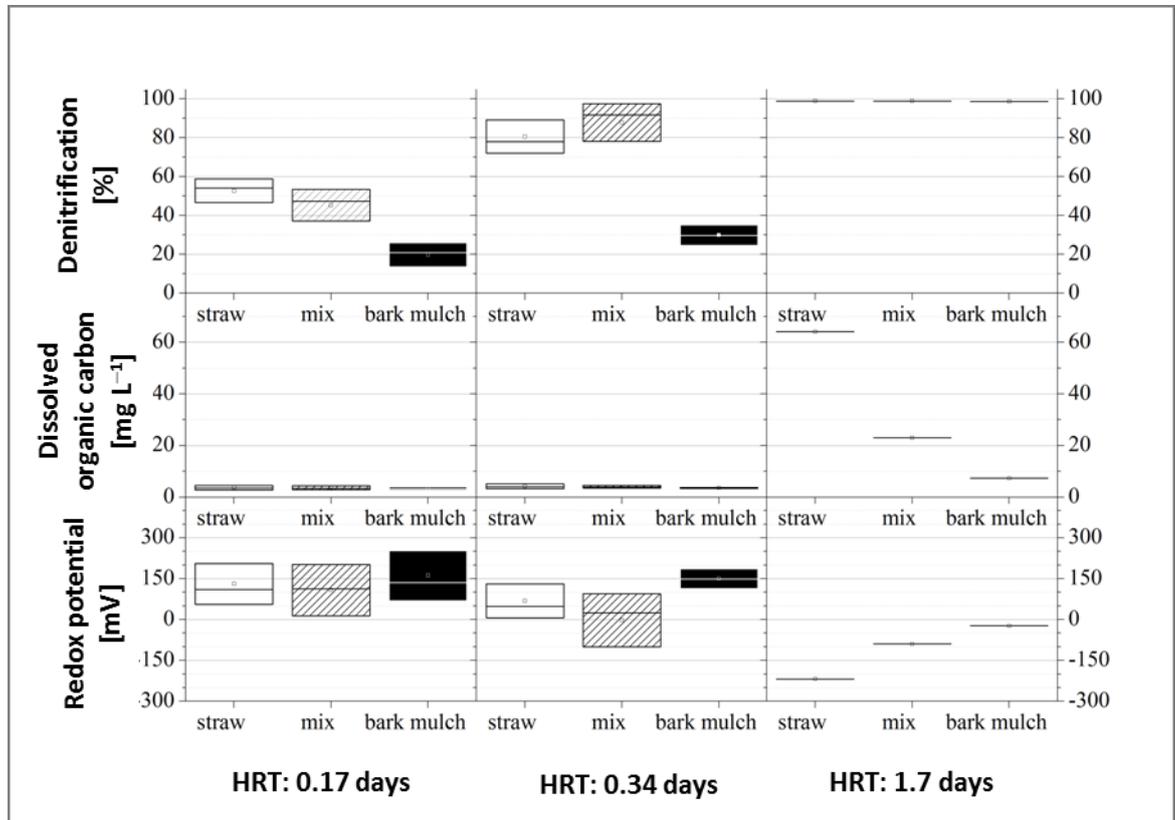


Figure 3-15: Effect of hydraulic residence time (HRT) on relative denitrification, dissolved organic carbon in the outflow and redox potential in the outflow in the columns with pure straw, pure bark mulch and the mixture of straw and bark mulch. The box plots show average (point), median (line) and standard deviation (vertical space between average and frame) of measurements between the 100th and the 300th day of the experiment.

At HRT of 1.7 days, the formation of DOC was sufficient for complete denitrification of 100 mg NO₃⁻ L⁻¹ in all substrates. The NO₃⁻ input was the limiting factor, unused organic carbon in straw was washed out and energetically less effective reactions, such as dissimilatory sulphate reduction, determined the redox potential. Bark mulch alone proved to be an adequate carbon source for denitrification at increased HRT. The denitrification potential was the same as in straw, but DOC was barely released.

Long-term studies with increased HRT (0.4 days) at the end of the column displacement experiments with atrazine and throughout the column displacement experiments with isoproturon put the results of the short-term experiments into perspective. Increased

DOC wash-out cannot be confirmed, neither a difference in DOC wash-out between straw and bark mulch. It seems to be a temporal effect of the HRT change on the microbiological equilibrium with higher impact in straw. The effect decreases and relativizes with time. However, it can be confirmed that relative denitrification increases with increasing HRT (Figure 3-11). Similar behaviour of organic carbon sources (sugar cane and reed) was observed by Wang and Wang (2012) for lower temperatures (12°C).

Effect of herbicide agents on denitrification

Denitrification was observed before and after the start of atrazine and bentazon dosing in the parallels of the straw-bark mulch column to observe possible interactions with herbicide agents. Denitrification performance did not change significantly after herbicide agent dosing. It is confirmed by other studies that the herbicide agents do not interfere with denitrification in the range of applied concentrations. Salhani et al. (1999) showed that with addition of low atrazine concentrations ($18 \mu\text{g L}^{-1}$) neither the course nor the efficiency of the denitrification process was affected. Ilhan et al. (2011) used 100-fold higher concentrations of atrazine in wood chips and did not observe negative effects on denitrification. Jørgensen et al. (2004) found in columns with till, which was degraded to natural DOC that bentazon rather improved denitrification, acting as a source of organic carbon.

In Figure 3-16 the denitrification performance over time in the straw-bark mulch column pair with and without isoproturon is presented. Differences in denitrification during the experiment were low and can be explained rather with differences in access to DOC than with an effect of the herbicide agent. Higher relative denitrification in the column without isoproturon during the first half year of the experiment correlates with higher DOC concentrations in the outflow of the column. Higher content of immediately available organic carbon, which was provided by enzymatic decomposition of the substrate after death of the plant (senescence), seems to be responsible. The organic substrates are highly heterogenic materials and variations in the columns, due to composition of the substrate and packing have to be expected. Isoproturon did not interfere with microbiological NO_3^- attenuation in the range of applied concentrations. Widenfalk et al. (2004) observed likewise no effect of isoproturon ($30 \mu\text{g L}^{-1}$) on denitrification rates in freshwater sediment. Persson (2000) found that isoproturon had

no negative impact on NO_3^- attenuation in lake sediment (maximal concentration: $530 \mu\text{g kg}_{\text{dry weight}}^{-1}$).

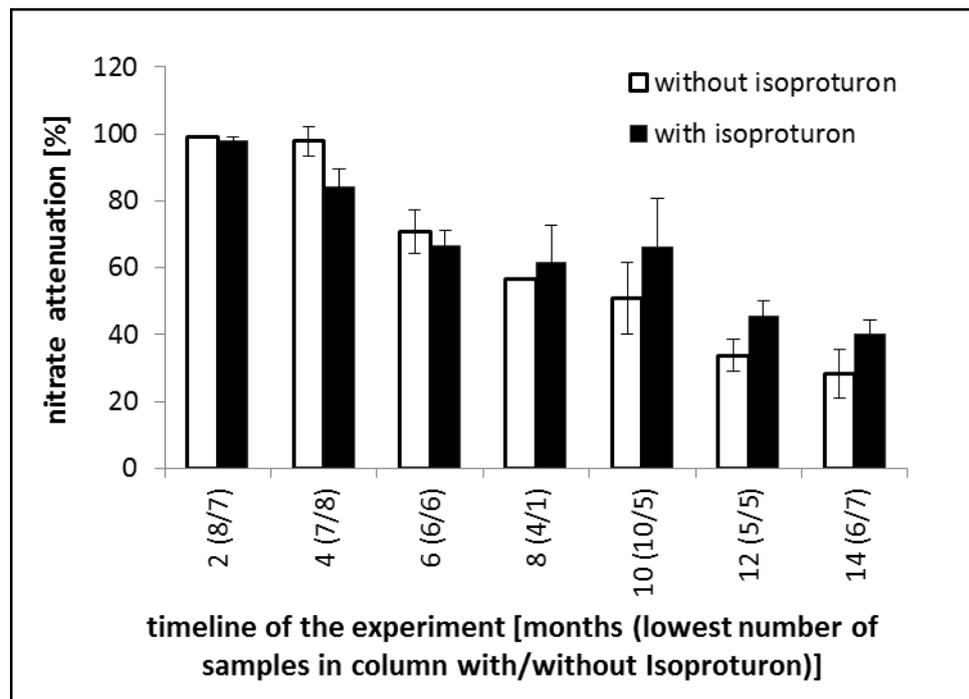


Figure 3-16: Effect of isoproturon on nitrate attenuation (denitrification) in straw-bark mulch column at hydraulic residence time of 0.4 days.

Effect of unsaturated stagnation

Like for the investigation of the HRT effect, a further short-term experiment was embedded into the main experiment. The impact of unsaturated phases on the availability of DOC and on the activity of denitrifying bacteria was studied in the straw-bark mulch column.

Figure 3-17 shows that after 3 weeks of drying (unsaturated, wet conditions from day 13 to day 34) and after 4 weeks of rewetting (saturated flow without addition of nutrients and herbicide agents from day 34 to day 63) denitrification reconstituted immediately. After restart of NO_3^- dosing, high denitrification performance was observed, which decreased gradually back within one month to the level before the dry phase. Strong depletion of the organic substrate and formation of a reservoir of readily available carbon during dry phase under wet but aerobic conditions can be assumed. After saturated flow was re-established, the surplus of organic carbon was washed out and after restart of NO_3^- dosing, it was used for denitrification. The enhanced DOC production under aerobic conditions carries along the risk of turning the organic

substrate into a source of nutrients (wash-out of DOC), when no or not sufficient electron acceptors, such as NO_3^- , are present.

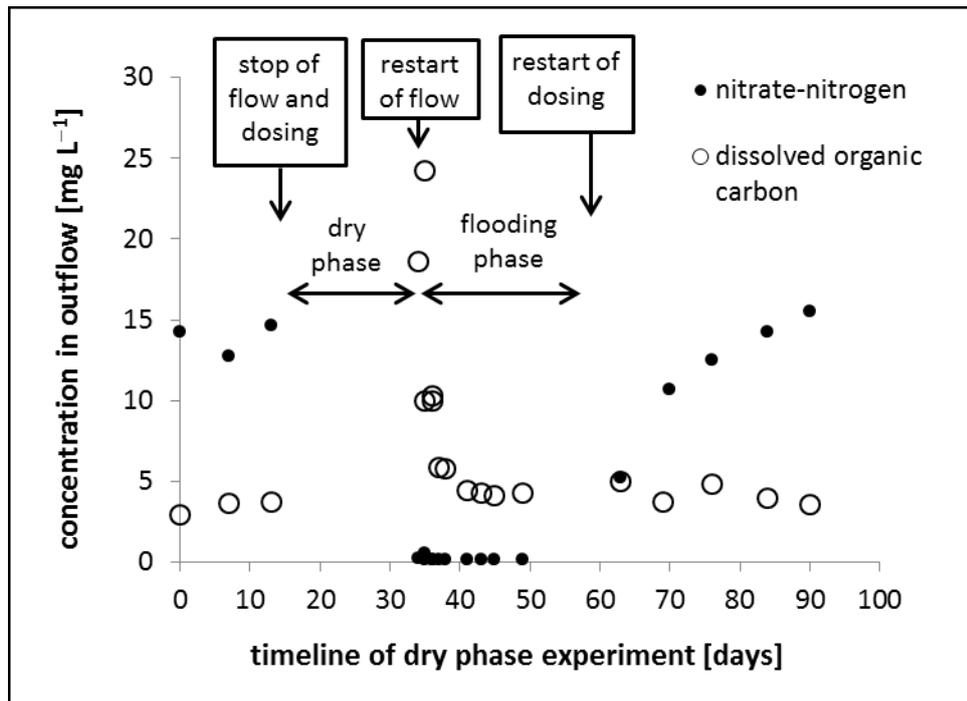


Figure 3-17: Effect of dry phase on nitrate attenuation (denitrification) in straw-bark mulch column. During dry phase substrate was wet, but not water-saturated. In flooding phase no nutrients or herbicide agents were dosed.

3.1.3.3 Utilization of the organic carbon source in laboratory columns

In an extra column displacement experiment without addition of herbicide agents the participation of the organic carbon source in competing respiratory reactions and wash-out was studied for 5 months to evaluate its efficiency in terms of denitrification. According to Equations 5, 6 and 7, lost carbon was estimated stoichiometrically from reduction of major electron acceptors (oxygen, NO_3^- and SO_4^{2-}) and from wash-out of organic carbon (Table 3-3).

Table 3-3: Carbon withdrawal by competing redox reactions and wash-out in straw-bark mulch columns.

Carbon withdrawing processes	Column			
	a		b	
	carbon loss			
	g	%	g	%
Oxygen reduction	0.76	5	1.26	9
Denitrification	7.14	45.5	6.71	47
Sulphate reduction	0.54	3.5	0.64	4
Wash-out	7.19	46	5.69	40

The stoichiometrical estimation of carbon withdrawal covered for 96% and 98% of actual carbon loss in column a and b, respectively. Wash-out of particular organic carbon was 4% and 8% of total organic carbon loss in column a and b, respectively.

In Figure 3-18 time dependent carbon loss by denitrification and wash-out in column a (HRT: 0.4 days) is depicted. It is demonstrated that the influence of wash-out on carbon loss was high only in the beginning. Excluding the first month, denitrification was responsible for 74 and 71% of carbon withdrawal in column a and b, respectively. After start phase the carbon source was efficiently used for denitrification. In average 4.4 g of the mixture of bark mulch and straw were used for the attenuation of one gram of nitrogen. The contribution of bark mulch to carbon supply for respiratory processes is estimated to about 20%. Soares and Abeliovich (1998) and Aslan and Türkman (2005) observed higher ratios between organic matter and nitrogen. Eleven and 24 g of straw, respectively, were necessary for the attenuation of one gram of nitrogen. They added fresh substrate when denitrification performance was sinking, so wash-out of organic carbon had higher effect on the carbon balance.

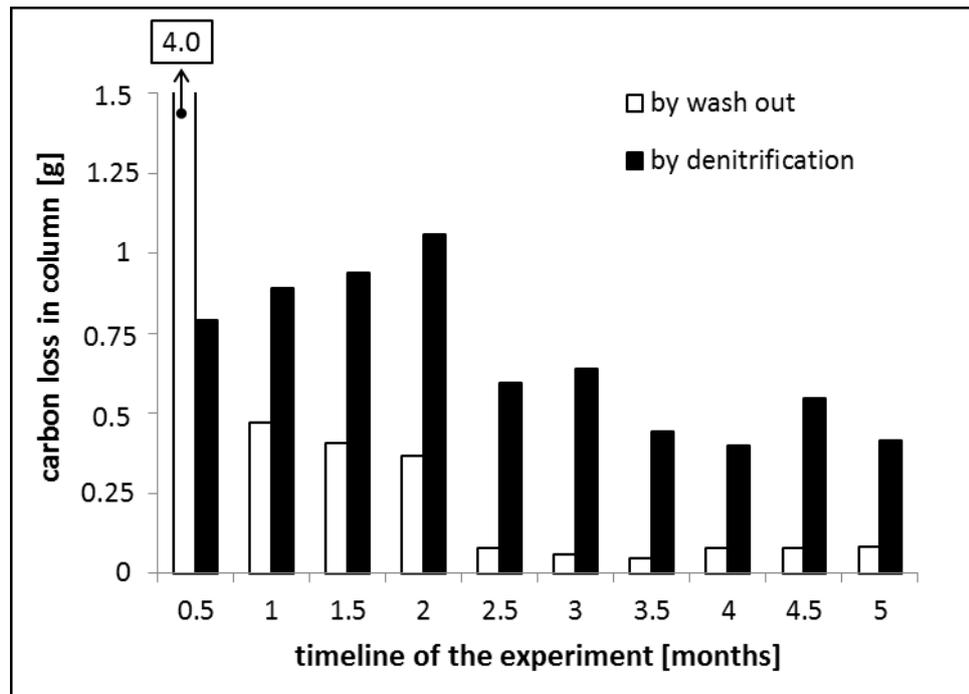


Figure 3-18: Contribution of nitrate attenuation (denitrification) and wash-out to reduction of organic carbon in straw-bark mulch column (column b) at hydraulic residence time of 0.2 days.

Next to the efficiency of the substrate as carbon source for denitrification, the influence of HRT on absolute denitrification was studied. Loads were kept stable by adjusting inflow concentrations at different HRT. Further the influence of different NO_3^- input loads on denitrification performance were investigated.

In Figure 3-19 the effect of HRT on absolute denitrification is depicted. In the first month at similar HRT in both columns (0.2 days), no difference in denitrification could be observed. With differentiation of HRT in the columns (0.4 days in a and 0.2 days in b) absolute denitrification rates did not differ for one month, probably due to sufficiently available organic carbon, since DOC in outflow was still increased. After that, denitrification is slightly favoured at 2-fold higher HRT, with a maximal difference of +15%. With halving of NO_3^- concentrations in inflow during the last 1.5 months, which resulted in decreased pressure on the carbon source, the difference in denitrification rates was decreasing to +11%. It seems that under conditions of the experiment the availability of organic carbon and the production rate of DOC have higher influence on absolute denitrification than HRT. It is possible that the effect of HRT on absolute denitrification becomes more influencing under more DOC-limited

condition, e.g. in longer processed substrates. Further long-term studies with more and wider differences in HRT are necessary.

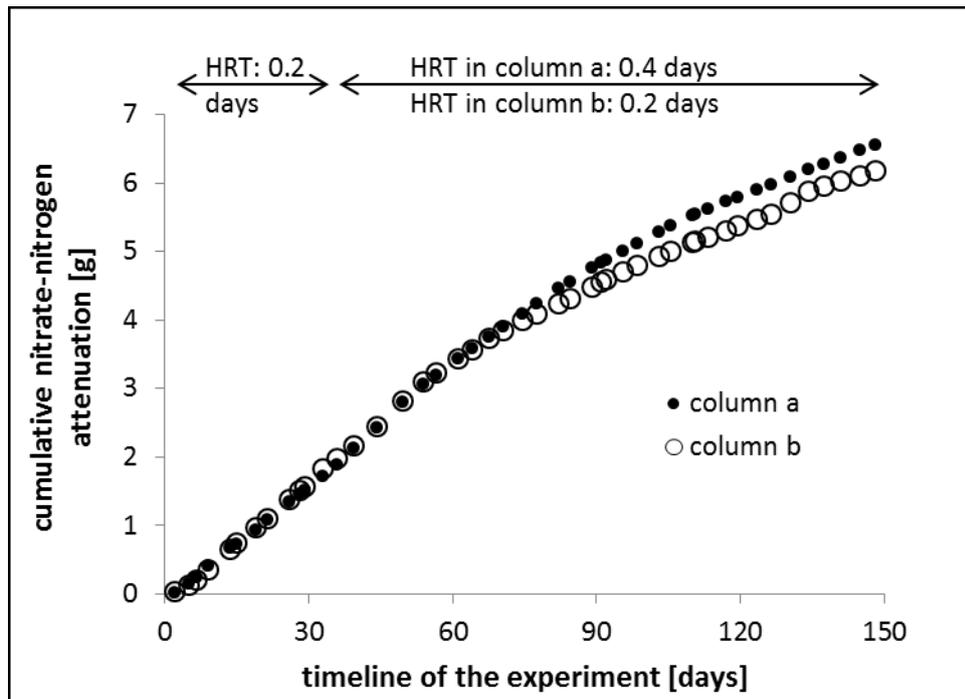


Figure 3-19: Effect of hydraulic residence time (HRT) on nitrate attenuation (denitrification). Nitrate input loads were equal in column a and b.

In Figure 3-20 the influence of NO_3^- input variations on absolute denitrification is shown. Sudden halving of input loads by halving of inflow concentration 1.5 months before the end of the experiment did not result in similar changes of denitrification. In fact, denitrification did barely respond to changes of inflow concentration. Schipper et al. (2010) has already mentioned low influence of inflow concentrations on denitrification performance. The continued gradual decrease of denitrification performance seems to be the result of the decreasing availability of organic carbon, which has already been observed before the change of inflow concentrations.

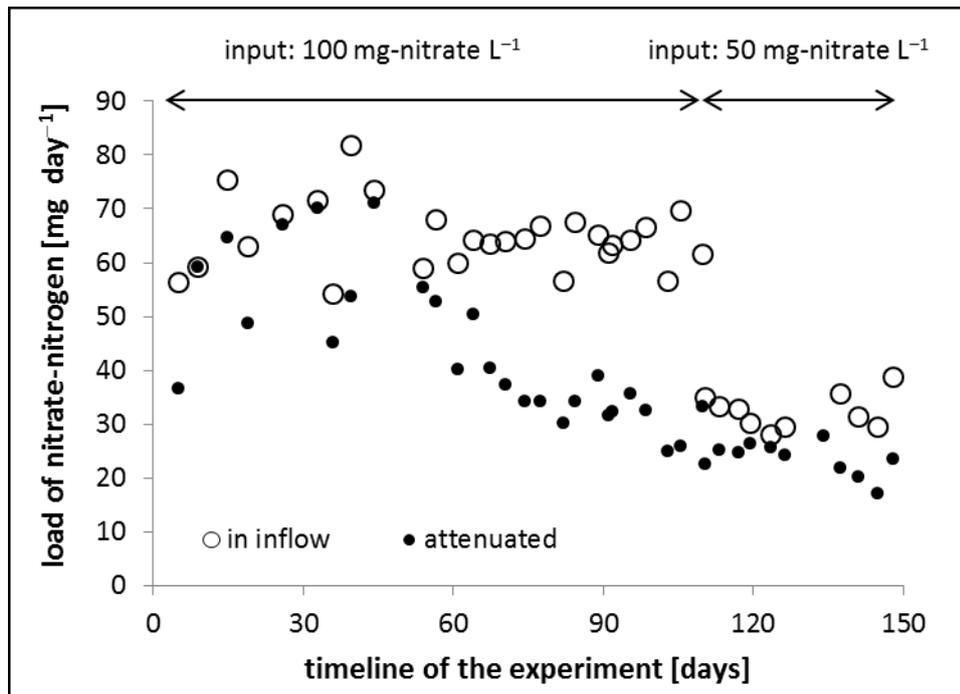


Figure 3-20: Effect of nitrate input on nitrate attenuation (denitrification) at hydraulic residence time of 0.2 days in a straw-bark mulch column (column b).

3.1.4 Summary of laboratory scale results

In laboratory column displacement experiments, straw and bark mulch were found to be suitable organic carbon sources, supporting instantaneous and efficient denitrification at high temperatures ($21\pm 1^\circ\text{C}$) and at low HRT (0.2 days). Under the conditions of the experiment, changes in NO_3^- input and HRT had low effect on absolute denitrification, when organic carbon and NO_3^- input were not limited. Hydraulic conductivity was assured for long-term period. The combination of both substrates diminished individual negative characteristics, such as DOC wash-out by straw and the resistance of organic carbon species in bark mulch against decomposition. With exception of the start phase, in which denitrification performance decreases drastically, the organic substrates seem to be a relatively steady carbon sources for constantly high and potentially long-lasting absolute denitrification (Figure 3-12 to Figure 3-14). Periodical contact with oxygen, as observed in unsaturated phases, improved degradation of the substrates and the availability of organic carbon on the upside, but increased the risk of DOC wash-out and reduced longevity of the carbon source on the downside. Denitrification was not disturbed by the use of the herbicide agents atrazine, bentazon and isoproturon in the range of studied concentrations.

The suitability of the substrates for herbicide agent attenuation under denitrifying and high flow conditions depends on specific herbicide agent characteristics. Bentazon was highly persistent against biotic degradation. It was barely attenuated, due to high solubility in water. Dissolved atrazine was partly degraded to metabolites, but predominantly adsorbed on the substrate. Atrazine attenuation in straw and bark mulch is substantial, supposedly in form of bound residues of atrazine and hydroxy-atrazine. The effect of the substrates on isoproturon attenuation is still uncertain. Straw and bark mulch contributed to retardation by adsorption, but biotic transformation seems to be the more influencing process. The behaviour of isoproturon in the column indicates the potential of effective degradation to metabolites by microorganisms or by co-metabolic processes.

3.2 Technical macro scale experiments

Results and discussions of the technical macro scale experiment are, in parts, accepted to be published in the Ecological Engineering – The Journal of Ecosystem Restoration (Krause Camilo (2015)).

3.2.1 Attenuation of herbicide agents and nitrate in straw-bark mulch bioreactors

3.2.1.1 Hydraulic characteristics of straw-bark mulch bioreactors

The bulk density of the straw-bark mulch mixture (0.1 g cm^{-3}) in the bioreactors was half of the value that was established in the laboratory scale column displacement experiments. The compression of the substrate was less effective, due to the dimensions of the technical macro scale experiment. The lower bulk density resulted in higher effective porosity in the bioreactors, as confirmed by tracer tests with sodium chloride, conducted at the onset of the experiments. The tests were repeated at the end of the experiment after 9 to 15 months to observe possible changes of hydraulic characteristics (Figure 3-21, Figure Appendix-4, Figure Appendix-5). The effective porosity in the bioreactors increased slightly over time, presumably due to decomposition of the straw that filled the pore space between bark mulch. The risk of favoured flow paths was increased at higher hydraulic residence times (HRT). In bioreactor 3 at HRT of 2.0 days, preferential flow was observed at the end of the experiment. On the other hand, preferential flow at the start of the experiment in bioreactor 5 was levelled out with time (Figure Appendix-5). In Figure 3-21 the tracer breakthrough curves in bioreactor 1 and 2 are presented exemplarily for the HRT span in the experiment.

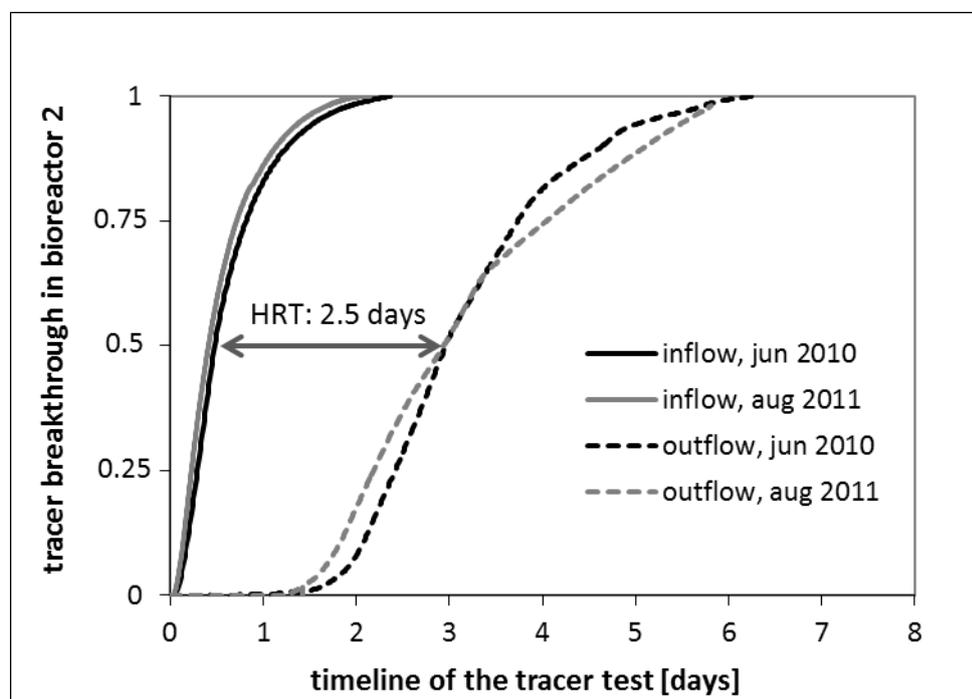
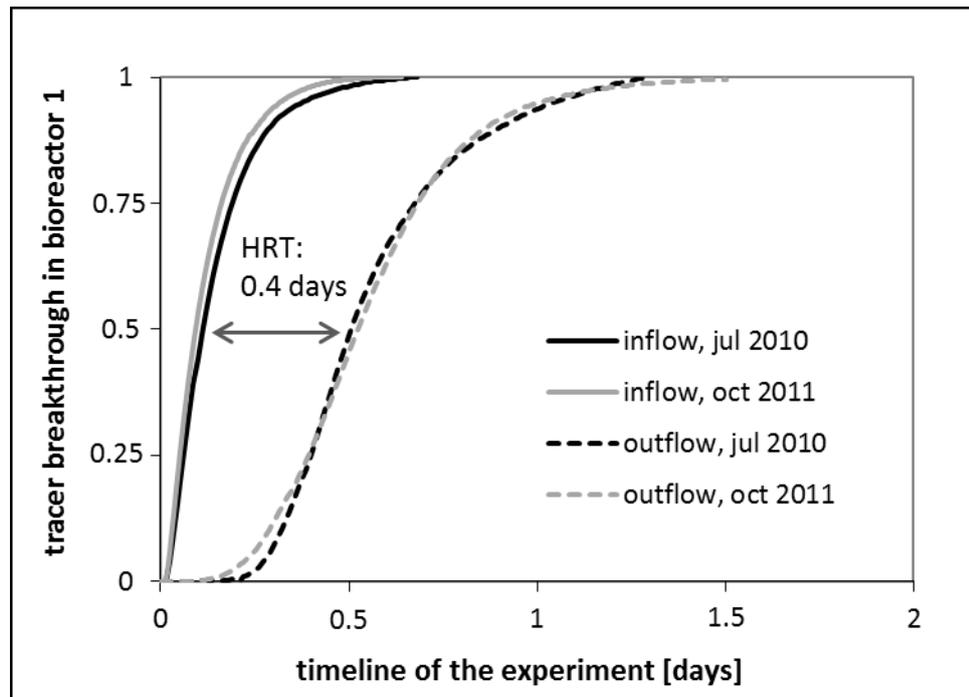


Figure 3-21: Estimation of hydraulic residence time (HRT) and structural stability in the straw-bark mulch bioreactors 1 and 2 with tracer breakthrough curves at the inflow and outflow of the bioreactors at the start and at the end of the experiment.

Average start value of effective porosity in the 5 bioreactors that were operated in total was $80 \pm 7\%$ (average \pm standard deviation) and average end value was $85 \pm 11\%$. Thus, it can be concluded that the longevity of high hydraulic conductivity in the mixture is

ensured under saturated conditions. In case the substrate is filled in and packed properly, the risk of preferential flow is low.

Under long-lasting unsaturated and wet conditions and under the weight of overlaying loads, like soil, the structure of straw is expected to be less resistant. However, it could be shown that the mixture of straw and bark mulch has the potential to preserve hydraulic stability. When straw is depleted, bark mulch can give structure to the filter and facilitates stable flow over time.

3.2.1.2 Attenuation potential of straw-bark mulch bioreactors

3.2.1.2.1 Atrazine

Effect of temperature and substrate aging

Figure 3-22 shows the development of atrazine attenuation in technical macro scale experiments. Relative atrazine attenuation correlated positively with temperature. Rates were maximal at highest temperatures with values of 48 and 100% and minimal at lowest temperatures with values of 15 and 52% at HRT of 0.4 and 2.5 days, respectively. In Figure 3-23 the corresponding values of absolute atrazine attenuation are shown.

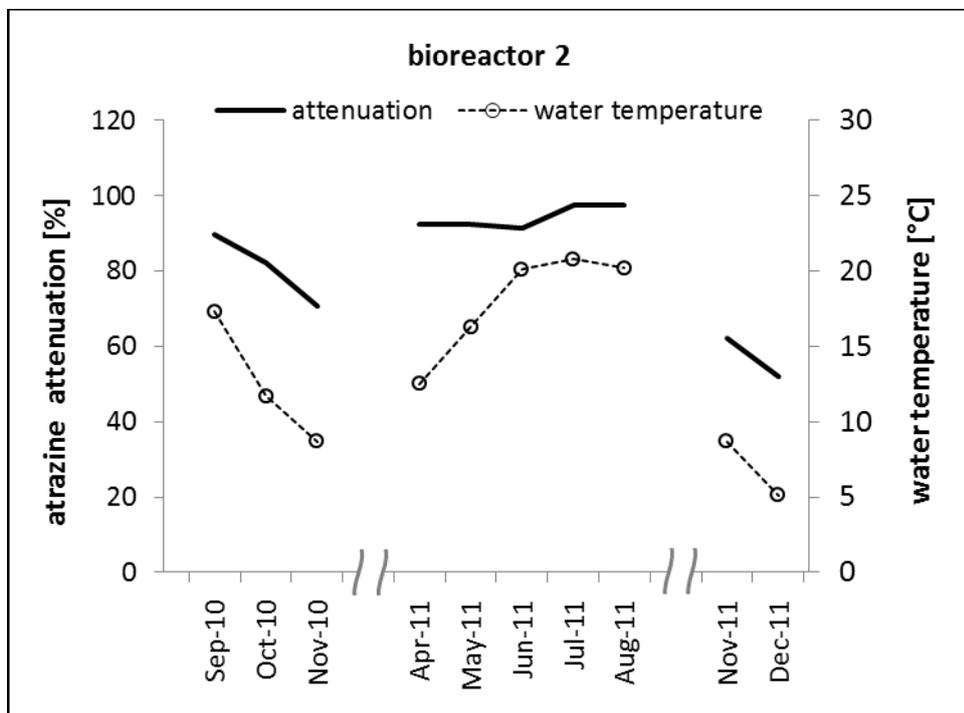
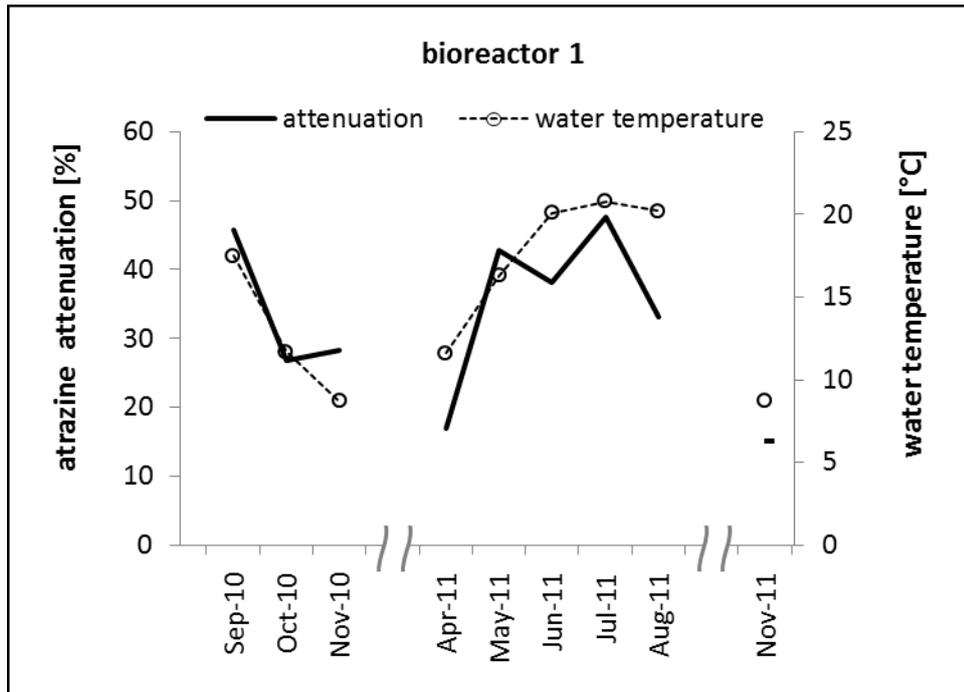


Figure 3-22: Relative atrazine attenuation (ratio of attenuation and inflow) in the bioreactors 1 and 2 at hydraulic residence times of 0.4 and 2.5 days, respectively.

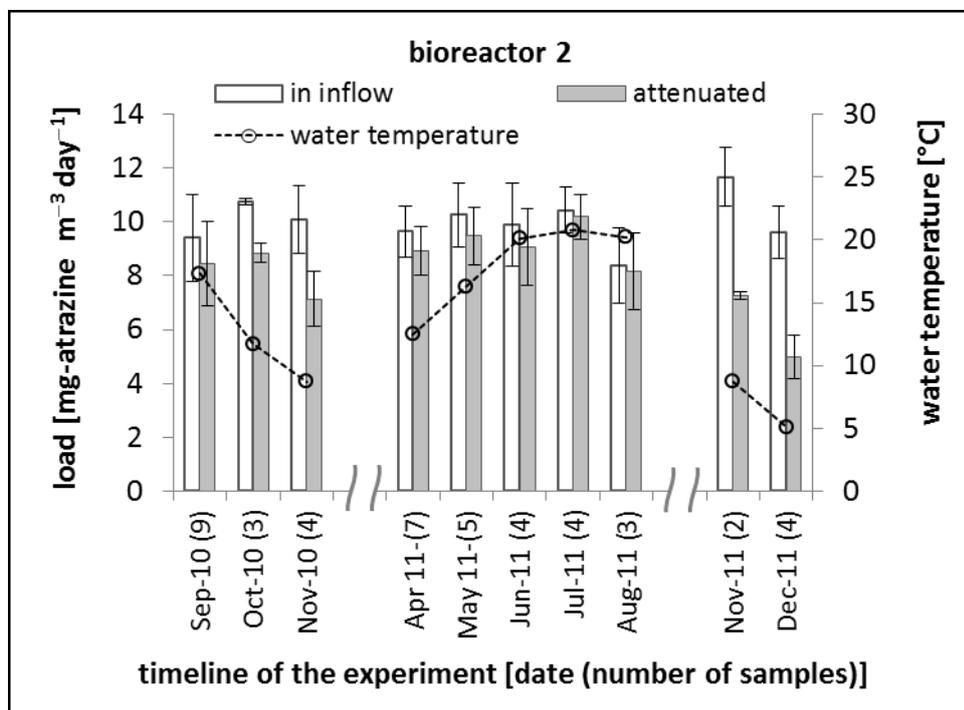
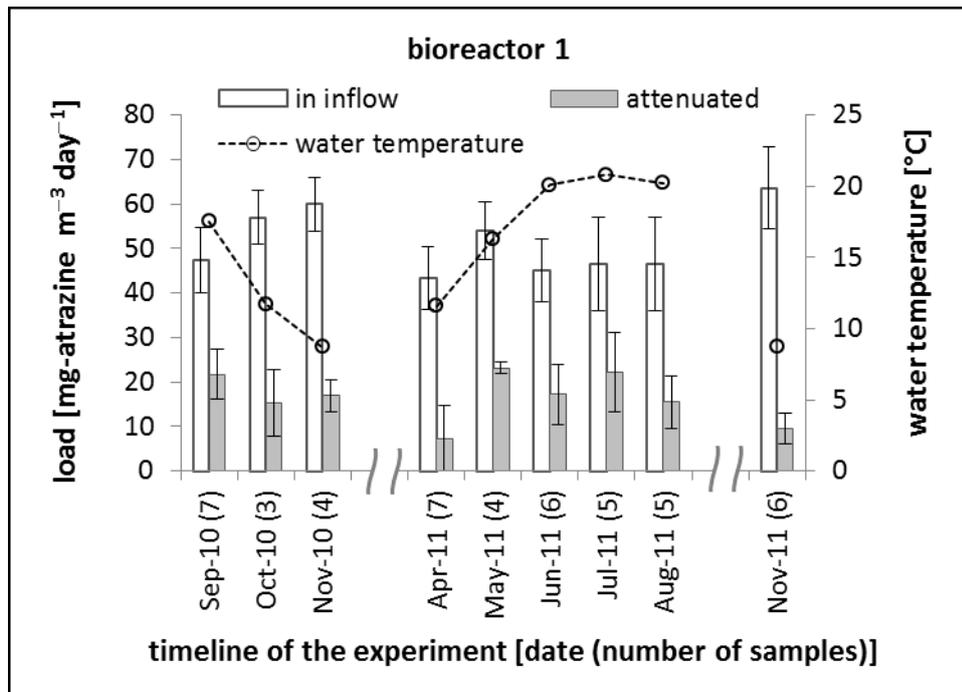


Figure 3-23: Absolute atrazine attenuation (attenuation of loads) in the bioreactors 1 and 2 at hydraulic residence times of 0.4 and 2.5 days, respectively.

The ability of the substrate to attenuate atrazine seems to be little influenced by substrate aging. Figure 3-24 shows that there is no clear shift of atrazine attenuation at same temperatures and different times of operation. The slight decrease of atrazine attenuation over time is probably the result of advancing saturation of binding sites.

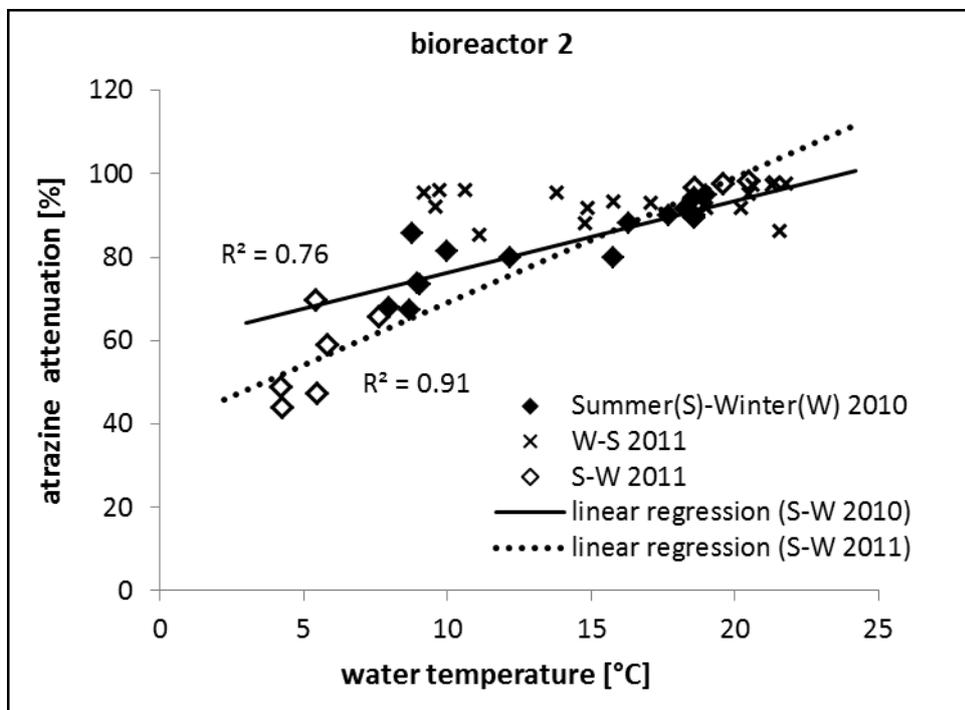
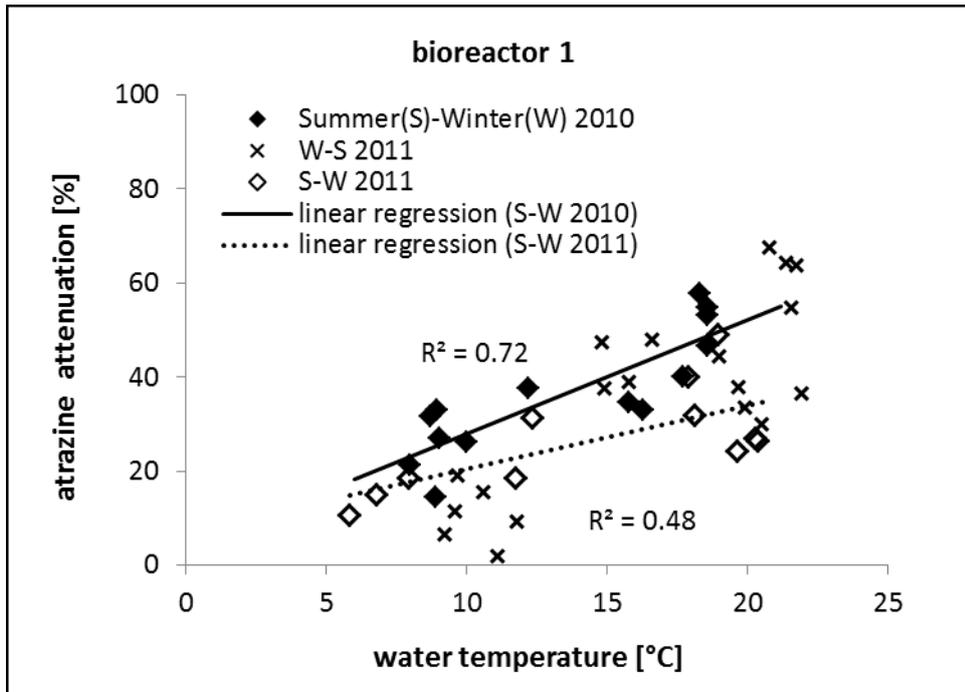


Figure 3-24: Correlation between relative atrazine attenuation and water temperature and effect of substrate aging on relative atrazine attenuation at hydraulic residence times of 0.4 and 2.5 days. Summer-Winter represents the period of decreasing water temperatures between summer and winter and W-S represents the period of increasing water temperatures between winter and summer.

Literature studies (Gendrault (2004)) indicate rather formation of bound residues instead of microbial degradation as the dominant attenuation mechanism. The formation of the metabolite hydroxy-atrazine in solution covers for about 13 and 20% of the attenuated atrazine load at HRT of 2.5 and 0.4 days, respectively (Figure Appendix-6).

The high relative hydroxy-atrazine production at HRT of 2.0 days in the short-term laboratory experiment (Figure 3-6), and with that a dominant contribution of degradation to atrazine attenuation, could not be confirmed in the long-term technical macro scale experiment. Release of deethyl-atrazine was marginal. Concentrations of the most relevant metabolite for drinking water production did not exceed $0.2 \mu\text{g L}^{-1}$.

The results are in accordance with other studies, which also showed positive correlation of atrazine adsorption and temperature. Schreiber et al. (2007) stated that atrazine adsorption to activated carbon increased with increasing temperature (atrazine concentration: 2 mg L^{-1} , water temperature: 5°C , 20°C , 35°C). Ovez and Höll (2008) found in batch experiments the same dependence also for low concentrations (atrazine concentration: $10 \mu\text{g L}^{-1}$, water temperature: 25°C , 35°C , 45°C). De Andrea et al. (1997) observed in batch experiments with Brazilian soil (atrazine concentration: 13 mg L^{-1} , water temperature: 10°C , 20°C , 30°C , 40°C) that formation of bound residues of atrazine and simultaneous chemical degradation (hydrolysis) to hydroxy-atrazine were directly related to temperature. The bound residues were predominantly hydroxy-atrazine (90%).

Effect of hydraulic residence time (HRT)

In Table 3-4, the atrazine attenuation in the technical macro scale bioreactors and in the laboratory column at temperatures of 9°C and 21°C are compared. The increased HRT affected positively the relative atrazine attenuation. At HRT of 2.5 days the evaluation of relative and absolute atrazine attenuation potential is hindered, because atrazine input limitations led at high temperatures to total atrazine attenuation. The investigation of atrazine attenuation in the first half of the bioreactor with a HRT of 2.5 days revealed that atrazine has already been attenuated after approximately one day.

The influence of HRT on absolute atrazine attenuation remains uncertain and subject to further studies. The overlay of possible input effect and HRT effect as well as the

influence of different scales prohibit sure assessment of single effects on absolute atrazine attenuation.

Table 3-4: Relative and absolute atrazine attenuation at different hydraulic residence times and in different scales at temperatures of 9 and 21°C. Absolute attenuation is presented as average \pm standard deviation.

Hydraulic residence time (scale)		0.2 days (laboratory)	0.4 days (laboratory)	0.4 days (technical)	1.25 days (technical) ^e	2.5 days (technical)
Atrazine attenuation [% of input (mg-atrazine m ⁻³ day ⁻¹) at	21°C	22 (15 \pm 5) ^a	45 (18 \pm 5) ^b	48 (22 \pm 9) ^c	94 (20 \pm 2) ^d	98 (10 \pm 1) ^d
	9°C	n.a.	n.a.	15 (10 \pm 3) ^c	49 (11 \pm 7) ^d	52 (5 \pm 1) ^d

^a column 1 (350th – 500th day of operation)

^b column 1 (530th – 670th day of operation)

^c bioreactor 1 (Jul 11, Nov 11)

^d bioreactor 2 (Jul 11, Nov 11)

^e first half of bioreactor 2

Effect of unsaturated stagnation

In analogy to laboratory experiments, the influence of a dry phase (Sept-2011) on atrazine attenuation was investigated also in the bioreactors. Unsaturated and wet conditions that were established during the dry phase were expected to be more favourable for microbial degradation of bound atrazine than saturated conditions. As in the laboratory scale, reduction of extractable bound atrazine during the dry phase was observed. However, the extractable amount of atrazine before and after the dry phase represented likewise only a small part of the amount of atrazine that was attenuated during the experiment (one to 2%).

Increased metabolite concentrations during flooding (Oct-2011) with pure process water after the dry phase did as well only cover for a marginal fraction of the attenuated atrazine (Figure Appendix-6). The short peak of hydroxy-atrazine in the range of 25% of atrazine inflow concentrations could also be the result of (i) wash-out of hydroxy-atrazine containing pore water or (ii) desorption of bound hydroxy-atrazine from the matrix, due to decomposition of the organic substrate.

Equally negligible short duration peaks of atrazine in the range of inflow concentrations, which were observed along with the hydroxy-atrazine peaks, support the

assumption that even under unsaturated and wet conditions microbial degradation of bound atrazine is of minor influence.

Conclusion

The results indicate adsorption as the dominant attenuation process and formation of bound residues in form of the parent compound and its metabolite hydroxy-atrazine, as observed in other studies under similar conditions (Gendrault et al. (2005), De Andrea et al. (1997)). Capriel et al. (1985) detected 9 years after atrazine application still 50% of bound residues in humic matter, mainly hydroxy-atrazine and hydroxy-atrazine metabolites, but also around 13% of initial atrazine and confirmed the stability of bound residues.

The effect of the dry phase as well as the fate of atrazine in the straw-bark mulch bioreactor cannot be stated conclusively. Further studies are required to answer open questions about (i) the effectiveness of the applied extraction method on aged bindings, (ii) the transformation of bound atrazine to bound hydroxy-atrazine as well as (iii) the participation of further possible metabolites at the substrate and in solution.

At least, a low risk of bound atrazine availability under saturated conditions can be assumed, as shown by continuous atrazine attenuation and leaching tests with pure process water at the end of the experiment. After 1.5 years of atrazine dosing, only a marginal wash-out of atrazine was observed. In bioreactor 1 at HRT of 0.4 days and in bioreactor 2 at HRT of 2.5 days, atrazine outflow concentrations decreased regressively from 6 to 1 $\mu\text{g L}^{-1}$ within 6 weeks and from 9 to 0.5 $\mu\text{g L}^{-1}$ within 3 weeks, respectively.

3.2.1.2.2 Isoproturon

Effect of temperature and redox conditions

In Figure 3-25, the relative attenuation of isoproturon (Dec-2011 to Jul-2012) is compared with water temperature. The low linear regression coefficient and a higher quadratic regression coefficient in bioreactor 4 at HRT of 0.5 days suggest influence of more environmental frame conditions than just temperature. In bioreactor 5, linear regression seems to be adequate to describe the correlation between relative attenuation of isoproturon and water temperature.

Observations in the bioreactors and in the laboratory column displacement studies suggest that isoproturon and atrazine have different attenuation pathways in organic substrates.

Isoproturon is of lower persistence under aerobic conditions than atrazine. Attenuation of isoproturon in the mixing cells was 35% in the bioreactor 4 at HRT of 0.5 days and 65% in bioreactor 5 at HRT of 2.0 days (Figure Appendix-8). The HRTs before inflow into the bioreactors were 3 and 14 hours, respectively. This is in contrast to observations of atrazine concentrations in analogue bioreactors, which decreased only by 4%. The attenuation of isoproturon in the mixing cell seems to be the result of microbial degradation under aerobic conditions. Other attenuation mechanisms, such as chemical degradation (Sørensen et al. (2003)), adsorption (no organic substrate in the mixing cell), photolytic degradation (sun and rain cover) and volatilization (see 2.1.3. Isoproturon/chemical, biological and physical characteristics) are expected to be of minor influence.

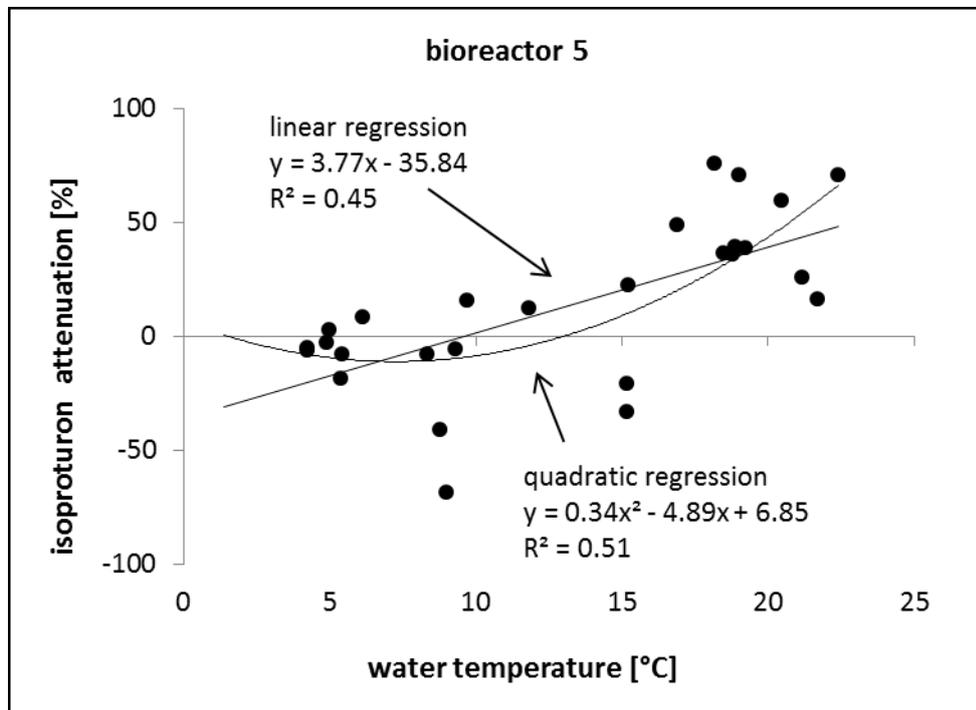
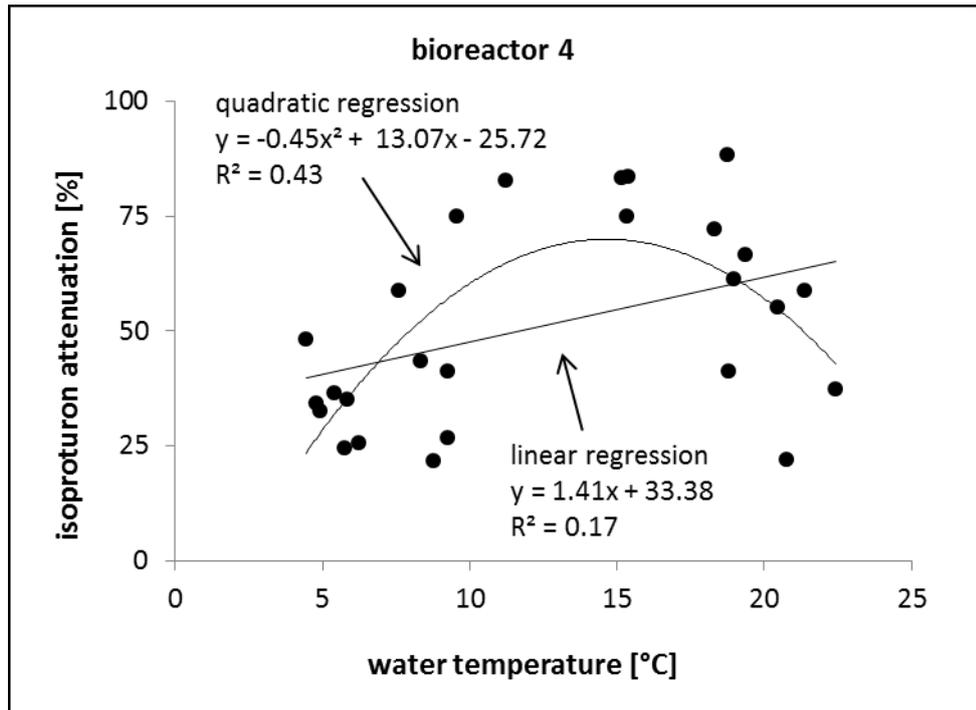


Figure 3-25: Correlation between relative isoproturon attenuation and water temperature in the bioreactors 4 and 5 at hydraulic residence time of 0.5 and 2.0 days, respectively (December 2011 to July 2012).

In Figure 3-26, it is demonstrated that during the starting phase (Oct-2011 to Nov-2011) isoproturon attenuation in the bioreactor 4 at HRT of 0.5 days was inhibited. In October 2011, NO_3^- was completely attenuated, due to a high amount of readily available organic carbon (Figure Appendix-10, Figure Appendix-11). The redox conditions were anoxic (Figure Appendix-12). In November 2011, the reservoir of readily available organic carbon was supposedly depleting and denitrification became more and more limited by the formation process of available organic carbon. In December 2011, conditions turned suboxic and relative isoproturon attenuation increased with increasing temperature until May 2012. In June 2012 and July 2012 at temperatures higher than 16°C , relative isoproturon attenuation decreased with increasing temperatures. The reason could be increasing denitrification, which caused oscillation between suboxic and anoxic conditions (Figure Appendix-12). Strong dissimilatory sulphate reduction in the same time range (May to July 2012) gives indication to at least anoxic zones in the bioreactor (Figure Appendix-7).

In bioreactor 4, the relative isoproturon attenuation under suboxic conditions correlates positively with temperature. Under anoxic conditions isoproturon attenuation seems to be inhibited. At high temperatures the increasing relative isoproturon attenuation with increasing temperatures seems to be counterbalanced by a reduction of the suboxic zone due to the increase of the anoxic zone in the bioreactor. With respect to observations in laboratory scale, degradation of isoproturon by adapted microorganisms or by co-metabolic reactions could be responsible for attenuation in the bioreactors under suboxic conditions.

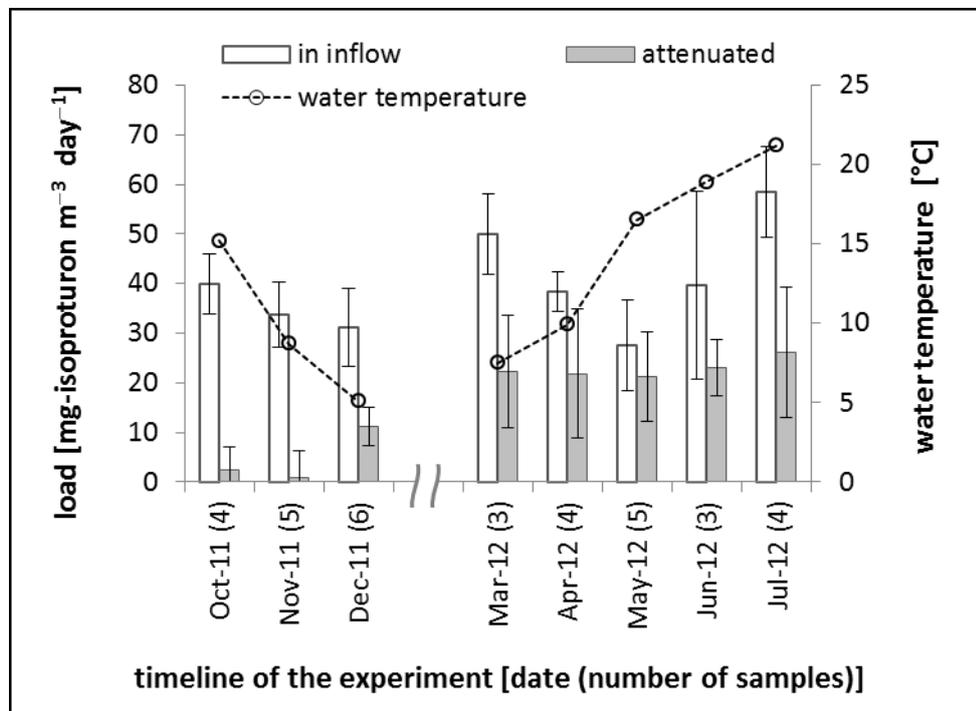
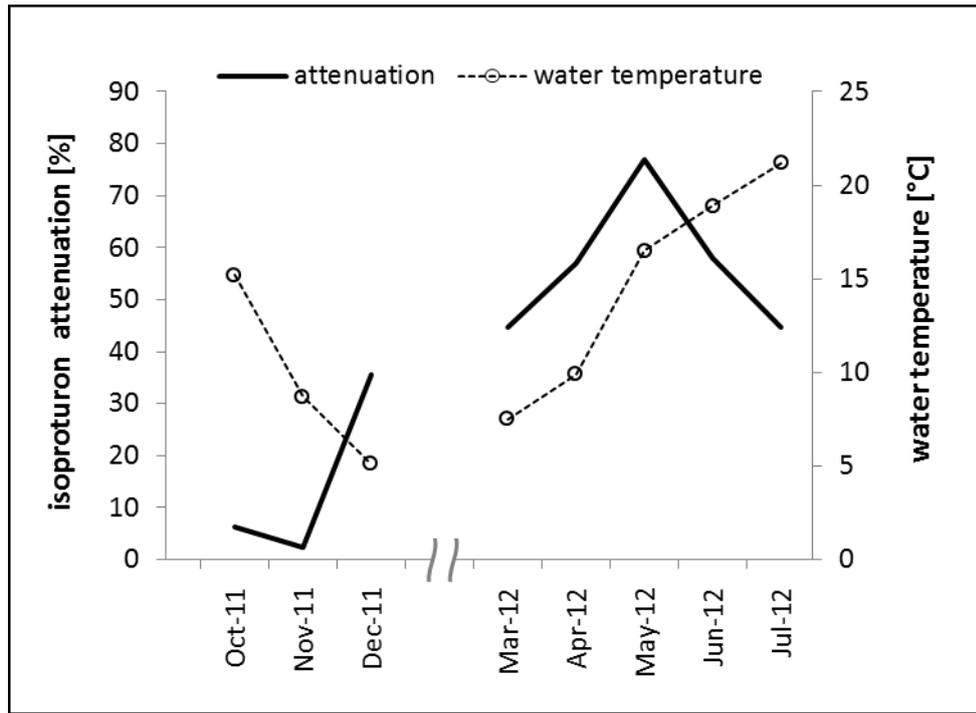


Figure 3-26: Relative isotropuron attenuation (ratio of attenuation and inflow) and absolute isotropuron attenuation (attenuation of loads) in bioreactor 4 at hydraulic residence time of 0.5 days.

Figure 3-27 presents the development of isoproturon attenuation over time in bioreactor 5. At HRT of 2.0 days, a part of the in September 2011 attenuated isoproturon was released again in October and November 2011 at lower temperatures. From December 2011 to April 2012 isoproturon was not attenuated in the bioreactor. At higher temperatures in May, June and July 2012, a moderate isoproturon attenuation could be observed.

In bioreactor 5, adsorption seems to be responsible for isoproturon attenuation in the beginning of the experiment. The attenuated isoproturon desorbed again, when isoproturon concentrations in the solution were low (Figure Appendix-8). The decrease of isoproturon concentration in the inflow of the bioreactor seems to be related to increasing isoproturon attenuation in the mixing cell, probably by degradation in the aerobic environment. The release of adsorbed isoproturon indicates that the herbicide agent did not form stable connections to the matrix of the organic substrates. In May, June and July 2012, high temperatures and adaptation of microorganisms or co-metabolic reactions could be the reason for isoproturon attenuation, predominantly in the suboxic zone in the first half of the bioreactor.

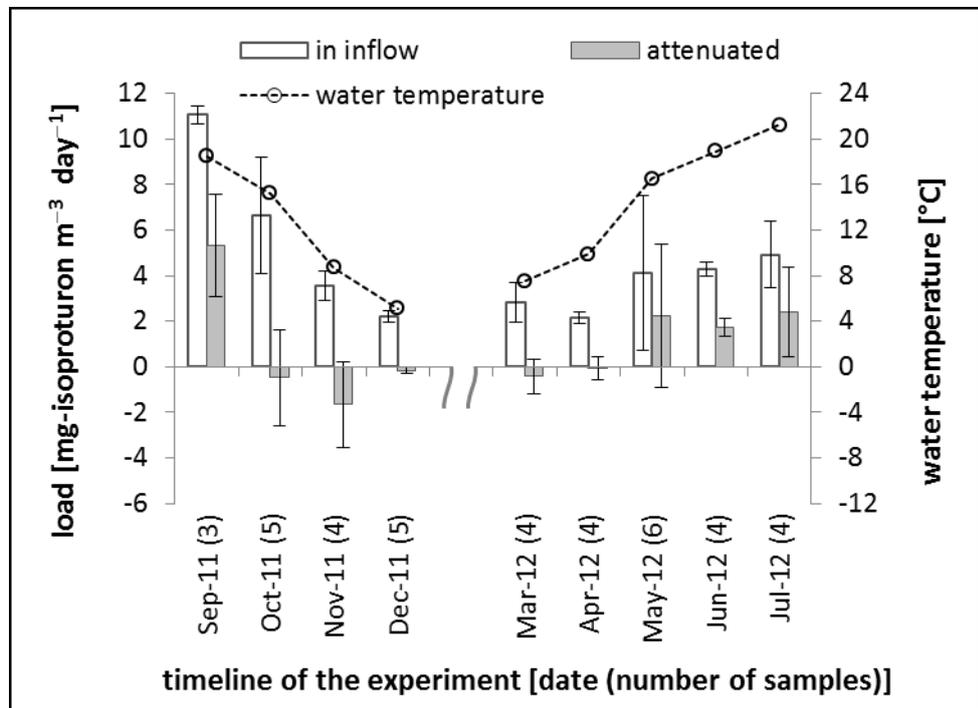
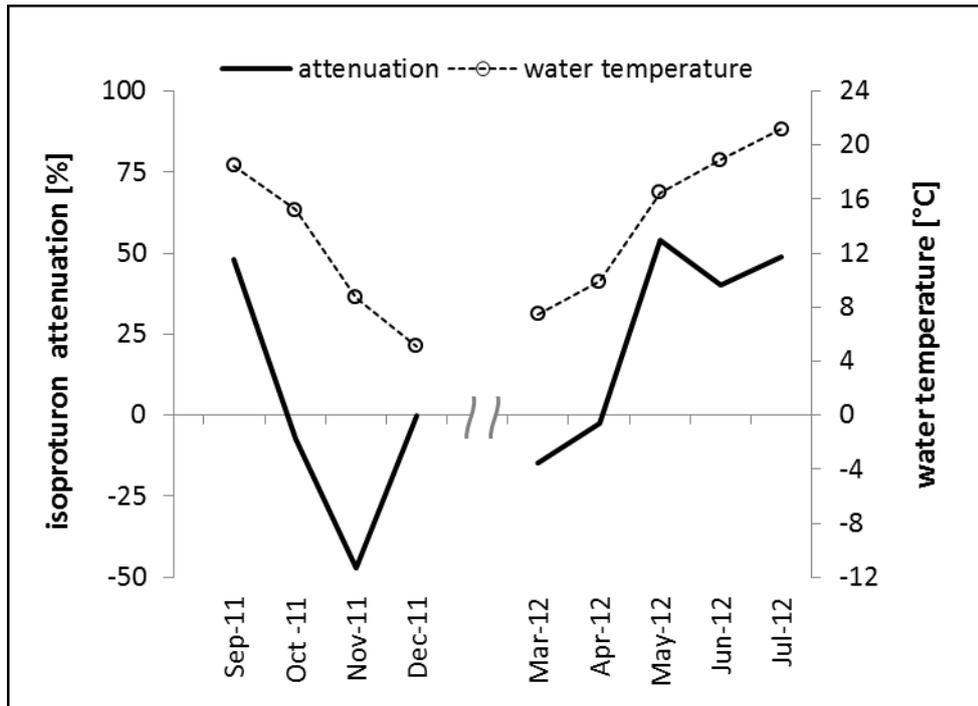


Figure 3-27: Relative isotoproturon attenuation (ratio of attenuation and inflow) and absolute isotoproturon attenuation (attenuation of loads) in bioreactor 5 at hydraulic residence time of 2.0 days.

A closer look at bioreactor 5 reveals that under anoxic conditions attenuation of isoproturon was suppressed and assumptions for influences on attenuation in bioreactor 4 are reasonable. In Table 3-5 the redox potential and isoproturon attenuation along bioreactor 5 are presented. At summerly temperatures the highest contribution to isoproturon attenuation is found in the mixing cell and in the first part of the bioreactor under aerobic to suboxic conditions. Only marginal isoproturon attenuation was observed in the second half of the bioreactor under anoxic conditions.

Table 3-5: Redox potential and corresponding isoproturon attenuation (average \pm standard deviation) in bioreactor 5 at hydraulic residence time of 2.0 days in June and July 2012.

Sampling points	mixing cell	outflow first half	outflow second half
Redox potential [mV]	Aerobic (+320 ^a)	Suboxic (+120 ^a)	Anoxic (-145 ^a)
Isoproturon attenuation from June to July 2012 [% of input]	64 \pm 8	9 \pm 3	2 \pm 5
Isoproturon attenuation in July 2012 ^b [% of input]	24 \pm 6	50 \pm 17	3 \pm 4

^a single measurement in July 2012

^b adjustment of inflow structure resulted in reduced residence time in the mixing cell

The inflow structure was modified to decrease the influence of aerobic conditions in the mixing cell on overall isoproturon attenuation at HRT of 2.0 days. Interestingly, the extent of joint isoproturon attenuation in mixing cell and bioreactor remained the same (75%).

Effect of hydraulic residence time (HRT)

In the bioreactors, isoproturon attenuation was in general lower at higher HRT (Figure 3-26, Figure 3-27). This behaviour contradicts experiences with adsorption experiments and indicates that adsorption is a less dominant attenuation process than expected.

The redox potential, which is amongst others controlled by the HRT in the bioreactor, seems to have the decisive influence on isoproturon attenuation. As shown in Table 3-5 successful isoproturon attenuation needs at least suboxic conditions. The HRT in the bioreactor does not present the HRT in the suboxic zone of the bioreactor. Therefore it is difficult to investigate the effect of the HRT on isoproturon attenuation under the

conditions of the experiment. At moderate temperatures, bioreactor 4 is entirely suboxic. At high temperatures suboxic and anoxic zones can be expected. In bioreactor 5 the suboxic zone is restricted to the first half of the bioreactor.

Conclusion

Isoproturon attenuation in organic substrates depends at first on the redox potential. Under suboxic conditions isoproturon attenuation seems to correlate positively with temperature. However, the influence of temperature cannot be completely isolated from the influence of isoproturon input (Figure 3-26). It remains an uncertainty about the effect of temperature. Anoxic conditions interfere with isoproturon attenuation. In the bioreactors isoproturon attenuation is optimal at high temperatures as long as redox conditions remain suboxic.

Although no positive influence of a higher HRT has been observed on isoproturon attenuation (Figure 3-25), adsorption and formation of bound residues of isoproturon and of isoproturon metabolites cannot be ruled out in organic substrates. In literature, it is emphasized that adsorption is an important attenuation processes. Tixier et al. (2002) stated that degradation of isoproturon to 4-isopropylaniline can be expected under denitrifying conditions. Johannesen et al. (2003) indicated that partial degradation of isoproturon to 4-isopropyl-aniline may lead to reduced mineralization of the herbicide agent, due to sorption of the aniline moiety. In their study, the FREUNDLICH coefficient of 4-isopropyl-aniline was 5-fold higher than the one of isoproturon.

The present study indicates that degradation, at least to metabolites, could be a possible attenuation path under the experimental conditions. On the basis of observations, attenuation seems to be influenced by factors that are important for microbial processes.

Conclusive evaluation of isoproturon attenuation potential in bioreactors is not possible before open questions are answered by further studies. Sustainable attenuation of isoproturon under conditions of the experiment has to be confirmed with focus on adaptation of possible degraders, potential mineralization rates and metabolites. Certain metabolites are more toxic than the parent compound (Tixier et al. (2002)).

3.2.1.2.3 Nitrate

The process that is mainly accountable for attenuation of nitrate (NO_3^-) in the bioreactors of the present study is expected to be denitrification (see 2.1.4. Nitrate and 3.1.3.2.4 Nitrate). In the following text the terms NO_3^- attenuation and denitrification are used synonymously. Relative denitrification describes the ratio of nitrogen from attenuated NO_3^- and nitrite (NO_2^-) and nitrogen from NO_3^- and NO_2^- in the inflow [%]. Absolute denitrification describes the attenuated loads of nitrogen from NO_3^- and NO_2^- [g-nitrogen m^{-3} day^{-1}]. The attenuation of NO_2^- and NO_3^- is subsumed as NO_3^- attenuation or denitrification. NO_2^- in the inflow originated from beginning NO_3^- transformation in the mixing cell and NO_2^- in the outflow was the result of incomplete transformation of NO_3^- into gaseous nitrogen species. In the present study NO_3^- attenuation, i.e. denitrification, is considered as transformation of dissolved inorganic nitrogen species in drainage water into gaseous nitrogen species.

Effect of temperature and substrate aging

The influence of temperature on denitrification in the range of 6 to 22°C in the technical macro scale is presented in Figure 3-28. When denitrification is not limited, it correlates linear and positive with water temperature, as observed in bioreactor 1 (HRT: 0.4 days) and from November to December 2011 in bioreactor 2 (HRT: 2.5 days).

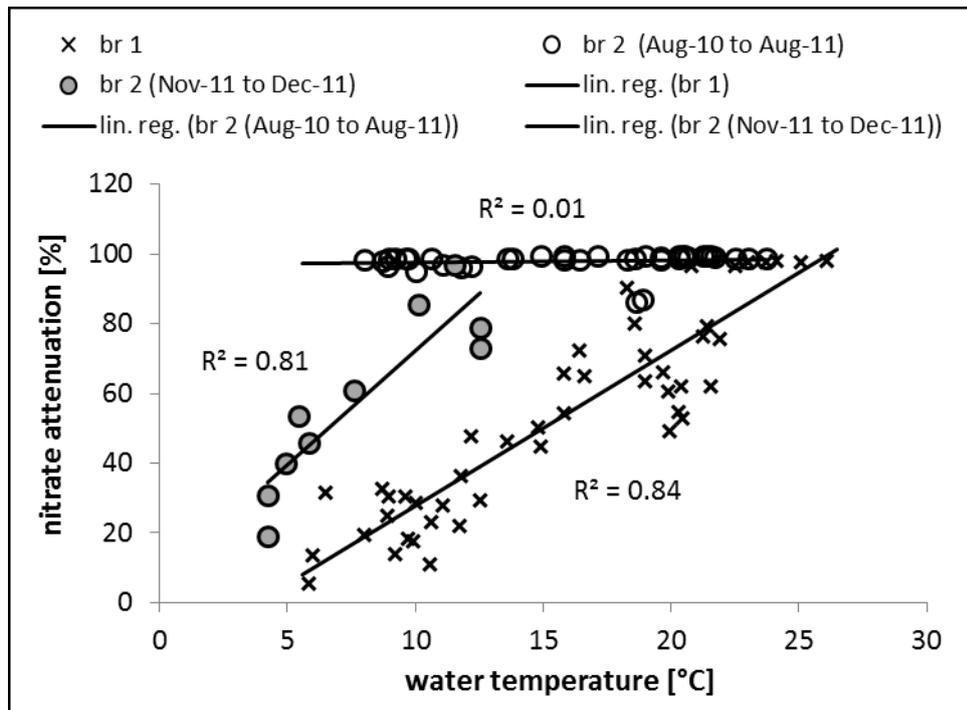


Figure 3-28: Correlation between relative nitrate attenuation (denitrification) and water temperature in the bioreactors (br) 1 and 2 at hydraulic residence time of 0.4 and 2.5 days, respectively. In bioreactor 2 the correlation is separately described by linear regression (lin. reg.) for the time intervals: August 2010 to August 2011 and November 2011 to December 2011.

In Figure 3-29 and Figure 3-30, relative and absolute denitrification in the bioreactors 1 and 2 at HRT of 0.4 and 2.5 days, respectively, are presented. High loads of readily available organic carbon on the surface of the substrate, formed during senescence of the plant, were responsible for complete denitrification after start of dosing. The wetting of the dry substrate with pure process water by initiation of flow resulted in concentrations of dissolved organic carbon (DOC) between 800 and 1100 mg L⁻¹ in the outflow. High start concentrations of DOC decreased within days to 100 mg L⁻¹ and within weeks to 10 mg L⁻¹. Dosing of NO₃⁻ was started after DOC concentrations in outflow had decreased to approximately 5 mg L⁻¹.

In bioreactor 2 at HRT of 2.5 days, no temperature effect was observed in the first year, due to limitations of denitrification by NO₃⁻ input. NO₃⁻ was completely attenuated in this phase. During the second winter at similar low temperatures (5 to 9°C) as in the first one, conditions in bioreactor 2 changed and NO₃⁻ concentrations in the outflow started to increase with decrease of temperatures. Responsible for the visibility of the temperature effect on denitrification at HRT of 2.5 days was assumably substrate aging, i.e. the decreasing availability of organic carbon over time.

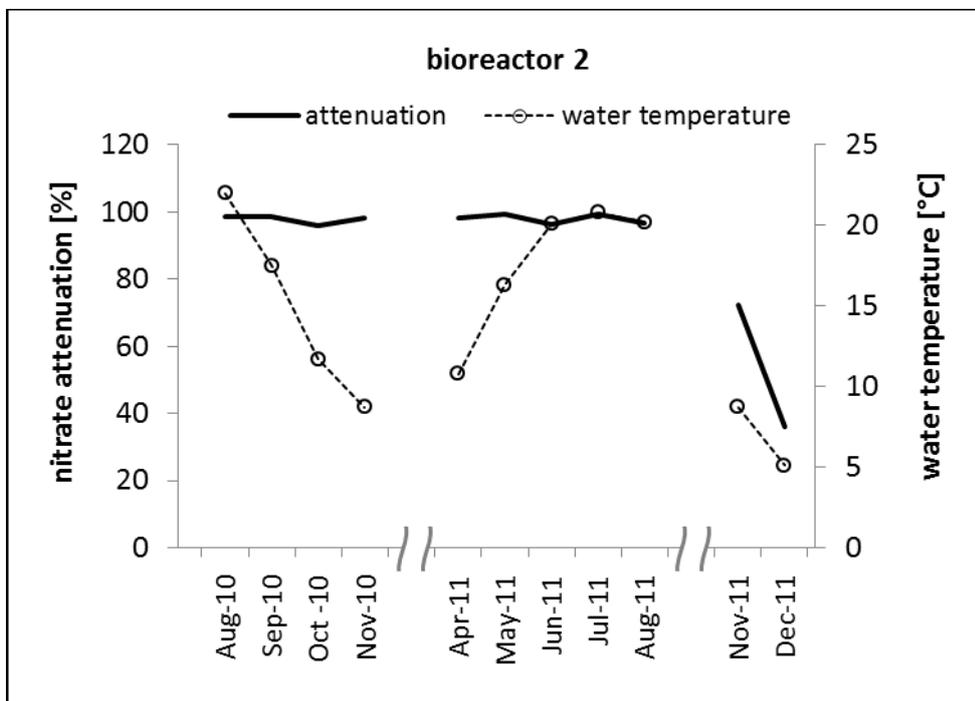
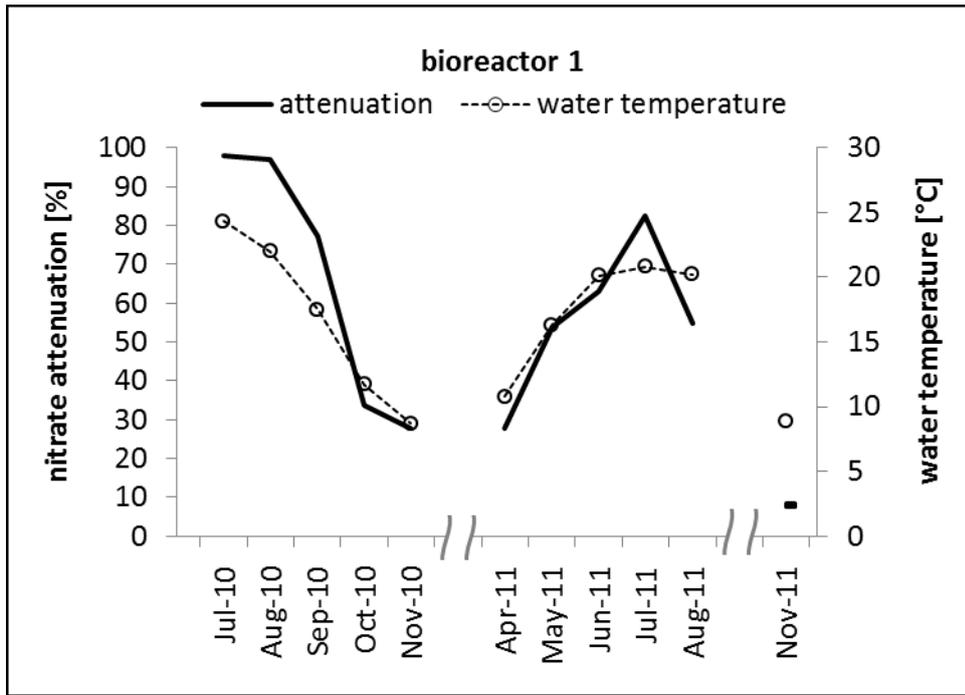


Figure 3-29: Relative nitrate attenuation/denitrification (ratio of attenuation and inflow) in the bioreactors 1 and 2 at hydraulic residence times 0.4 and 2.5 days, respectively.

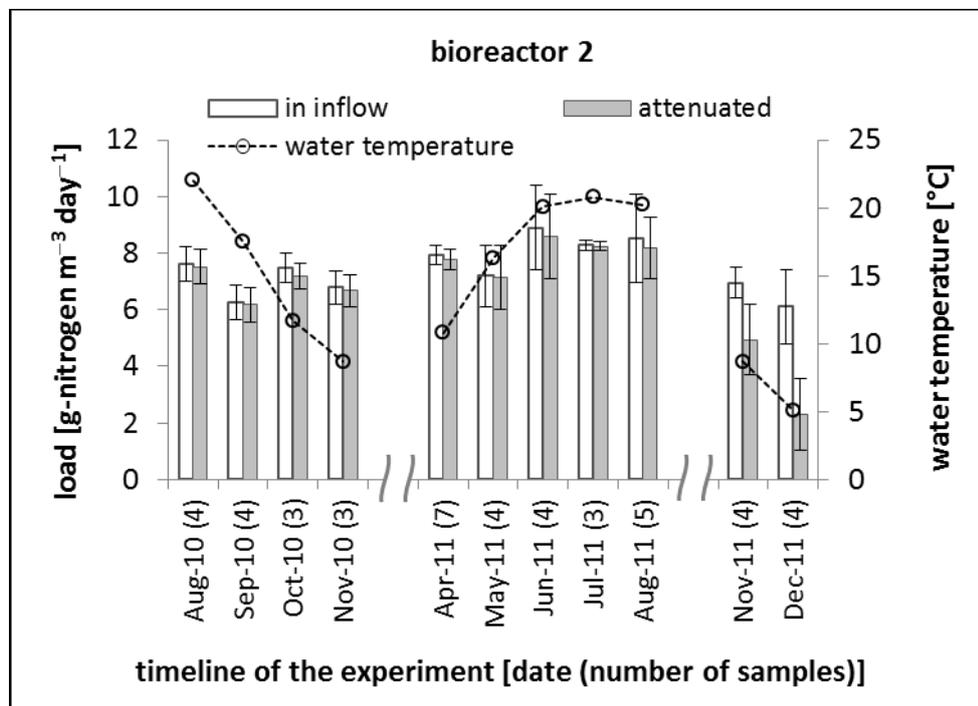
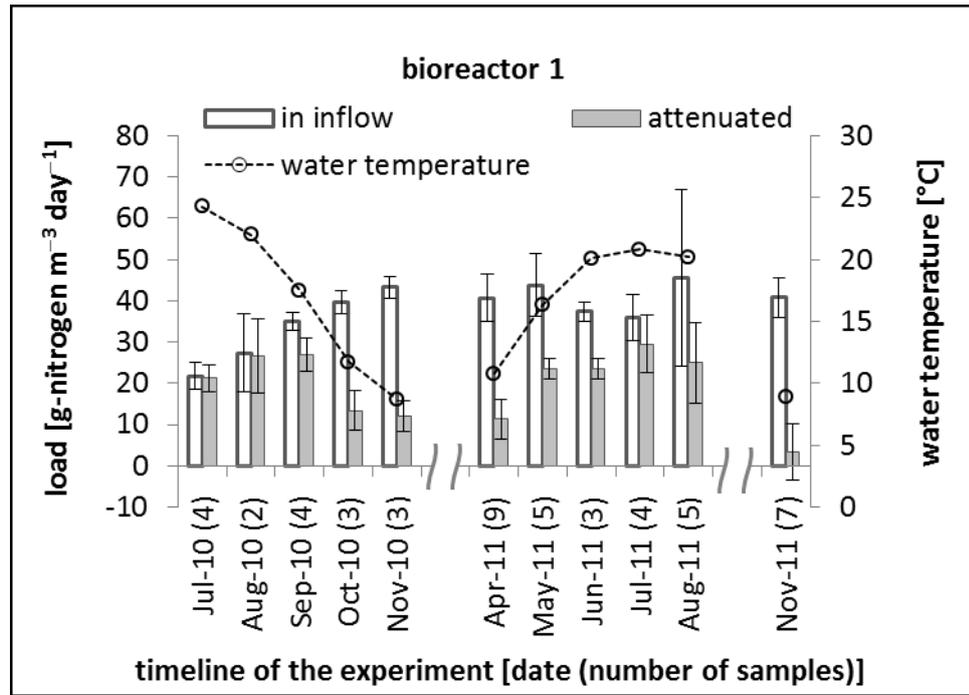


Figure 3-30: Absolute nitrate attenuation/denitrification (attenuation of loads) in the bioreactors 1 and 2 at hydraulic residence times of 0.4 and 2.5 days, respectively.

The redox potential can be used as a rough indicator for the occurrence of denitrification processes. In Figure 3-31 the development of the redox potential during most of the experiment in the bioreactors 1 at HRT of 0.4 days and 2 at HRT of 2.5 days is presented. Denitrification can be observed at a redox potential below +500 mV. When the redox potential is below +220 mV, NO_3^- is completely attenuated and denitrification is limited by NO_3^- input (Kretzschmar (2010)). The increase of redox potential in bioreactor 2 at HRT of 2.5 days in the winter 2011/12 reflects the lack of sufficient, available organic carbon for total denitrification.

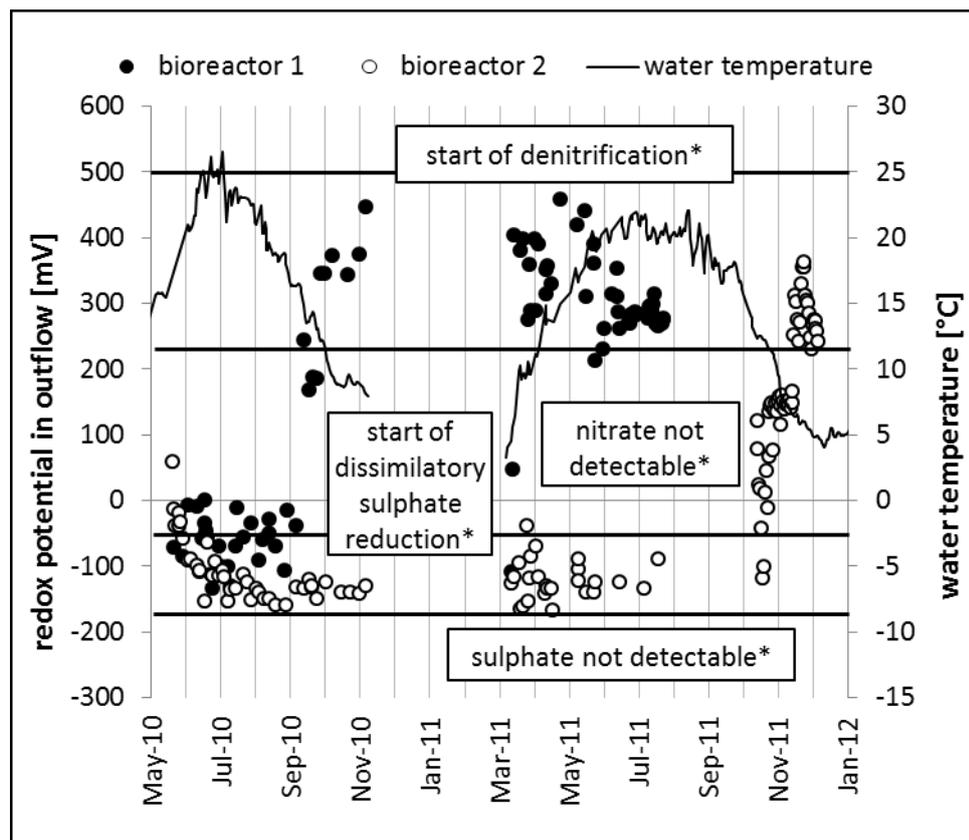


Figure 3-31: The redox potential in the outflow of the bioreactors 1 and 2 as indicator for nitrate attenuation at hydraulic residence times of 0.4 and 2.5 days, respectively (*experimental limits for different redox reactions at neutral pH value by Kretzschmar (2010)).

In Figure 3-32, the influence of substrate aging on denitrification is presented. At HRT of 0.4 days a steady decrease of relative denitrification can be observed from the second half of 2010 to the second half of 2011 for the complete temperature range. In bioreactor 2 at HRT of 2.5 days substrate aging was first observed in the second half of 2011. Denitrification, first limited by NO_3^- input, became limited by DOC formation.

The decomposition process of the substrate slowed down with time, because the ratio of more resistant and less resistant organic carbon species was increasing.

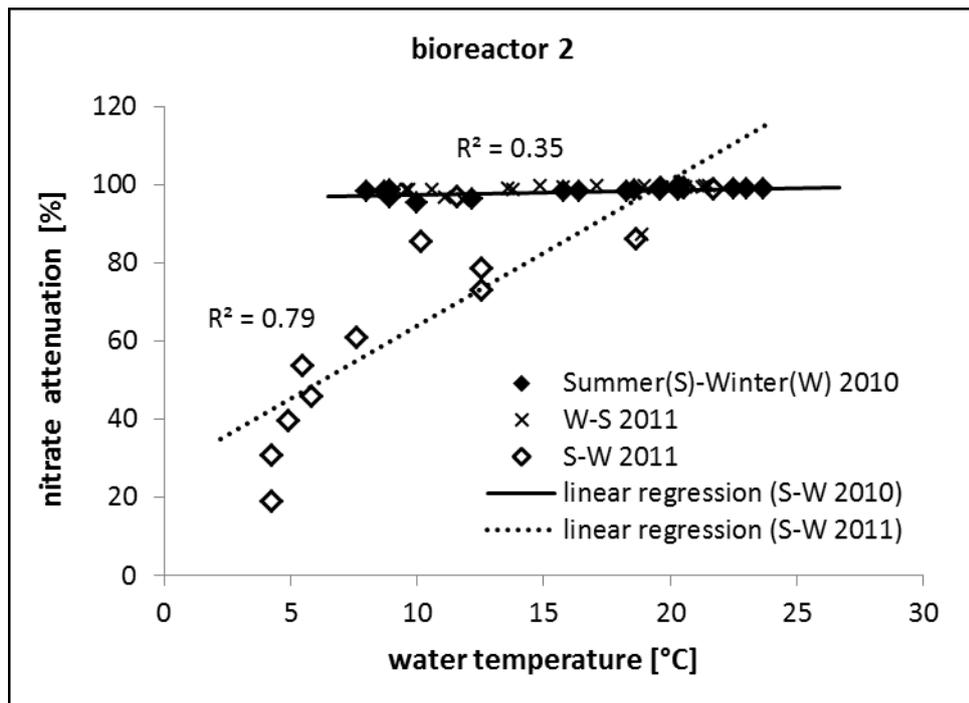
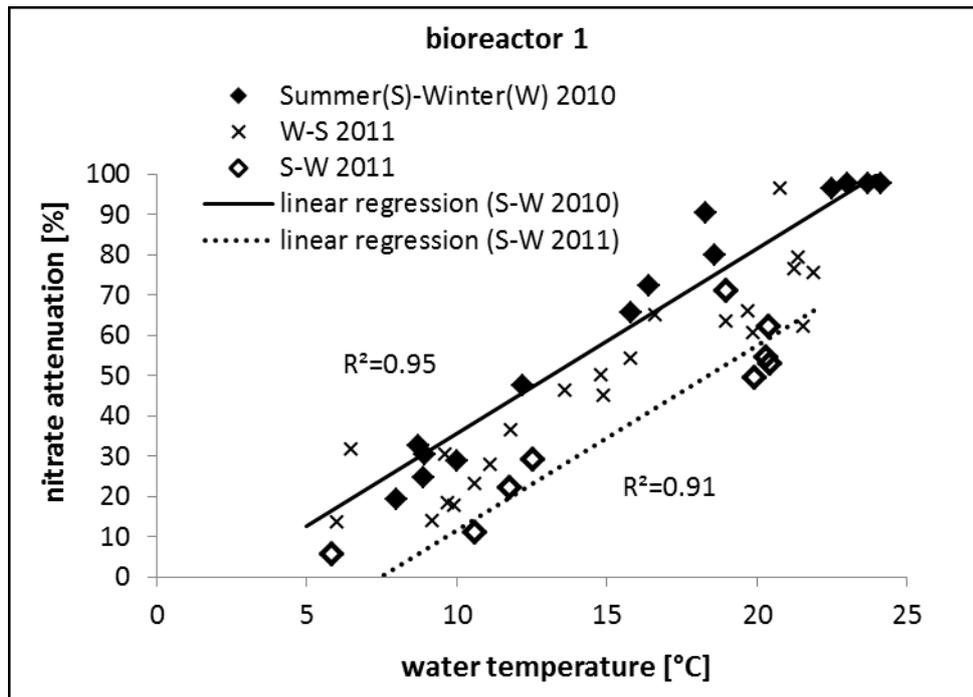


Figure 3-32: Effect of substrate aging on relative nitrate attenuation/denitrification (ratio of attenuation and inflow) in the bioreactors 1 and 2 at hydraulic residence times of 0.4 days and of 2.5 days. Summer-Winter represents the period of decreasing water temperatures between summer and winter and W-S represents the period of increasing water temperatures between winter and summer.

Denitrification in the bioreactors 1 and 2 with atrazine was compared with denitrification in the bioreactors 4 and 5 with isoproturon to assess, whether the influence of substrate age or temperature was stronger. In the bioreactors, the same substrate mixes were used under similar conditions, such as flow rate and temperature, but with different substrate ages during winter. The bioreactors 1 and 2 were started in summer and the bioreactors 4 and 5 in autumn. In general, the denitrification results in the bioreactors 4 and 5 in 2011/12 confirmed the results of the bioreactors 1 and 2 in 2010/11. In bioreactor 5 at HRT of 2.0 days, NO_3^- was completely attenuated during the first year, independent on temperature. In bioreactor 4 at HRT of 0.5 days, relative and absolute denitrification were similarly dependent on temperature as in bioreactor 1 at HRT of 0.4 days. However, comparing the results in detail (Figure 3-30 and Figure Appendix-11), it can be observed that in fresher substrate higher denitrification performance can be observed even at lower temperatures, as summarized in Table 3-6.

Table 3-6: Influence of water temperature and substrate age on nitrate attenuation (denitrification) in bioreactors 1 and 4 at hydraulic residence times of 0.4 and 0.5 days, respectively.

Bioreactor	Water temperature	Substrate age	Absolute denitrification	Relative denitrification
	°C	months after start of dosing	g-nitrogen m^{-3} day^{-1}	% of input
4	9	2	25	59
1	9	5 (1 st winter)	12	28
1	9	17 (2 nd winter)	3.5	8
4	5	3	21	45

The interference of the different herbicide agents (isoproturon in the fresher and atrazine in the older substrate) should be insignificant in comparison to temperature. Literature studies and laboratory scale experiments at HRT between 0.2 and 2.0 days demonstrated that denitrification was not affected by herbicide agents in the experiment. No negative effect of atrazine on denitrification at HRT of 2.0 to 2.5 days was observed, comparing the denitrification rates in the bioreactor 2 with atrazine and bioreactor 3 without atrazine (Figure Appendix-9). Increased outflow concentrations in bioreactor 3 at low

temperatures are expected to be the result of preferential flow and lower HRT of the matrix flow (Figure Appendix-4).

Effect of hydraulic residence time (HRT)

In Table 3-7, average denitrification rates in the technical macro scale bioreactors and in the laboratory column at temperatures of 9 and 21°C are compared. In the bioreactors at HRT of 2.0 to 2.5 days additional samples were taken in the middle of the reactor to study denitrification at intermediate HRT (1.0 to 1.25 days).

Table 3-7: Relative and absolute nitrate attenuation (denitrification) at different hydraulic residence times and at temperatures of 9 and 21 °C. Absolute nitrate attenuation is presented as average ± standard deviation.

Hydraulic residence time (scale)		0.2 days (laboratory)	0.4 days (laboratory)	0.4-0.5 days (technical)	1.0-1.25 days (technical) ^g	2.0-2.5 days (technical) ^g
Denitrification [% of input (g-nitrogen m ⁻³ day ⁻¹)] at	21°C	46(22±6)^a	69(17±2)^b	82(38±9)^c to 82(30±7)^d	97(17±1)^e to 99(19±2)^f	99(8±1)^f to 100 (9±1)^e
	9°C	-	-	8(3.5±7)^d to 59(25±4)^c	29(5±2)^f to 69(12±3)^e	72(5±1)^f to 98(8±1)^e

^a 100th-300th day of operation (with atrazine from day 188)

^b 100th-260th day of operation (with isoproturon from the start)

^c bioreactor 4 (Nov-11, Jul-12) – fresher substrate

^d bioreactor 1 (Jul-11, Nov-11)

^e bioreactor 5 (Nov-11, Jul-12) – fresher substrate

^f bioreactor 2 (Jul-11, Nov-11)

^g denitrification limited by nitrate input

As observed in the laboratory, higher HRT had positive influence on relative denitrification in technical macro scale. NO₃⁻ was completely attenuated after less than one day at high temperatures (21 °C). At low temperatures, total denitrification was observed after 2.0 days in fresh substrate.

Different relative denitrification rates in laboratory and in technical macro scale at similar HRTs and temperatures have to be explained with different experimental conditions, especially in terms of availability of organic carbon. In technical macro scale, denitrification was favoured, because transformed organic carbon in the saturated zone of the bioreactor was consistently replaced by fresh substrate from the overlaying, dry buffer zone.

In the bioreactors the evaluation of the HRT effect on absolute denitrification is not possible, due to complete NO_3^- attenuation at HRT of 2.0 to 2.5 days.

Effect of unsaturated stagnation

The dry phase resulted in short-term increase of DOC wash-out after re-initiation of flow with pure process water. As observed in the laboratory, the decomposition of the organic carbon was enhanced under wet and aerobic conditions. Maximal DOC concentrations in the outflow were around 50 mg L^{-1} . They decreased to the level of inflow concentrations (around 4 mg L^{-1}) within 3 days in bioreactor 1 and within 3 weeks in bioreactor 2. Denitrification was not delayed after restart of nutrient dosing. Different from laboratory was only that denitrification after the dry phase was not higher than before the dry phase, probably due to an overlapping temperature effect. Water temperatures after the dry phase were about $10 \text{ }^\circ\text{C}$ lower than before.

3.2.1.3 Utilization of the organic carbon source in straw-bark mulch bioreactors

The approach to determine the loss of organic carbon in the substrate by its estimation from major respiratory reactions and by wash-out was successfully tested in the laboratory. The stoichiometrically calculated organic carbon covered for 96 to 98% of the actual reduction of the organic carbon source. In the technical macro scale experiment, it was not possible to measure the actual carbon loss. On the basis of experiences in laboratory scale, the efficiency of denitrification, using the organic substrate as carbon source, was estimated in the bioreactors. The withdrawal of carbon by parallel respiratory and other carbon reducing processes, such as oxygen reduction, denitrification, dissimilatory sulphate reduction and wash-out, is shown in Figure 3-33.

The substrate has to be thought of as reactor and organic carbon source for different redox processes. In the front zone carbon is used for the reduction of oxygen in the inflow. When oxygen is depleted, denitrification becomes the dominant process and when NO_3^- is transformed, dissimilatory sulphate reduction mainly uses the organic carbon source. The surplus of produced but unused DOC is washed out.

The contribution of observed redox reactions and wash-out to organic carbon use in the bioreactors is summarized in the following paragraphs as average values for the whole period. Oxygen reduction contributed to carbon consumption with 11 and 3% at HRT of 0.4 and 2.5 days, respectively. At anoxic conditions, SO_4^{2-} was used as electron acceptor by microbial processes and reduced to H_2S . Dissimilatory sulphate reduction was expected to start, after NO_3^- was completely removed. Interestingly, dissimilatory sulphate reduction occurred even, when denitrification was not limited by NO_3^- input. It can be assumed that the transition between the processes was gradual and the redox reactions took place parallel in niches of micro- and macro-scale. At HRT of 0.4 days, dissimilatory sulphate reduction used only 18% of the organic carbon and denitrification was the major DOC consumer with 61%. At HRT of 2.5 days the contribution of dissimilatory sulphate reduction to carbon use was 50%. Only 33% of the available organic carbon were used for denitrification, due to limitation of the suboxic respiration process by NO_3^- input, which led to total NO_3^- attenuation.

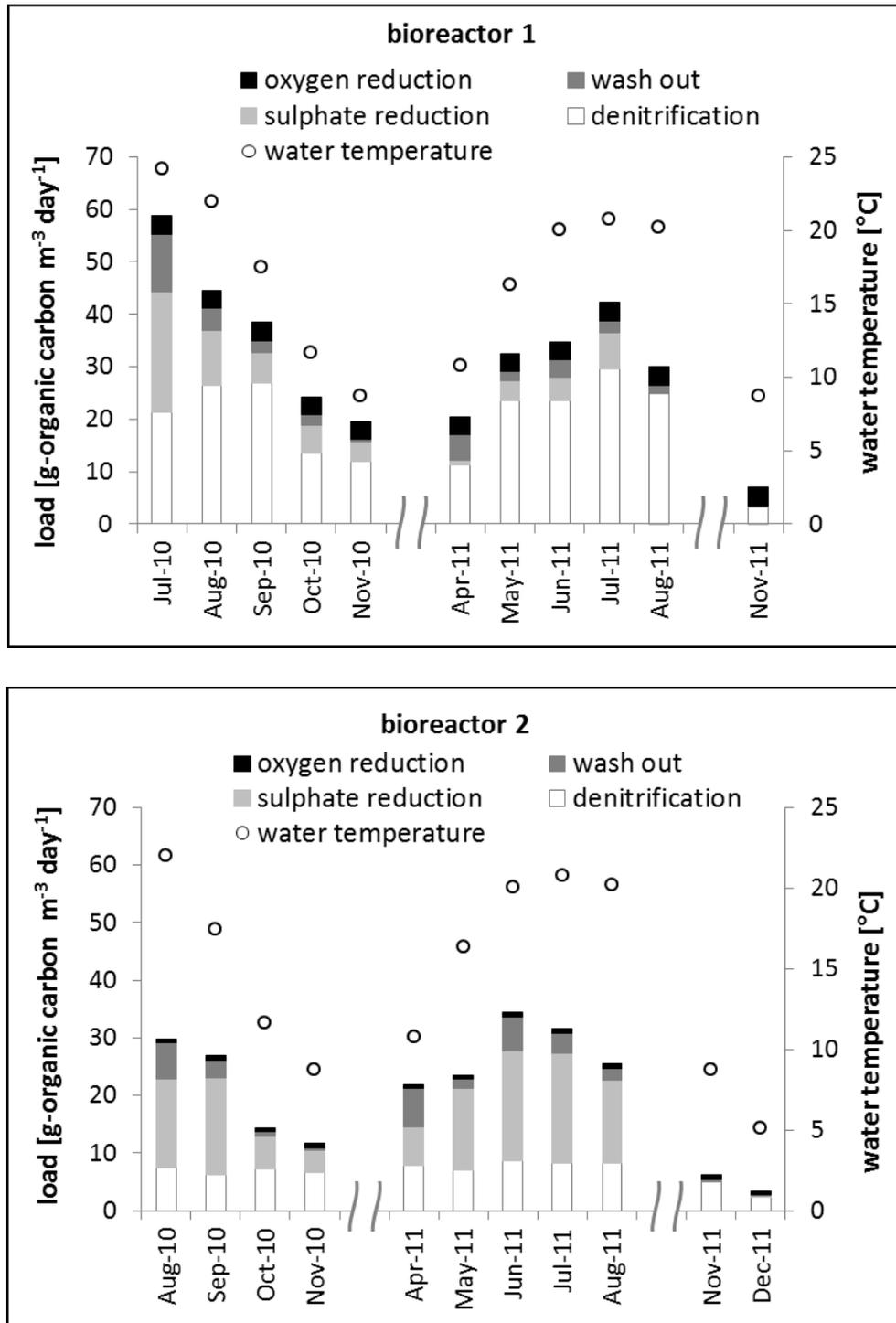


Figure 3-33: Contribution of the organic carbon source to major redox reactions and wash-out in the bioreactors 1 and 2 at hydraulic residence times of 0.4 and 2.5 days, respectively.

Total wash-out was similar at both HRTs with 13% and 10% of available organic carbon at HRT of 2.5 and 0.4 days, respectively. Increased wash-out was observed after start and stagnation phases (at both HRT) and at high temperatures at HRT of 2.5 days, when electron acceptors in the inflow were nearly used up.

The results of a separate contemplation on DOC withdrawal in bioreactor 2 in the first and the second half with HRT of 1.25 days in each half are shown in Figure 3-34.

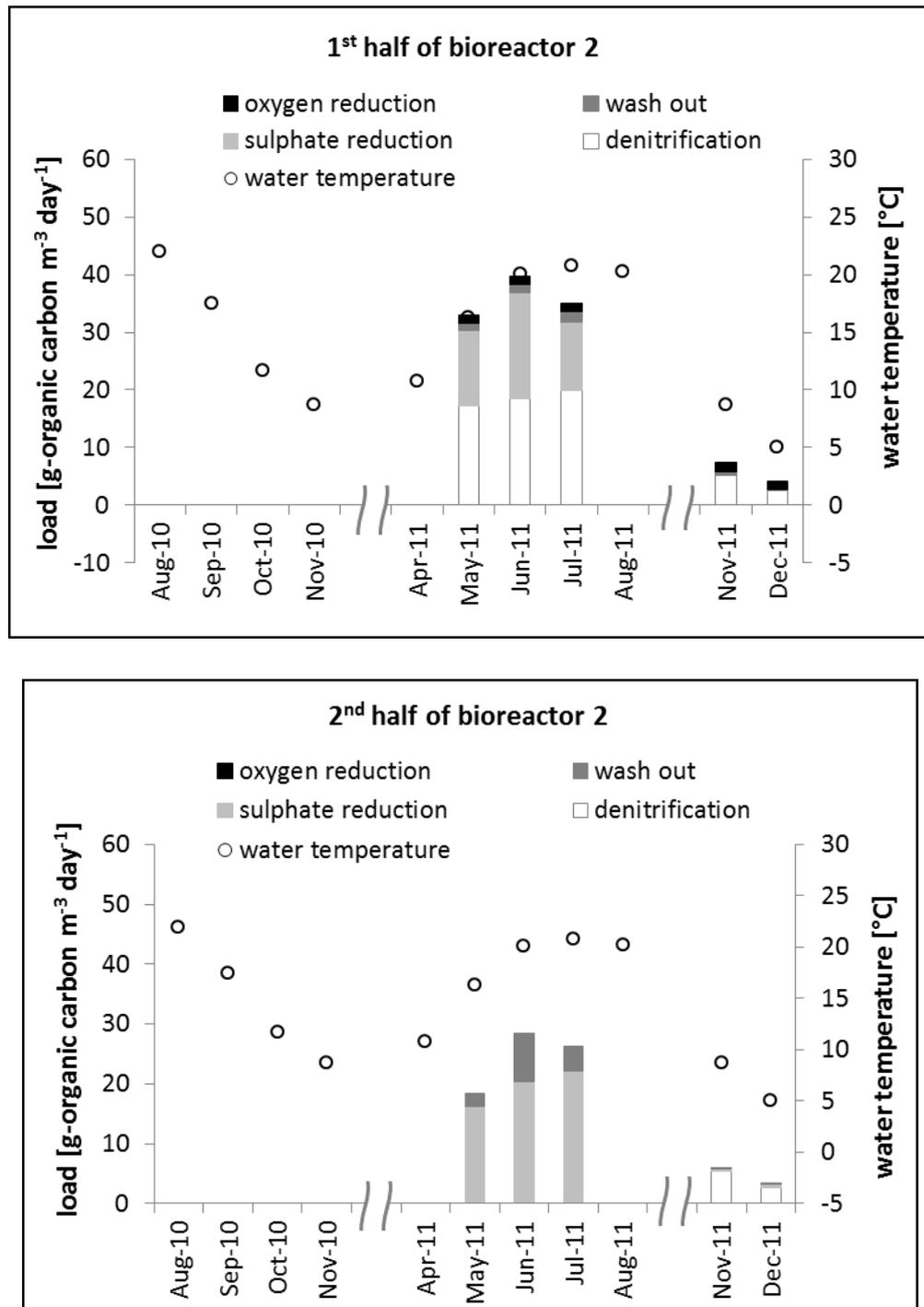


Figure 3-34: Contribution of the organic carbon source to major redox reactions and wash-out in the first and the second half of bioreactor 2.

Considering the respiratory reactions, which are predominantly responsible for organic carbon reduction in the substrate, the temperature effect on microbiological processes

becomes visible in bioreactor 2 (HRT: 2.5 days), in which the denitrification process was limited by total NO_3^- attenuation.

The results indicate that the organic carbon source was consumed in dependence on the energetic yield of major respiratory processes. Carbon use was higher in the first half of the bioreactor, where oxygen reduction and denitrification were the domination redox reactions.

Further the organic substrate seems to be degraded in dependence on available electron acceptors. While in the second half of the bioreactor even the dissimilatory sulphate reduction process is limited by SO_4^{2-} input at high temperatures, the DOC wash-out remains comparatively low. It can be expected that the organic carbon source is relatively stable in absence of electron acceptors. With lower SO_4^{2-} concentrations in the inflow, the functionality of the substrate as organic carbon source for denitrification would be ensured even longer than observed in the experiments.

Limitations of the denitrification process by NO_3^- input have to be avoided to guarantee efficient organic carbon source utilization for NO_3^- attenuation, especially when competing electron acceptors are present.

3.2.2 Summary of technical macro scale results

Technical macro scale experiments confirmed that the mixture of bark mulch and straw provides high hydraulic conductivity on a long-term basis.

Atrazine attenuation correlates linear and positive with water temperature. Atrazine concentrations were reduced by 15 to 48% at HRT of 0.4 days and by 52 to 98% at HRT of 2.5 days in the temperature range of 9 to 21°C over a period of 1.5 years. Corresponding absolute atrazine attenuation yielded 10 to 22 mg-atrazine $\text{m}^{-3} \text{day}^{-1}$ at HRT of 0.4 days and 7 to 10 mg-atrazine $\text{m}^{-3} \text{day}^{-1}$ at HRT of 2.5 days.

Technical macro scale experiments substantiated assumptions on the base of laboratory experiments that atrazine attenuation is predominantly driven by adsorption, only a minor part of the attenuated atrazine could be recovered as dissolved metabolite (hydroxy-atrazine). The metabolite deethyl-atrazine was of no concern. Atrazine seems

to create stable bound residues at the substrate matrix, partially in form of hydroxy-atrazine, as indicated in literature.

The risk of desorption and salting out is low, given the observations that atrazine was permanently attenuated during the 1.5 year experiment and only marginally washed out after stop of dosing in the end. Unfortunately, the composition of bound residues is uncertain and the bound atrazine can be set free after decomposition of the binding sites. Under the experimental conditions, substrate aging had low negative influence on atrazine attenuation.

In conclusion, the risk remains that fractions of atrazine will be set free with time. On the other hand, there is a good chance for long-lasting attenuation and partial transformation to a high extend.

For isoproturon, the one year-lasting technical macro scale experiments reinforced the indications of laboratory experiments that biotic degradation could be an influencing process in attenuation, next to adsorption. Attenuation of isoproturon seems to be linked to the energetic efficiency of respiratory processes, indicated by the redox potential. The decrease of the redox potential into the anoxic zone, as result of complete denitrification, interfered with isoproturon attenuation. Under suboxic conditions isoproturon attenuation seems to correlate positively with temperature. However, evaluation of isoproturon attenuation in the bioreactor has to be considered provisory. Further investigations with focus on metabolites are necessary.

Relative isoproturon attenuation was 36 to 77% at HRT of 0.5 days in the temperature range of 5 and 17°C and 0 to 49% at HRT of 2.0 days in the temperature range of 5 and 21°C. Corresponding absolute isoproturon attenuation yielded 11 to 21 mg-isoproturon $\text{m}^{-3} \text{day}^{-1}$ at HRT of 0.5 days and 0 to 2 mg-isoproturon $\text{m}^{-3} \text{day}^{-1}$ at HRT of 2.0 days.

At hydraulic residence time (HRT) of 0.4 days, relative denitrification correlated positively with water temperature. At HRT of 2.5 days, total denitrification was observed during the first year, even at low temperatures in the first winter. In the second winter, relative denitrification decreased with decreasing temperature, due to substrate aging.

NO_3^- concentrations were reduced by 8 to 82% at HRT of 0.4 days and by 72 to 100% at HRT of 2.5 days in the temperature range between 9 and 21°C over a period of 1.5 years.

Denitrification performance decreased with increasing substrate age, i.e. decreasing availability of organic carbon. In fact, substrate age had higher influence on denitrification than temperature. In fresher, less processed substrate, higher denitrification was observed at lower temperatures (45% at HRT of 0.5 days at 5°C).

Attenuated loads of NO_3^- and NO_2^- yielded between 3 g-nitrogen $\text{m}^{-3} \text{ day}^{-1}$ (9°C) and 30 g-nitrogen $\text{m}^{-3} \text{ day}^{-1}$ (21°C) at HRT of 0.4 days and between 5 g-nitrogen $\text{m}^{-3} \text{ day}^{-1}$ (9°C) and 8 g-nitrogen $\text{m}^{-3} \text{ day}^{-1}$ (21°C) at HRT of 2.5 days.

At HRT of 0.4 days, efficient use of the organic substrate and high denitrification rates in comparison with comparable studies were observed. About 61% of the organic carbon were metabolized by denitrification

At HRT of 2.5 days, denitrification was more effective (complete NO_3^- attenuation) but less efficient in the scope of organic carbon use, as long as the denitrification process was limited by NO_3^- input. Under these conditions the carbon source was substantially used for dissimilatory sulphate reduction and only about 33% of the available organic carbon were used for denitrification. When denitrification was no longer limited by NO_3^- input and became dependent on temperature, due to substrate aging, the carbon use efficiency of denitrification increased to 77%.

Under the experimental conditions, denitrification seems to correlate positively with the production rate of available organic carbon, which depends on temperature, HRT, presence of electron acceptors, energy yield of redox reactions and resistance of organic carbon species.

The wash-out of organic carbon during operation was low, even when electron acceptors, such as oxygen, NO_3^- or SO_4^{2-} , were completely attenuated. It is indicated that the solid organic carbon source has to be located in the reactor for efficient use of dissolved organic carbon (DOC) in the denitrification process. Microorganisms seem to dissolve the organic substrate only when organic carbon in solution is not sufficient in

relation to present electron acceptors, which are used for respiration. In absence of electron acceptors, the organic carbon source is spared.

The implementation of a bioreactor, which supplies organic carbon to microbiological processes, is also accompanied by a series of risks for the receiving waters. An obvious drawback of the labile carbon source, which is responsible for the enhanced denitrification, is the risk to turn into a source of organic carbon after start of flow and after saturated and unsaturated stagnation phases. Also, wash-out of acidity during start phase was observed, which is originated in bark mulch. The value of pH increased from 4.5 to 6 within one month at HRT of 2.5 days and from 4.5 to 7 within 1.5 months at HRT of 0.4 days.

At low temperatures during first winter and following spring, increased concentrations of nitrite (NO_2^-), a fish toxin, ranged from 3 up to 9 mg L^{-1} . This indicates that the reduction process from NO_3^- to dinitrogen (N_2) was disturbed at low HRT (0.4 days) and further intermediate products, such as nitrous oxide (N_2O), which acts as greenhouse gas, were released. Interestingly, the release of NO_2^- did not exceed 0.4 mg L^{-1} in the second winter, maybe due to adaptation of microorganisms.

Total denitrification was observed at HRT of 2.0 to 2.5 days and concentrations of NO_2^- in the outflow did not exceed 0.5 mg L^{-1} . On the other hand, complete attenuation of NO_3^- in combination with a labile carbon source enables the formation and release of methane (CH_4), another potent greenhouse gas. However, CH_4 is less effective than N_2O .

With respect to the total nitrogen balance over a watershed, Moorman et al. (2010) noted that, if NO_3^- is not efficiently treated in a bioreactor, more N_2O may be released downstream under less efficient conditions.

When SO_4^{2-} is present in the inflow, hydrogen sulphide (H_2S) is formed in the bioreactor. H_2S is a fish toxin. H_2S has also negative effect on denitrification, because it disturbs the last two transformation steps from NO_3^- to N_2 (Sørensen et al. (1980), Pan et al. (2013)) and decreases the ratio of $\text{N}_2/\text{N}_2\text{O}$. Dissimilatory sulphate reduction also enables mercury methylation (Christianson (2011)). According to Clifford and Liu

(1993), biological denitrification brings along oxygen demand, turbidity and additional bacteria in the receiving waters.

However, according to Schipper et al. (2010), the adverse effect potential of mitigation systems depends on the ratio of drainage- and receiving water discharge and on the nature and quality of receiving waters.

Possible adverse effects have to be investigated quantitatively in further studies to be able to compare their negative impact with the improvements, the measure offers in return in terms of contaminant attenuation.

Chapter 4

Conclusion and Recommendations

The conclusions and recommendations have been produced by the author and were partly published earlier in Krause Camilo et al. (2013) or are, in parts, accepted to be published in the *Ecological Engineering – The Journal of Ecosystem Restoration* (Krause Camilo (2015)).

Efficient mitigation systems should be designed to enhance denitrification as well as attenuation of herbicide agents. Nitrate (NO_3^-) is still the main relevant contaminant in agriculture in terms of biodiversity and drinking water production. But, the relevance of herbicide agents is increasing, due to, e.g. development of resistances in targeted herbs. The fate of herbicide agents in natural compartments is often incompletely understood and its contamination of raw waters, for drinking water use, is of growing concern.

The positive influence of organic carbon addition on NO_3^- attenuation is well known and comprehensively studied for low-flow conditions in subsurface flow or ground water. Resistant organic carbon sources, such as bark mulch, wood chips, saw dust, etc., are successfully tested and proven to be suitable for long-lasting denitrification in mitigation systems, under the premise of a steady, low discharge of drainage water. Unfortunately, periodic high flow events reduce drastically the overall efficiency of common mitigation systems. Thereby, the risk of higher contaminant loads and of bypassing untreated water is increasing. Under high flow conditions, the use of less resistant organic carbon sources, such as straw, weed, sugar cane, corncobs, etc., is necessary.

The experiments of the present study demonstrate that the combination of substrates with readily available organic carbon (e.g. straw) and substrates with stable structure and high effective porosity (e.g. bark mulch) are a good solution for effective denitrification under high flow conditions. The mixture of straw and bark mulch has the potential (i) to attenuate comparatively high loads of NO_3^- at low hydraulic residence times (HRT), even at low temperatures, and (ii) to keep high effective hydraulic conductivity for a long time.

Also, a supportive environment for effective removal of atrazine and isoproturon from solution seems to be provided. The two preferentially aerobically degraded herbicide agents, with high leaching potential, are substantially attenuated by adsorption and degradation under suboxic to anoxic conditions. It can be assumed that herbicide agents,

which belong to the same chemical groups as atrazine (triazines, e.g. simazine and terbutylazine) and isoproturon (phenylurea herbicide agents, e.g. diuron, chlortoluron and linuron), would be similarly attenuated in the organic substrates. Herbicide agents with chemical properties like bentazon, on the other hand, would not be affected in a straw-bark mulch bioreactor under conditions, as established in the experiment.

The finite fate of the herbicide agents, atrazine and isoproturon, in the bioreactors cannot be revealed in this study. Uncertainties remain about their grade of degradation and about the extent of metabolites in solution and on the matrix. At least, the diffuse herbicide agent contamination seems to be transformed into a punctual problem, which can be solved with less effort in following proceedings.

The utilization of organic substrates, as organic carbon sources for the denitrification process, involves the risk of disadvantageous side effects on the quality of downstream waters, e.g. oxygen demand, turbidity, odour and contamination with by-products, e.g. excess of organic carbon (dissolved and particular), microorganisms, nitrite and hydrogen sulphide. Hydrogen sulphide is a fish toxin and has the potential to hinder the formation of N_2 , by disturbing the denitrification process on the level of nitric and nitrous oxides. However, precautionary measures during planning and appropriate management of mitigation systems can minimize or prevent described disadvantages.

It can be assured that bioreactors are the most efficient designs for successful denitrification and herbicide agent attenuation in mitigation systems, as literature study and experiments have shown until the present time.

According to the experimental conditions (input: $100 \text{ mg-NO}_3^- \text{ L}^{-1}$, 25 to 30 $\mu\text{g-atrazine L}^{-1}$, 20 to 25 $\mu\text{g-isoproturon L}^{-1}$, water-saturated reactor, continuous flow) and results, a HRT of around 0.5 days would be an effective ratio between reactor volume and inflow in mitigation systems. Fifteen to 48% (9 to 21°C) of atrazine and 36 to 77% (5 to 17°C) of isoproturon were attenuated under these high flow conditions. Around 60% of the organic carbon that was traceably metabolized for respiratory means by microorganisms or washed out, was utilized for efficient denitrification. If oxygen and SO_4^{2-} concentrations in the inflow were lower the efficiency could increase up to 90%. Values of minimal and maximal relative denitrification between the temperatures of 9 and 21°C were 8 and 82%, respectively. The substrate age is very important for denitrification. In

fresher, less processed substrate, 45% of dosed NO_3^- were attenuated at a temperature of 5°C.

Table 4-1 to Table 4-3 show the expected mitigation system dimensions and accompanying contaminant attenuation performances on basis of technical macro scale results at HRT of around 0.5 days. Estimations refer to a 10 ha field. Concentrations of 100 mg- $\text{NO}_3^- \text{ L}^{-1}$, 27 μg -atrazine L^{-1} and 20 to 25 μg -isoproturon L^{-1} in the drainage water and a drainage coefficient of 12 mm were taken into account.

Table 4-1: Expected dimensions of straw-bark mulch bioreactors and nitrate attenuation (denitrification) on the basis of different temperatures and treated percentages of flow at HRT of 0.5 days (area: 10 ha, drainage coefficient: 12 mm^a, effective porosity in organic substrate: 80%, inflow concentration: 100 mg-nitrate L^{-1}).

Percentage of treated flow [%]	Volume of filter [m ³]	Fraction of field area for filter, when depth is 1 m [%]	Summer (water temperature: 21°C)		Winter (water temperature: 9°C)	
			Denitrification [kg-nitrogen day ⁻¹] ^b	Denitrification [%]	Denitrification [kg-nitrogen day ⁻¹] ^b	Denitrification [%]
100	750	0.8	23	82	2.5 to 19 ^c	8 to 59^c
50	375	0.4	11	41	1.3 to 10 ^c	4 to 30 ^c
25	188	0.2	6	20	0.6 to 5 ^c	2 to 15 ^c

^a Drainage coefficient: depth of water, a drainage system should remove from a field in 24 hour period; in humid climate zones without surface inflow, common values are between 6 and 18 mm for mineral and organic soils, respectively.

^b Under the premise of a discharge of 12 mm per day

^c Higher value results from addition of fresh substrate short before drop of temperatures

Table 4-2: Expected dimensions of straw-bark mulch bioreactors and atrazine attenuation on the basis of different temperatures and treated percentages of flow at HRT of 0.5 days (area: 10 ha, drainage coefficient: 12 mm^a, effective porosity in organic substrate: 80%, inflow concentration: 27 µg-atrazine L⁻¹).

Percentage of treated flow [%]	Volume of filter [m ³]	Fraction of field area for filter, when depth is 1 m [%]	Summer (water temperature: 21°C)		Winter (water temperature: 9°C)	
			Attenuation [g-atrazine day ⁻¹] ^b	Attenuation [%]	Attenuation [g-atrazine day ⁻¹] ^b	Attenuation [%]
100	750	0.8	17	48	8	15
50	375	0.4	8	24	4	8
25	188	0.2	4	12	2	4

^a Drainage coefficient: depth of water, a drainage system should remove from a field in 24 hour period; in humid climate zones without surface inflow, common values are between 6 and 18 mm for mineral and organic soils, respectively.

^b Under the premise of a discharge of 12 mm per day

Table 4-3: Expected dimensions of straw-bark mulch bioreactors and isoproturon attenuation, based on different temperatures and treated percentages of flow at HRT of 0.5 days (area: 10 ha, drainage coefficient: 12 mm^a, effective porosity in organic substrate: 80%, inflow concentration: 20 µg-isoproturon L⁻¹ (winter) and 25 µg-isoproturon L⁻¹ (summer)).

Percentage of treated flow [%]	Volume of filter [m ³]	Fraction of field area for filter, when depth is 1 m [%]	Summer (water temperature: 17°C)		Winter (water temperature: 5 °C)	
			Attenuation [g-isoproturon day ⁻¹] ^b	Attenuation [%]	Attenuation [g-isoproturon day ⁻¹] ^b	Attenuation [%]
100	750	0.8	16	77	8	36
50	375	0.4	8	39	4	18
25	188	0.2	4	19	2	9

^a Drainage coefficient: depth of water, a drainage system should remove from a field in 24 hour period; in humid climate zones without surface inflow, common values are between 6 and 18 mm for mineral and organic soils, respectively.

^b Under the premise of a discharge of 12 mm per day

HRTs lower than 0.5 days are critical for contaminant attenuation at low temperatures (5°C). With higher HRTs, the volume of the reactor or the bypass of untreated water is increasing. At HRT higher than one day, the risk of anoxic conditions with negative effects, as described above (and in detail in 3.2.2 Summary of technical macro scale

experiments), rises. At HRT higher than 2.0 days, the risk of complete denitrification is increased, with the result that the organic carbon source could be inefficiently utilized. Due to NO_3^- input limitations, only 33% of the organic carbon, which was traceably metabolized in respiratory processes or washed out during the bioreactor experiments, were used for denitrification.

Horizontal water-saturated bioreactors, with a homogeneous mixture of bark mulch and straw, could be implemented in the bank of surface waters at the tile drain outflow or in existing drainage swales. The design can also be adapted, according to site conditions. At steep slope with high water head difference between drainage outflow and receiving water, a vertical flow bioreactor, which needs less surface area, can be considered. In case, there is no area to implement the bioreactor on site, an in-stream reactor within the receiving water could be a solution (Robertson and Merkley (2009)). Preferable substrates would be bark mulch or comparable, under the premise that flow is constant and low. On condition that low and high flow events are alternating, the bioreactor could be built in layers with substrates of different characteristics. A bottom layer of a resistant organic carbon source, such as bark mulch, saw dust or wood chips, treats the base flow. In an upper layer, which consists of a mixture of labile (straw, sugar cane, reed, corn cobs) and resistant organic carbon sources (see above), the high flow would be processed. Due to relatively rapid depletion of the labile substrate under periodically unsaturated and wet conditions, the upper layer would need to be exchanged more frequently. The exchange interval depends on the loads of NO_3^- and competing electron acceptors and on climate conditions on site, which determine the degradation rate of the organic substrate under aerobic to anoxic conditions. Since the homogeneous mixture of the labile and resistant organic carbon sources could cause a technical problem, the substrates can be added in thin horizontal layers.

The addition of the readily accessible organic substrate should be accomplished in one of the following scenarios to minimize the effect of bioreactor-born contaminations or to optimize the attenuation of agriculture-born contaminants in receiving waters. When NO_3^- concentrations in drain flow are high, free organic carbon of fresh substrate is efficiently used by denitrification. When temperatures are low, fresh substrate still enables high denitrification. In the rainy season under high flow conditions, dilution of washed out organic carbon from the fresh substrate in the receiving water is higher.

Generally, the combination of surface wetlands and bioreactors should be considered. The efficiency of existing wetlands can be increased by implementation of a bioreactor downstream, which results in lower need of surface area or reactor volume for the wetland. In addition the negative effects of the bioreactor can be mitigated.

For example, in wetland studies HRT was in average 10 days and denitrification was in average $185 \text{ mg-nitrogen m}^{-2} \text{ day}^{-1}$ (Périllon and Matzinger (2010)). In the present bioreactor study at HRT of 0.4 days, minimal denitrification of $3.5 \text{ g-nitrogen m}^{-3} \text{ day}^{-1}$ at low temperatures (Table 3-7) translates into $875 \text{ mg-nitrogen m}^{-2} \text{ day}^{-1}$, taking into account a bioreactor height of 0.4 m. Wetlands without carbon addition need at least 5 times the surface area of the bioreactor for the same denitrification performance. Utilizing parts of the wetland to increase storage volume for the bioreactor would prevent bypass of untreated water. Furthermore, the bioreactor could be kept saturated to reduce decomposition of the organic carbon sources and to increase longevity of the bioreactor.

The interval of substrate exchange under saturated conditions can be exemplarily estimated under the premise of the following data. The annual groundwater recharge rate is 200 mm in Berlin (Germany). The average annual denitrification performance is around 50 % at HRT of 0.4 days. The average NO_3^- input is 100 mg L^{-1} . Five to 10 g of straw were needed to denitrify one gram of nitrogen (the present study, Soares and Abeliovich (1998)). Taking into account the bioreactor volume for 100% treated flow in Table 4-1, straw had to be refilled at best after 12 to 25 years.

However, enhanced sulphate reduction in times of NO_3^- input limitation, e.g. under low flow conditions, would shorten exchange intervals. Under unsaturated conditions, the decomposition of straw would be accelerated, due to higher oxygen input. According to results of the present study, it is recommended to add fresh substrate to the bioreactor every two years. Attenuation performance would benefit from adapted microorganisms in the established, remaining substrate and from fresh readily available organic carbon, especially under critical conditions, such as low temperatures or high discharge.

A surface wetland upstream could be useful for herbicide agent attenuation by aerobic and photolytic or hydrolytic processes as well as for sedimentation of eroded soil particles to prevent clogging of the bioreactor. A surface wetland downstream of the

bioreactor could improve the quality of the outflow by DOC reduction and oxygen enrichment.

As always, a successful design has to be adapted to site-specific conditions.

According to the results of the experiments in the present study, the amendment of conventional mitigation systems with organic substrate for efficient and simultaneous attenuation of NO_3^- and certain herbicide agents at short HRTs can be recommended. It has to be underlined that conditions in the experiment are worst-case considerations, since concentrations of the contaminants are above average and continuously added. In real systems, the longevity of the organic substrate, as efficient organic carbon source and as adsorbent, is expected to be higher.

On condition that the negative effects of bioreactors with organic substrates can be minimized, the studied design has the potential to be a valuable and effective tool in the set of best management practices, which reduce agricultural contamination in surface waters.

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Appendix

Literature review

Table Appendix 1: Nitrate attenuation (denitrification) and hydraulic residence time in field experiments in systems that mitigate agricultural pollution (Périllon and Matzinger (2010))

Type of mitigation system	Denitrification	Hydraulic residence time	Water temperature	Reference
	g-nitrogen m^{-3} of reactor day^{-1}	days	°C	
Surface flow wetland (without carbon addition)	0.37 ^a	9.5	n.a.	Review of 25 studies by Périllon and Matzinger (2010)
Horizontal subsurface flow system with straw	1.98	5 to 23	n.a.	de Haan et al. (2010)
Wood chip denitrification wall	2.3	n.a.	7 to 9	van Driel et al. (2006a)
Wood chip denitrification wall	0.62	2 to 32	10	Jaynes et al. (2008)
Wood chip upflow reactor	1.2 to 5	1 to 5.6	9 to 13	van Driel et al. (2006b)
Wood chip bioreactor in stream	0.33 to 6.66	0.8	3 to 14	Robertson and Merkley (2009)

^a under the premise, depth of the wetland would be 0.5 m.

Atrazine (laboratory scale)

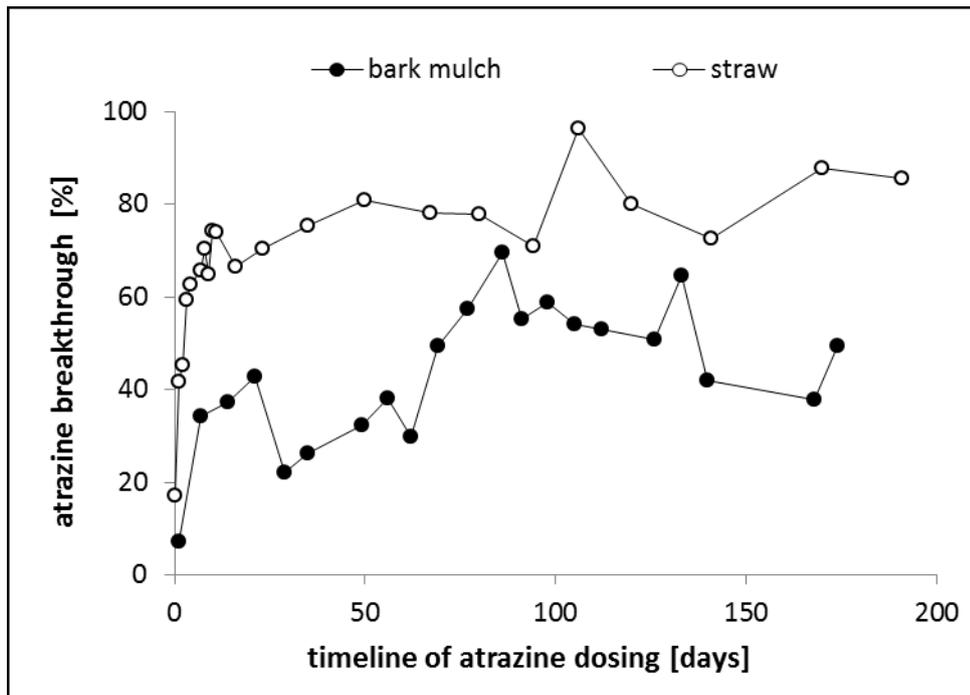


Figure Appendix-1: Breakthrough curves of atrazine in pure straw and in pure bark mulch at hydraulic residence time of 0.2 days.

Construction (technical macro scale)



Figure Appendix-2: Pictures from the construction site of the bioreactors – 1st part



Figure Appendix-3: Pictures from the construction site of the bioreactors – 2nd part

Hydraulic residence times (technical macro scale)

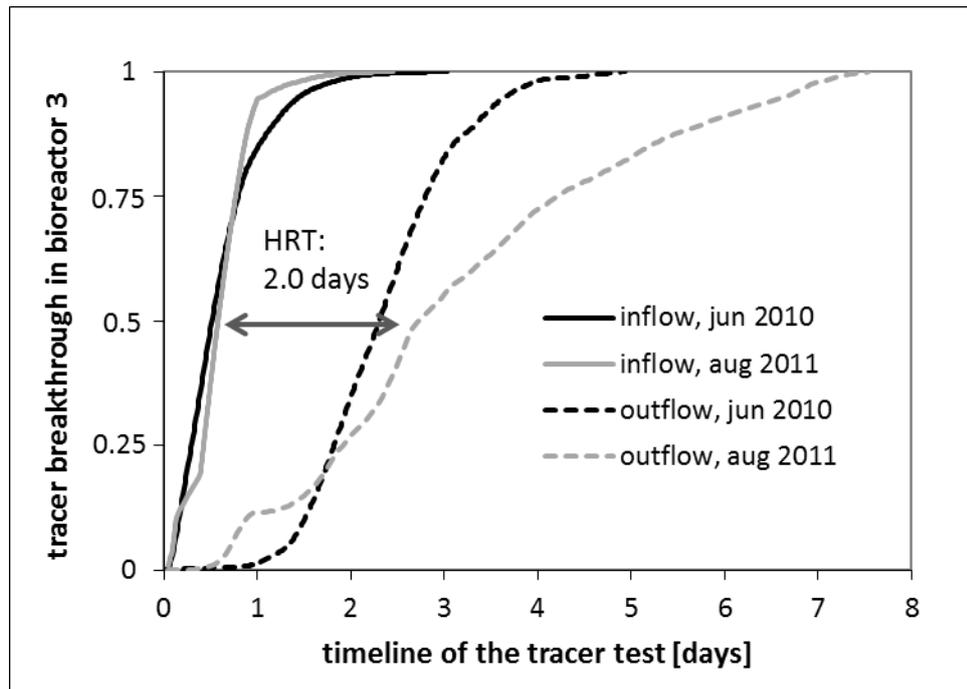


Figure Appendix-4: Estimation of hydraulic residence time (HRT) and structural stability in straw-bark mulch bioreactor 3 with tracer breakthrough curves at the inflow and outflow of the bioreactor at the start and at the end of the experiment.

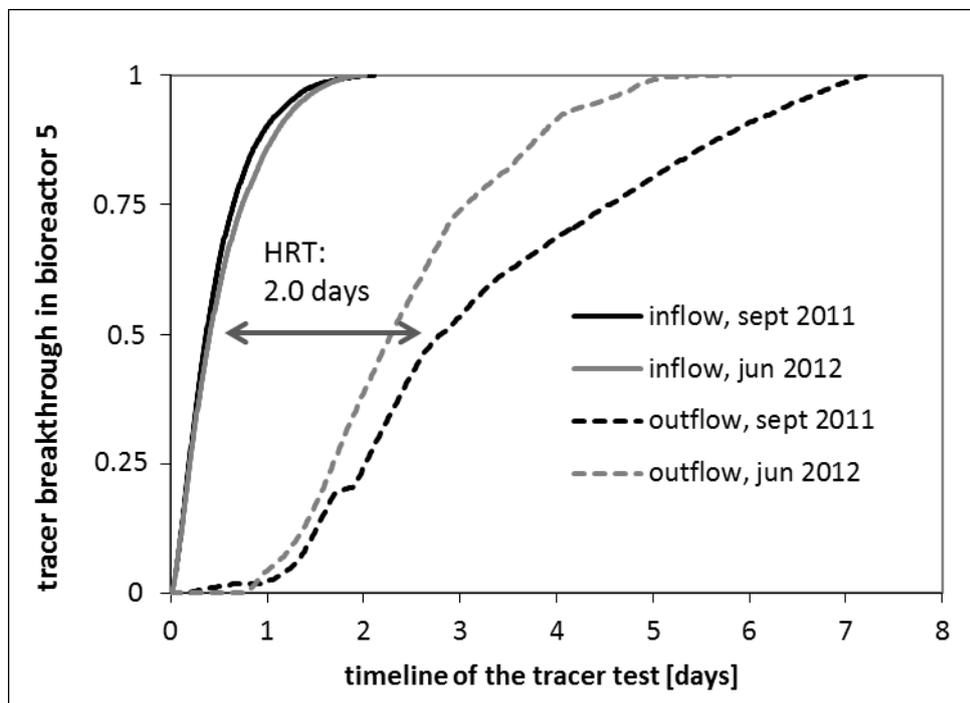
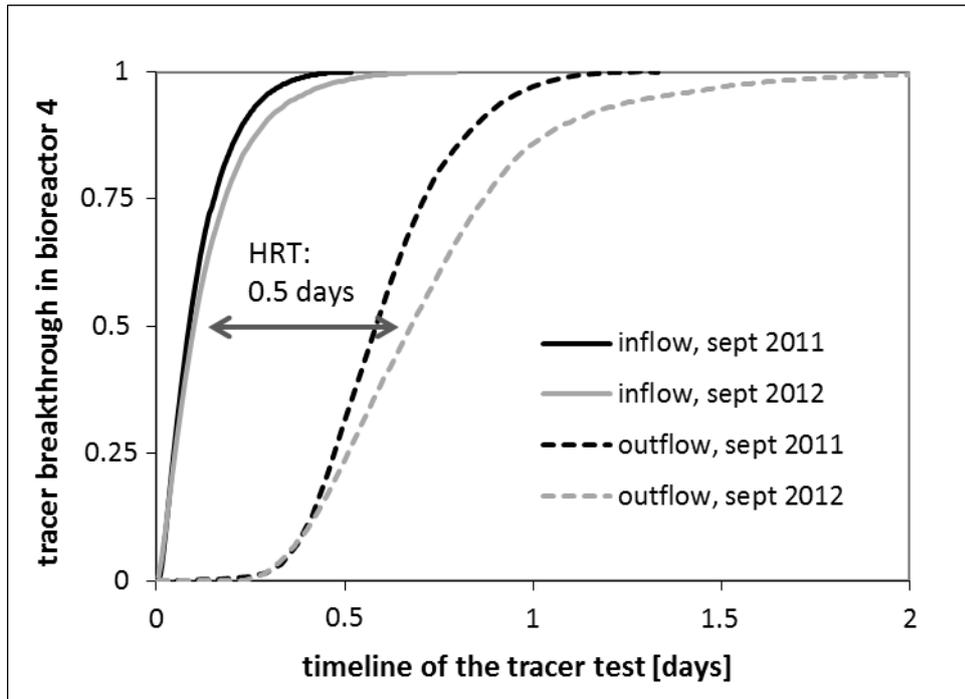


Figure Appendix-5: Estimation of hydraulic residence time (HRT) and structural stability in the straw-bark mulch bioreactors 4 and 5 with tracer breakthrough curves at the inflow and outflow of the bioreactors at the start and at the end of the experiment.

Atrazine (technical macro scale)

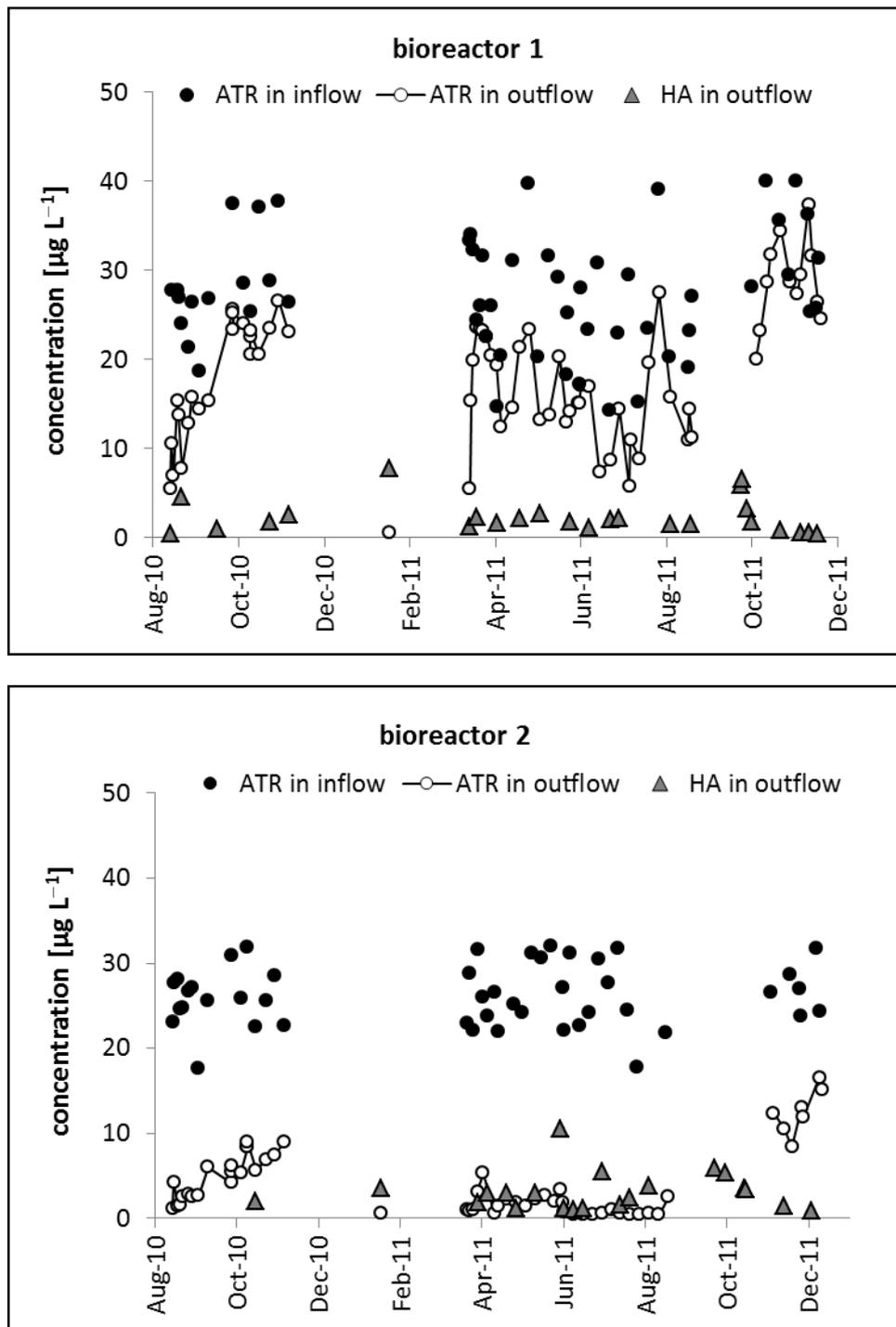


Figure Appendix-6: Inflow- and outflow concentrations of atrazine (ATR) and outflow concentrations of hydroxy-atrazine (HA) in the bioreactors 1 and 2.

Isoproturon (technical macro scale)

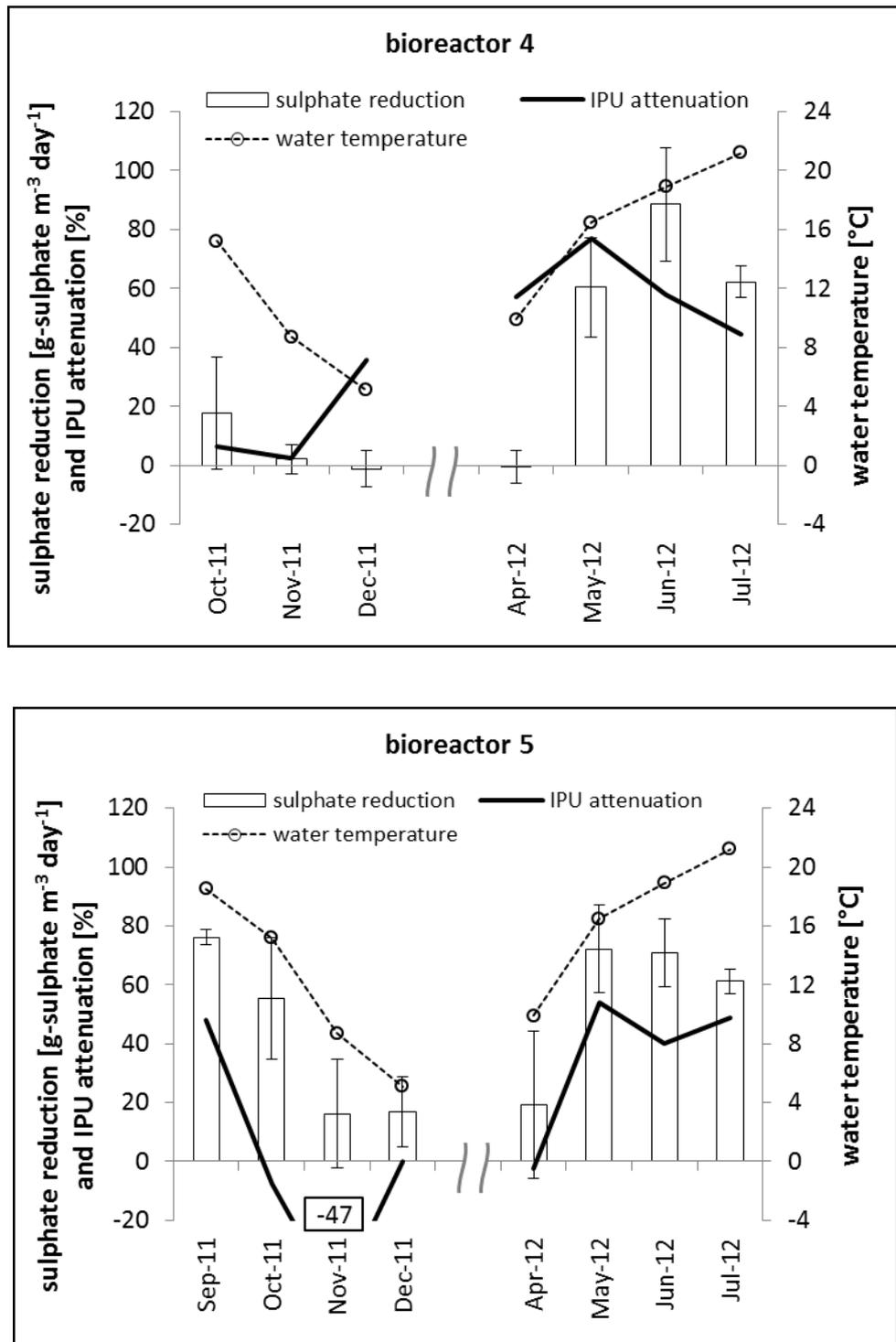


Figure Appendix-7: Relation of isoproturon (IPU) attenuation, water temperature and redox conditions. Dissimilatory sulphate reduction represents redox conditions. No to low dissimilatory sulphate reduction indicates moreover suboxic conditions. High dissimilatory sulphate reduction indicates predominant anoxic conditions.

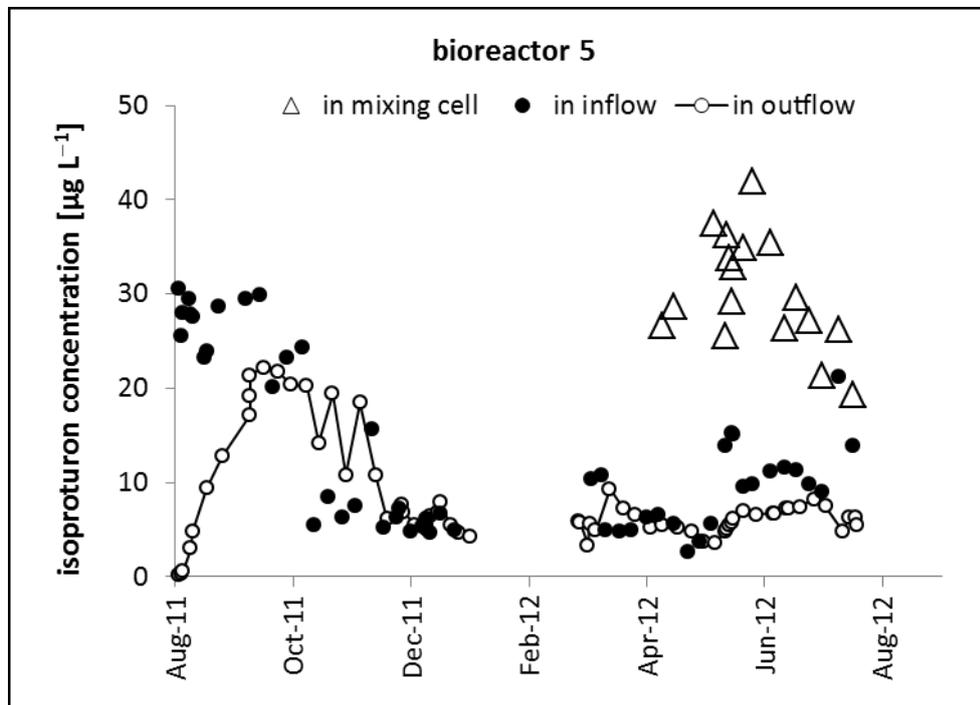
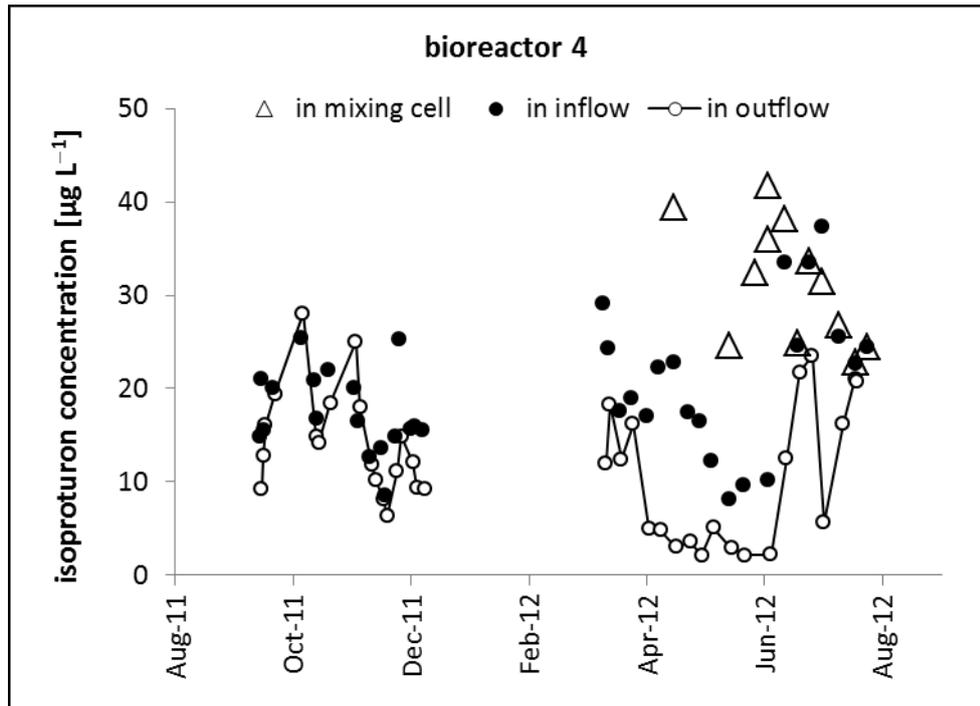


Figure Appendix-8: Inflow- and outflow concentration of isotroturon at hydraulic residence times of 0.5 and 2.0 days in the bioreactors 4 and 5, respectively.

Nitrate (technical macro scale)

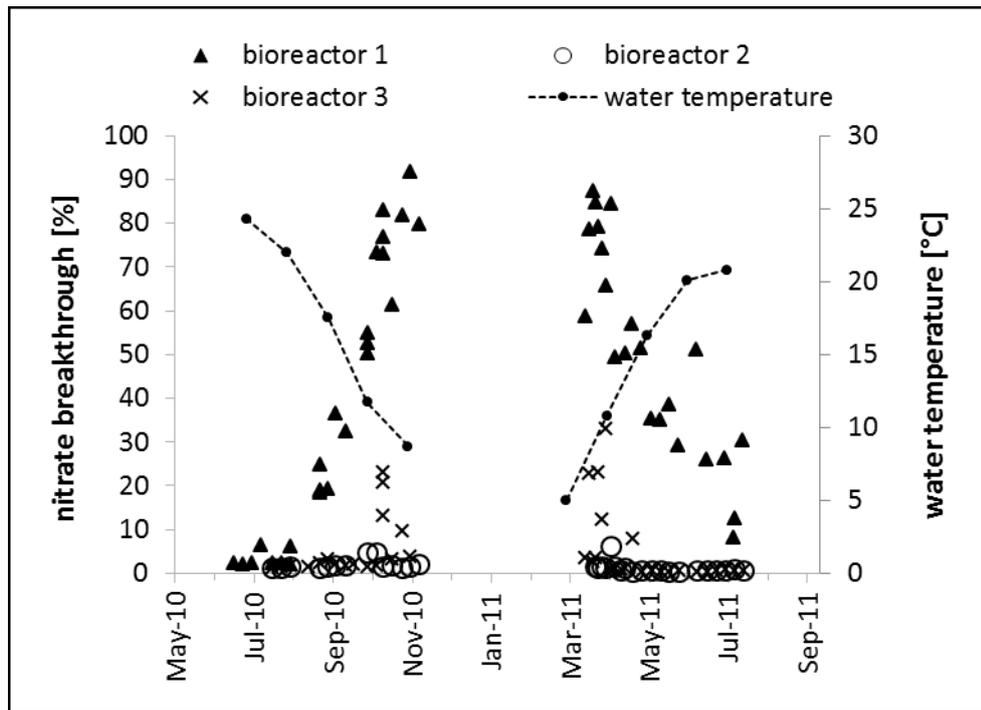


Figure Appendix-9: Breakthrough curves of nitrate in the bioreactors 1 and 2 (with atrazine) at hydraulic residence times of 0.4 and 2.5 days, respectively, and in bioreactor 3 (without atrazine) at hydraulic residence time of 2.0 days.

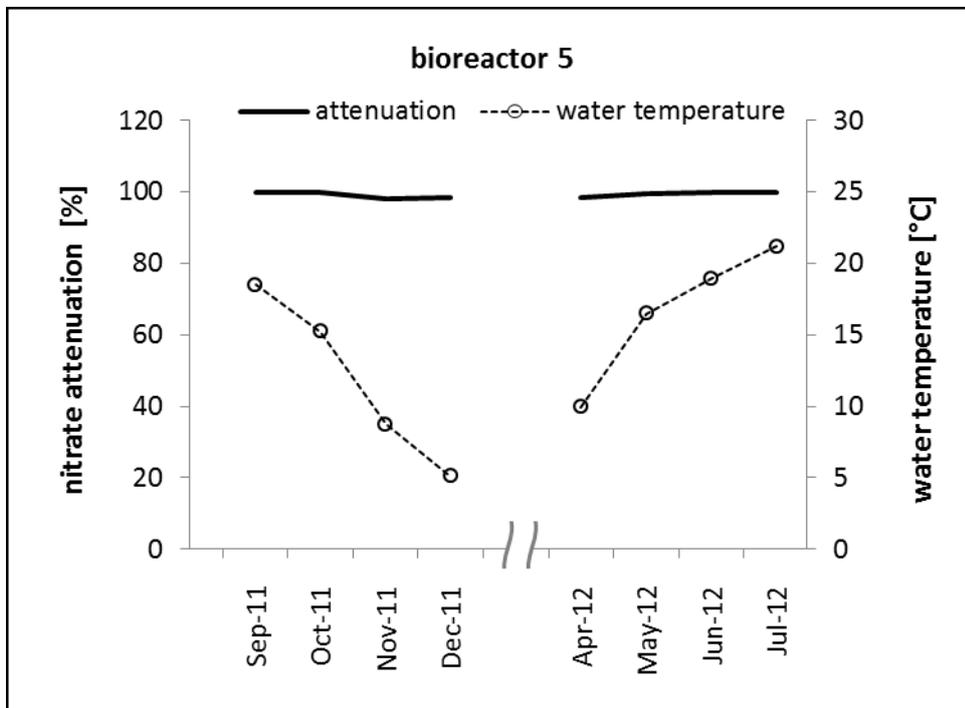
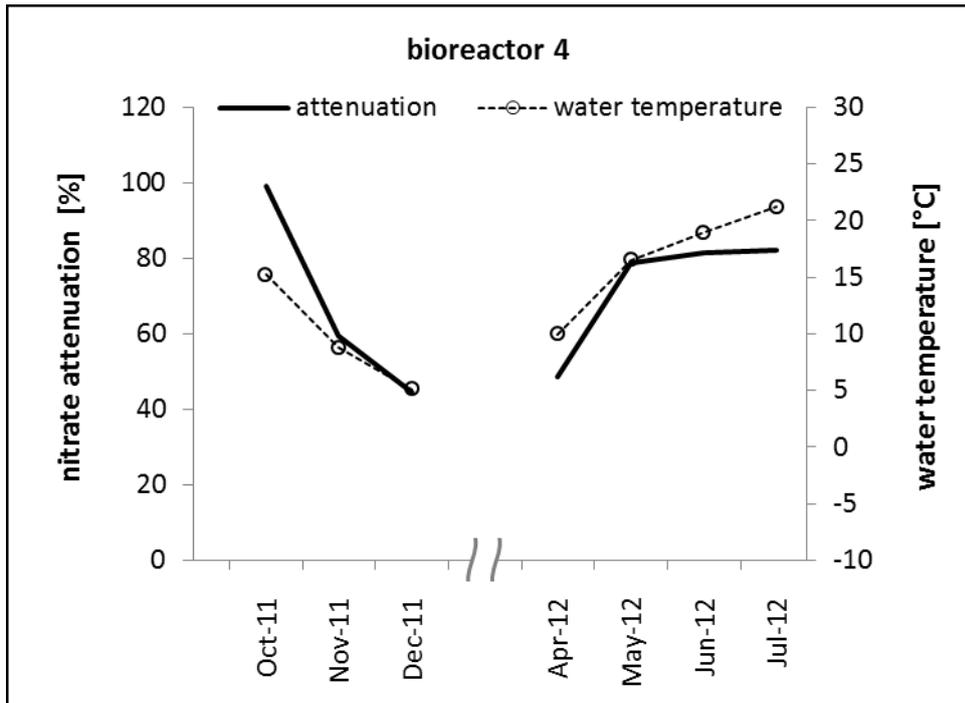


Figure Appendix-10: Relative nitrate attenuation/denitrification (ratio of attenuation and inflow) in the bioreactors 4 and 5 with hydraulic residence times of 0.5 and 2.0 days, respectively.

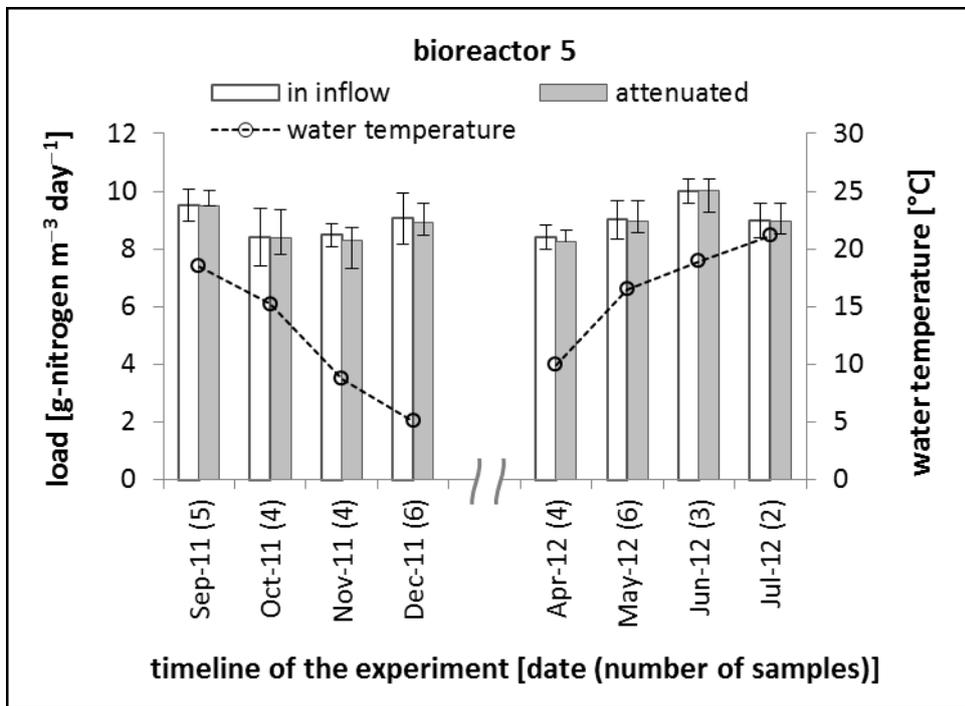
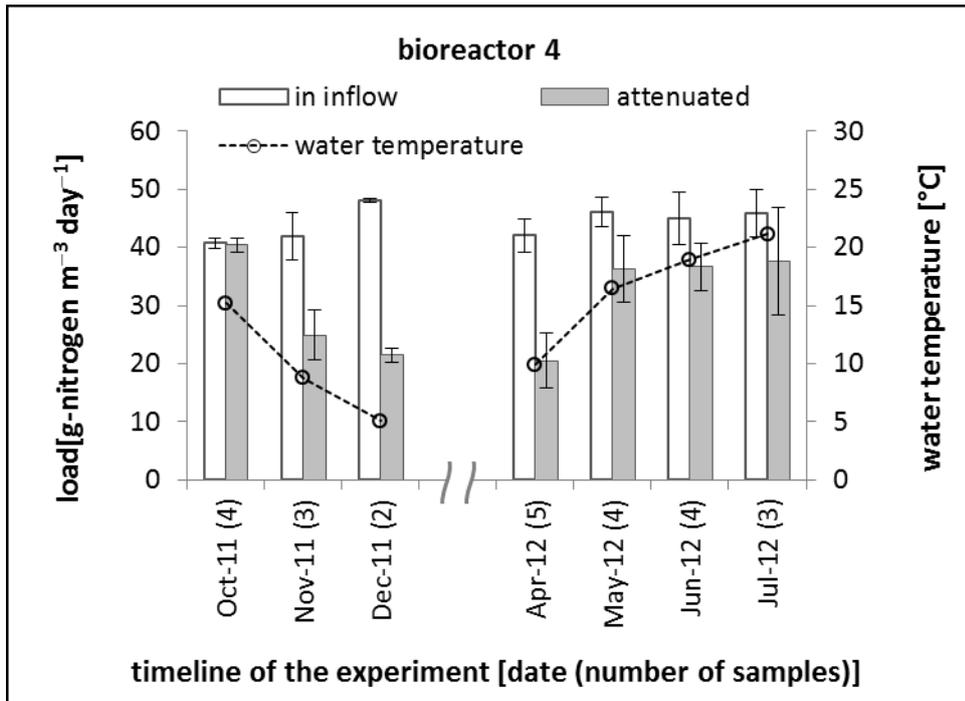


Figure Appendix-11: Absolute nitrate attenuation/denitrification (attenuation of loads) in the bioreactors 4 and 5 with hydraulic residence times of 0.5 and 2.0 days, respectively.

Redox potential (technical macro scale)

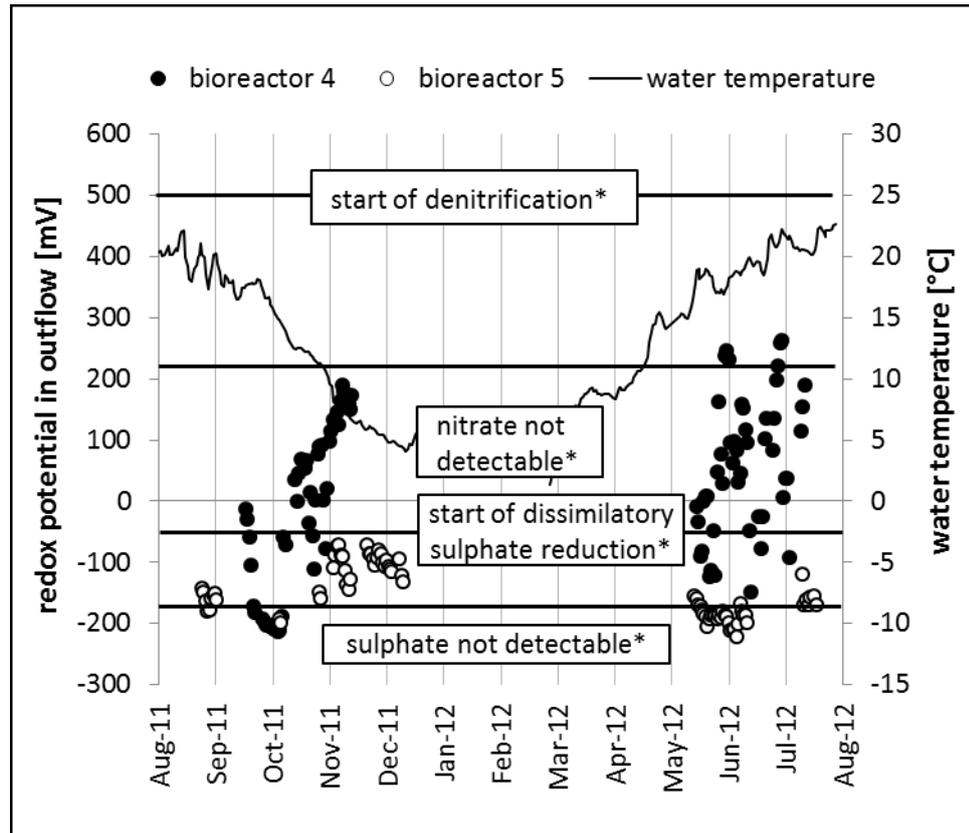


Figure Appendix-12: The redox potential in the outflow of the bioreactors 4 and 5 as indicator for nitrate attenuation (denitrification) at hydraulic residence times of 0.5 and 2.0 days, respectively (*experimental limits for different redox reactions at neutral pH value by Kretzschmar (2010)).