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Department of Environmental Engineering

PhD Thesis
September 2013

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Preface

This PhD thesis is based on work carried out at the Department of Environmental Engineering at the Technical University of Denmark in the period from August 2009 to June 2013. The thesis was funded by the GOODWATER project of European Commission's Marie Curie Initial Training Network for young scientists under grant no. 212683. The project was supervised by Professor Hans-Jørgen Albrechtsen and Professor Poul L. Bjerg.

The PhD thesis comprises a synopsis of the work and three scientific papers. In the synopsis, the papers are referred to by roman numbers (e.g. **Paper I**).

I. Levi, S., Hybel, A. M., Bjerg, P. L., Albrechtsen, H. J., 2013. Stimulation of aerobic degradation of bentazone, mecoprop and dichlorprop by oxygen addition to aquifer sediment. Submitted to Environmental Pollution.

II. Levi, S., Jepsen, T. S., Bjerg, P. L., Albrechtsen, H. J., 2013. Potential for aerobic biodegradation of bentazone in aquifer sediments. Submitted to Pest Management Science.

III. Levi, S., Qiu, S., Bjerg, P. L., Elsner, M., Albrechtsen, H. J., 2013. Anaerobic degradation of dichlorprop in landfill leachate affected groundwater based on laboratory batch experiments. Technical note.

During the PhD study, the work was presented at international meetings and conferences. This has led to the following two abstracts:

Levi, S., Hybel, A.M., Bjerg, P.L. and Albrechtsen, H.-J. Aerobic degradation potential of the herbicides mecoprop, dichlorprop and bentazone in groundwater from chalk aquifers. IWA Specialist Groundwater Conference, September 8 – 10, 2011, Belgrade, Serbia. Conference article in proceeding and oral presentation.

Levi, S., P.L. and Albrechtsen, H.-J. Effects of biodegradation of mecoprop, dichlorprop and bentazone by changing the redox conditions from anaerobic to aerobic in sandy aquifer. 8th International Symposium of Subsurface Microbiology, September 11 – 16, 2011, Garmisch-Partenkirchen, Germany. Conference article in proceeding and poster presentation.

In this online version of the thesis, the papers are not included but can be obtained from electronic article databases e.g. via www.orbit.dtu.dk or on request from:

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Thanks to my co-authors, my colleagues, office mates, friends and all the people from administration and reception from DTU Environment.

A huge hug goes to my friends Chiara, Gamze, Karolina, Elham, Ursula, Patricia, Anne-Marie, Ana, Odell, Gizem, Thomas, Katerina and friends outside of Denmark for bringing love and laughs to my life.

Very special thanks to Erkin for your support and love and all my family for their encouragement. Finally, I dedicate my work to my uncle, Moiz Abensoa. You will always be remembered wherever you are now.

July 2013

Suzi Levi

Abstract

Groundwater contamination by pesticides is a widespread environmental problem and a major threat to drinking water supplies. Diffuse source contamination of groundwater that enters from an extensive area is characterized by low pesticide concentrations (nanogram-microgram per liter) in large volumes of water. It is regarded as one of the major threats to groundwater quality originating from agriculture, roads and railways. These large volumes of water in combination with the low concentration cause difficulties in preventing contamination of drinking water supplies and this is a challenge to develop remediation solutions.

Abstraction fields often include several wells. Even if only one of the wells is contaminated, this water mixes with uncontaminated groundwater from the other wells and causes excessive volumes of water to be treated at the waterworks. An alternative approach to the treatment of contaminated groundwater at waterworks is enhanced *in situ* aerobic bioremediation in the anaerobic aquifers at the contaminated well. This may be achieved by increasing the oxygen content in aquifers with pumping strategies in or around drinking water abstraction wells. With that approach, aerobic groundwater from the upper part of the aquifer may mix with deeper anaerobic groundwater, increasing the oxygen content and creating favorable conditions for biodegradation. However, factors such as oxygen consumption related to organic matter, reduced inorganic species present in the sediment and precipitation of iron followed by the growth of iron bacteria leading to bioclogging reduces the efficiency of bioremediation of contaminated aquifers.

The overall scope of this PhD study was to investigate biostimulated degradation potentials of pesticides at low concentrations in groundwater contaminated by diffuse sources in or around water abstraction fields. This approach could lead to more efficient *in situ* remediation solutions and protection of groundwater as a drinking water supply.

Herbicides are generally expected to be difficult to be degraded under anaerobic conditions, but prone to biodegradation under aerobic conditions. Laboratory batch experiments were conducted with anaerobic aquifer material and groundwater collected near an operating drinking water abstraction field to study the potential for stimulating biodegradation of pesticides (bentazone, mecoprop and dichlorprop) at environmentally relevant concentrations (1 $\mu g\ L^{-1}$) by addition of oxygen. Addition of oxygen stimulated mineralization not only at

high oxygen concentrations but also at substantially lower concentrations (< 2 mg L^{-1}). Biostimulation in terms of enhanced oxygen concentrations around abstraction well fields was found to be a potential remediation solution for pesticides. Furthermore, bentazone mineralization was first time found in aquifer sediments at low oxygen concentrations.

Enhanced biostimulation by adding nitrate or nutrients was also seen as potential technologies, however, in the case of nitrate, it was suggested that a mixed oxygen/nitrate system could be used for the contaminants to be degraded under microaerophilic conditions.

A literature review regarding degradation of bentazone in topsoils and aquifer sediments supported laboratory experiments that were performed with groundwater and aquifer sediment. Bentazone was degradable in aquifer sediments under aerobic conditions. Furthermore, we were able to transfer bacteria with the capability to degrade bentazone over a wide range of bentazone concentrations from the aquifer material to laboratory experiments.

Laboratory microbial studies, aiming to support the observations of transformation of dichlorprop to 4-CPP by different analytic methods at an old landfill, were conducted to investigate anaerobic degradation of ^{14}C-labeled dichlorprop. Concentrations of dichlorprop and 4-CPP with groundwater from the same field site under anaerobic conditions were also measured by analytical chemical analysis. Dichlorprop was recalcitrant in groundwater samples and 4-CPP concentration remained constant during the incubation period. This illustrates the complexity of the field site, characterized by clay-till, with varying redox conditions and residence times providing a higher degradation potential in the field than could be transferred to the laboratory.

In conclusion, this PhD has developed our understanding on degradation processes of pesticides in aquifer systems. We have shown that biostimulation by oxygen addition even at relatively low concentrations is a promising remediation technology for groundwater contaminated by pesticides at abstraction well fields. The results of this thesis contribute to our understanding of the development of treatment strategies to protect drinking water wells and suggest an alternative solution to closure of abstraction wells due to pesticide contamination.

Dansk resumé

Grundvandsforurening med pesticider er et udbredt miljøproblem og en trussel mod vandforsyningen. Grundvandsforurening fra diffuse kilder er kendetegnet ved lave koncentrationer (nanogram-mikrogram per liter) og store vandvoluminer. Denne type forurening, der stammer fra landbrug, veje og jernbaner, betragtes som en af de største trusler mod grundvandskvaliteten. De lave forureningskoncentrationer i kombination med de store mængder vand gør det vanskeligt at forebygge forurening af drikkevandsforsyninger og skaber store udfordringer for udviklingen af afværgeteknikker.

Kildepladser omfatter ofte flere boringer. I tilfælde hvor kun én af boringerne er forurenet, vil det forurenede vand efter oppumpning blive blandet med uforurenet grundvand fra de andre boringer, under transporten til vandværket. Et alternativ til behandling af forurenet grundvand på vandværker er stimuleret *in situ* aerob bioremediering i de anaerobe magasiner i indvindingsområdet. Dette kan muligvis opnås ved at øge iltindholdet i grundvandsmagasinerne vha. smarte pumpestrategier. Med denne fremgangsmåde kan aerobt grundvand fra den øvre del af grundvandsmagasinet blandes med dybere anaerobt pesticidforurenet grundvand, hvormed iltindholdet øges og der skabes gunstige betingelser for bionedbrydning. Men faktorer som iltforbrug relateret til organisk stof, reducerede uorganiske forbindelser i sedimentet og udfældning af jern efterfulgt af vækst i jernbakterier, der forårsager bioclogging, reducerer effektiviteten af bioremediering af forurenede grundvandsmagasiner.

Det overordnede formål med dette PhD-studie var at undersøge potentialet for biostimuleret nedbrydning af pesticider ved lave koncentrationer i grundvand ved kildepladser, der er forurenet via diffuse kilder. Denne fremgangsmåde kan potentielt føre til mere effektive *in situ* remedieringsløsninger og bedre beskyttelse af grundvandet.

Herbicider forventes i almindelighed at være vanskeligt nedbrydelige under anaerobe forhold, hvorimod mulighederne for nedbrydning er væsentligt højere under aerobe forhold. Batcheksperimenter blev udført i laboratoriet med grundvand og sediment fra et anaerobt magasin i nærheden af en aktiv kildeplads, for at undersøge mulighederne for stimuleret bionedbrydning af pesticider (bentazon, mechlorprop og dichlorprop) ved miljørelevante koncentrationer (1 µg L^{-1}) via tilsætning af ilt. Tilsætning af ilt stimulerede mineralisering selv ved relativt lave koncentrationer (<2 mg L^{-1}). Biostimulering i form af forhøjet iltkoncentration omkring kildepladser blev identificeret som en

potentiel afværgeteknologi for pesticider. Desuden har vi for første gang rapporteret bentazon-nedbrydning i grundvandssediment ved lave iltkoncentrationer.

Stimuleret bioremediering ved tilsætning af nitrat eller næringsstoffer blev også betragtet som potentielle afværgeteknologier, men mht. nitrat blev det foreslået, at et blandet ilt/nitrat-system kan anvendes til at nedbryde de forurenende stoffer under mikroaerofile betingelser.

Bentazon blev nedbrudt i grundvandssediment efter tilsætning af ilt. Desuden var vi i stand til at overføre bakterier fra grundvandssediment hvor bentazon blev nedbrudt, til en anden forsøgsopsætning, hvor de kunne nedbryde et bredt spektrum af bentazonkoncentrationer.

For at understøtte observationer med forskellige analytiske metoder af omdannelse af dichlorprop til 4-CPP, udført på en gammel losseplads, blev potentialet for anaerob nedbrydning af 14C-mærket dichlorprop undersøgt ved mikrobiologiske laboratorie-undersøgelser med anaerobt grundvand fra samme feltlokalitet. Dichlorprop var var svært nedbrydeligt i grundvandsprøverne og 4-CPP koncentrationen forblev konstant i inkubationstiden. Dette illustrerer de komplekse forhold på feltlokaliteten, hvor varierende redoxforhold og opholdstider kombineret med heterogene geologiske forhold (fx moræneler) resulterede i et højere nedbrydningspotentiale end det var muligt at overføre til laboratoriet.

Samlet set har dette PhD-projekt udviklet vores forståelse af pesticiders nedbrydnings-processer i grundvandsmagasiner. Vi har vist, at biostimulering ved tilsætning af ilt selv ved relativt lave koncentrationer er en lovende afværgeteknologi for kildepladser, hvor grundvandet er forurenet med pesticider. Resultaterne fra denne afhandling bidrager til vores forståelse af remedieringsstrategier til beskyttelse af drikkevandsboringer og foreslår således en alternativ løsning til lukning af indvindingsboringer som løsning på problemer med pesticidforurening.

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1 Introduction

1.1 Background and motivation

Groundwater is an extremely important source for drinking water production in many countries including Denmark, where it undergoes a simple treatment consisting of aeration and rapid sand filtration before distribution to the consumers (Jørgensen and Stockmarr, 2009). Therefore, groundwater protection is a matter of great concern. Pesticides are a group of xenobiotic organic compounds that, despite their benefits, may pose a potential threat to the quality of groundwater and other water resources (Scheidleder et al., 1999; Pazos et al., 2003; Schipper et al., 2008). The presence of pesticide residues in soil and water is a potential risk for both humans (Otto et al., 2007) and aquatic ecosystems (Dabrowski and Schulz 2003; McKnight et al., 2012). The frequent detection of pesticides in Danish groundwater has led to the close-down of drinking water wells (Miljøstyrelsen, 2011).

Pesticides with high water solubility, low tendency to sorb to the soil and low degradability have the highest potential for leaching through the soil into the subsurface and thereby causing groundwater contamination (Navarro et al., 2007). This contamination arises from various sources e.g. agricultural use, spraying of private gardens and public areas, landfills, machine pools, market gardens and accidental spills, which are diffuse sources and point sources (Freeze & Cherry, 1979; Christensen et al., 2000; Milosevic et al., 2012). Diffuse sources are characterized by relatively low pesticide concentrations in large volumes of water that enter the environment from an extensive area, whereas point sources comprise relatively high concentrations in smaller volumes of water and enter the environment from a localized site. These characteristics make development of remedial actions for pesticides in groundwater from diffuse sources difficult.

Pesticides and their residues from diffuse sources are generally detected at low concentrations, usually in nano- to microgram per liter concentration range, in contaminated aquifers (Kolpin et al., 2000; Thorling et al., 2012). The fate, degradation potential and remediation of pesticides at high concentrations (50-100 μg L^{-1}) are relatively well studied in laboratory and field experiments (Tuxen et al, 2006; Broholm et al., 2001). However, assessment of degradation at environmentally relevant low concentrations (0.1-1 μg L^{-1}) as those commonly found in groundwaters is more difficult and still needed (Gozdereliler, 2012). The detailed knowledge on microbial processes and degradation potentials at low concentrations is important in order to allow for a better and efficient remediation strategy to remove pesticides in aquifers.

Besides the physiochemical properties of the pesticides and redox conditions, different processes such as dilution, transport, dispersion, sorption and abiotic and biotic degradation may also affect the distribution and concentration of pesticides in soil and subsurface environments. However, biodegradation, the microbially facilitated process, is the dominant process for complete removal of contaminants from the environment (Alexander, 1981). Although many organic contaminants are biodegradable under both aerobic and anaerobic conditions in contaminated subsurface environments, degradation potentials are higher under aerobic conditions since anaerobic rates of metabolism are slower than aerobic rates (Vink and van der Zee, 1997; Bondarenko and Gan, 2004; Charnay et al., 2005). In addition, less energy is available for ATP production in anaerobic processes compared to aerobic processes (Champ et al., 1979).

The conventional treatment to remove pesticides at waterworks is filtration by granular activated carbon (Heijman et al., 2002). Biological removal of pesticide was also shown in rapid sand filters at waterworks (Corfitzen et al., 2009; Hedegaard, 2012; Zearley and Summers, 2012). Treatment by granular activated carbon can produce high water quality. However different water qualities (e.g. with or without pesticides) that are pumped from different wells will mix before the treatment at the waterworks (Figure 1). This may result in (i) contamination of clean water which is not contaminated with pesticides at wells, (ii) treating excessive volumes of water, (iii) lowering pesticide concentrations by dilution which may challenge the remediation process. An alternative approach is the remediation of the water in contaminated wells before it reaches to waterworks. Enhanced *in situ* aerobic bioremediation can be a method of degrading pesticides in groundwater. This can be achieved by "biostimulation" where addition of nutrients and oxygen or other electron acceptors are applied in order to stimulate microbial growth (Vidali, 2001). Biostimulation techniques for treating contaminated aquifers from a point source with high concentrations of organic contaminants such as pesticides have been shown by Tuxen et al. (2006). Nevertheless, detailed knowledge on the bioremediation of pesticides at low concentrations in aquifers resulted from diffuse source is scarce. A thorough understanding of the behavior of pesticides under different redox conditions present in aquifers and their interaction with amendment of different electron acceptors may create a solid basis for biostimulation of pesticide degradation in subsurface environments. With that perspective, the framework of research focus is on the biostimulated degradation processes for contaminants in transition zones where different water type mixes and regarded as a place of high microbial activity due to steep redox gradients between anaerobic and aerobic groundwater.

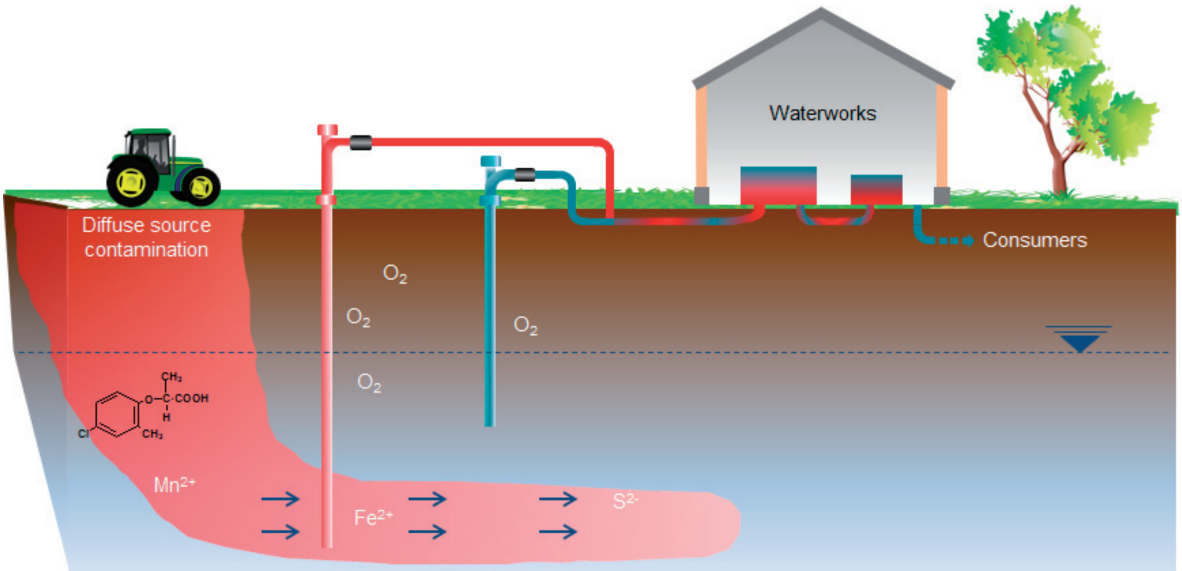


Figure 1. A sketch showing different water qualities which are contaminated (red) and uncontaminated (blue) that pumped from different wells are mixing at the waterworks before distributed to the consumers.

In this thesis, the herbicides mecoprop (RS) 2-(2-methyl-4-chlorophenoxy) propionic acid, dichlorprop (RS)-2-(2,4-dichlorophenoxy) propionic acid) and bentazone (3-isopropyl-1*H*-2, 1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide) were used as model compounds. The three specific herbicides with different physical and chemical properties were selected due to their frequent use worldwide and common detection in groundwater at the selected field site in Denmark. Bentazone was chosen due to the strong need for more information as the literature regarding potential degradation of bentazone in aquifer materials is very limited. Two phenoxy alkanoic acids; mecoprop and dichlorprop were chosen based on the detailed knowledge of their fate in aquifers.

1.2 Objectives and research questions

The **overall aim** of this PhD study was to investigate degradation potential and assess feasible bioremediation solutions for low contaminant concentrations arising from diffuse sources in or around water abstraction wells by enhancing oxygen levels in the aquifers with smart pumping strategies. Achieving and implementing this approach may lead to a better drinking water quality that reaches to the waterworks before distribution to the consumers. Furthermore, a specific objective for bentazone, was to get an overview of the published literature regarding its degradation in topsoils and particularly in aquifer materials and to investigate bentazone degradation in sandy and chalk aquifer sediments through laboratory batch experiments.

The main research questions addressed in the thesis are:

i. What is the degradation potential for the selected pesticides with and without oxygen addition at different concentrations in sandy aquifer material and in groundwater? How do the increased oxygen concentrations affect the degree of mineralization?

ii. Is it possible to enhance pesticide degradation in aquifers before contaminated groundwater reaches to waterworks? Is oxygen stimulation a feasible bioremediation technology for pesticide-contaminated groundwater instead of implementation of traditional treatment?

iii. Does addition of nitrate to anaerobic aquifer material have a positive effect on pesticide biodegradation?

iv. Is bentazone, one of the persistent compounds in subsurface environments, degradable in aquifer materials under aerobic and anaerobic conditions?

v. Is it possible to transfer observed degradation potential for dichlorprop in the field to the controlled laboratory experiments?

2 Groundwater contamination

Groundwater is an important natural resource for the human society. It plays an important role as a water supply for drinking water production as well as for irrigation and industrial purposes. In US, 75% of water supply is derived from groundwater (Zektser and Everett, 2004) whereas; in Europe 70% and in countries like Austria and Denmark drinking water is entirely provided through groundwater (Navarrete et al., 2008). Access to clean drinking water from public supply is one of the biggest challenges of the coming decades. This requires high groundwater quality that must meet standards with respect to micropollutants such as pesticide concentration above the threshold value of 0.1 $\mu g\ L^{-1}$ set by the EC Directive (2006/118/EC).

2.1 Groundwater contamination by pesticides

Contamination of groundwater by pesticides, one of the largest groups of xenobiotic compounds, is a widespread environmental problem. Extensive use of pesticides in agriculture that are in some instance persistent increases the risk of leaching into underlying aquifers. Their presence in groundwater may be a major threat to drinking water supply, especially in countries where groundwater often constitutes important drinking water sources. Worldwide concern of growing stress on groundwater quality has led to an increased emphasis on a better understanding of groundwater contamination. Pesticides and their residues are detected in groundwater typically in the concentration range of micrograms per liter (Kolpin et al., 1997; 2000). A survey of groundwater by the United States Geological Survey showed that herbicides were detected in about 50% and residues were detected in about 75% of the wells sampled (Kolpin et al., 1998).

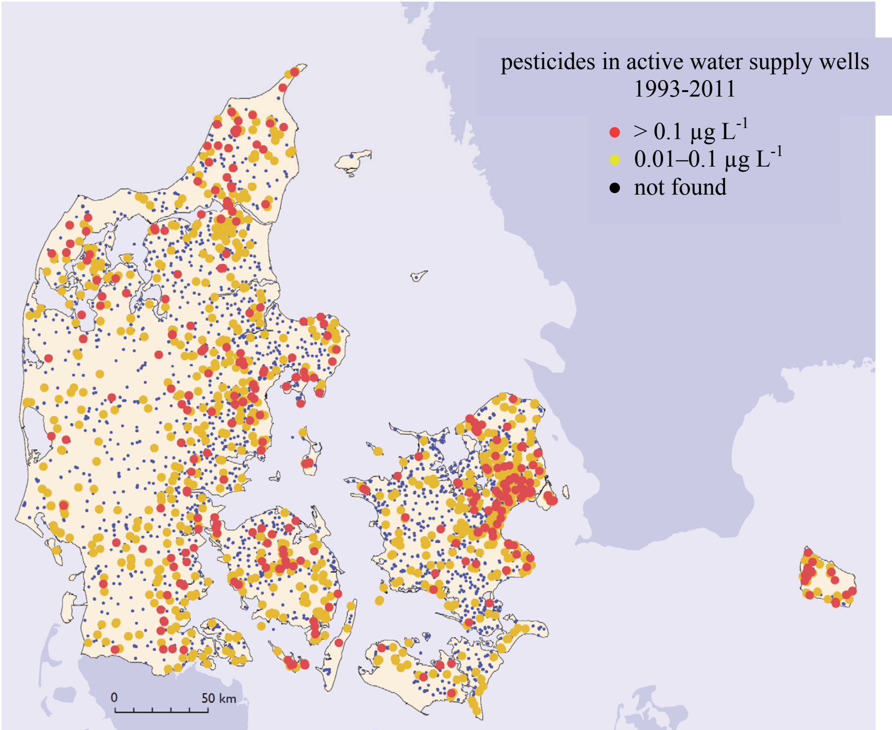
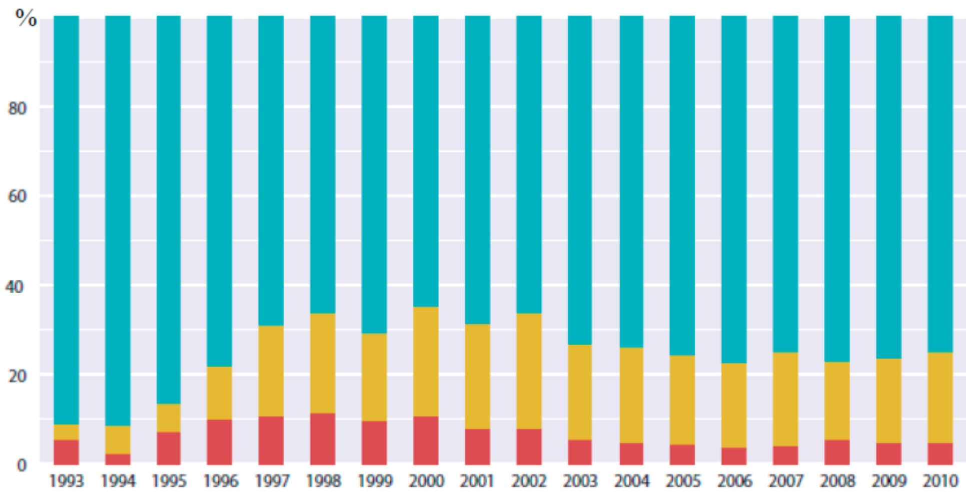


Figure 2. Detection of pesticides and their metabolites in active water supply wells during 1993-2011. Wells are included in map if pesticides or metabolites were found at least once in the well during that period (Thorling et al., 2012).

Increasing number of pesticides has been detected in groundwaters in several countries e.g. in the United Kingdom (Reid et al., 2003), in the United States (Gilliom et al., 2007) and in Europe (Thorling et al., 2012). In an ongoing Danish Groundwater Monitoring Programme, pesticide residues were detected in 40% of monitoring wells and 23% of the active waterworks wells. Figure 2 shows findings of pesticides in Danish water supply wells during 1993-2009.

In 2009, pesticides were detected in 19% of the active Danish drinking water wells and the maximum allowable concentrations for drinking water which is 0.1 µg/L in EU (EC, 2006) was exceeded in 4.3% of the wells (Thorling et al., 2012). Their occurrence in Danish groundwater has massively increased since 1993 and this has resulted in the closure of many drinking water wells. On average, 150 wells are closed each year due to pesticide contamination (Brüsch, 2007). The three selected pesticides (bentazone, mecoprop and dichlorprop) in this thesis are among the most frequently detected compounds in the groundwater monitoring program between 1999 and 2011 (Thorling et al., 2012). The distribution of pesticides in monitoring wells between 1993 and 2011 (Figure 3) highlights the detection of pesticides at low concentrations (0.01 – 0.1 µg L^{-1}) being one of the major problems in Denmark.



	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
no findings	130	2007	879	750	930	1193	1426	1202	1182	859	1211	1247	1238	1024	1075	1144	1200	1270
0,01–0,1 µg/l	5	139	66	115	271	406	396	452	403	335	350	358	327	251	301	259	297	337
>0,1µg/l	7	43	69	92	139	197	185	192	128	95	86	76	66	44	51	75	68	76

Figure 3. Distribution of pesticide concentration ranges in monitoring wells in the years (1993-2010). The indicator does not contain the same wells from year to year, since these are analyzed in a rotation of up to five years.

Furthermore, it is recognized that well depth is an important parameter governing the risk of contamination of drinking water wells. It is shown by Danish National Borehole Database *Jupiter* (Gravesen and Fredericia, 1984) that both occurrence and concentration of pesticides are related to the well depth. In the case of mecoprop, no contamination was found in deep wells (120-150 m), while detectable amounts of mecoprop were observed in shallower wells (< 40 m) with concentrations exceeding the threshold drinking water value (Figure 4). This may be supported by the fact that groundwater in well-confined and deep aquifers usually contains older and cleaner water that has not been contaminated. In the Netherlands, pesticide residues were detected in 27-55% (minimal and maximal estimation) of the monitoring wells in upper groundwater (1-7 meter below surface (mbs)) at concentrations above 0.1 µg L^{-1}, whereas this percentage was 15-40% in groundwater at a depth of 7-20 mbs (Schipper et al., 2008). These patterns in shallow groundwater are strongly related to the occurrence of pesticides in vulnerable abstraction wells that are used for the production of drinking water. The significant relation of the occurrence of herbicides to the well depth was also reported by Kolpin et al. (1997) where they showed decreasing in herbicide concentrations with increasing well depth.

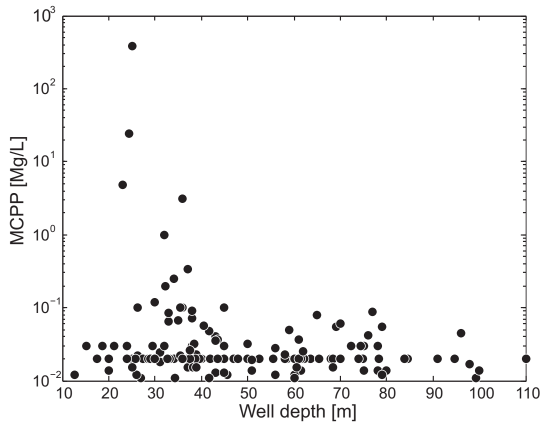


Figure 4. Relationship between mecoprop concentrations and well depth in Zealand, Denmark. Modified from Malaguerra, (2011).

2.2 Point source and diffuse source contamination

Pesticide contamination can arise from various point sources such as leachates from many old landfills and machine pools (Helweg, 1994; Baun et al., 2004; Christensen et al., 2001; Kjeldsen et al., 2002; Christensen et al., 2000; Milosevic et al., 2012). Leachates of inorganic and organic contaminants from these landfills may have influence on the groundwater quality and risk to drinking water resources.

Pesticides are often detected in high concentrations in groundwater where large spills and landfills close to the wells (Harrison et al., 1998); typically between 10 to 250 µg L^{-1} concentrations (Gintautas et al., 1992; Lyngkilde and Christensen, 1992; Zipper et al., 1998; Baun et al., 2003). Assessment of attenuation processes at point source sites depends on the redox conditions (Bjerg et al., 1995). However, it is difficult to estimate the pesticide contamination originated from point sources since it is being diluted during the transportation to the wells.

The largest diffuse source of pesticide contamination is believed to be agriculture (Johnson et al., 2001; Sørensen et al., 2003; Dolan et al., 2012), followed by roads and railways. Diffuse source contamination is generally characterized by the presence of pesticides in groundwater at nanogram-microgram per liter concentrations. Diffuse source contamination is regarded as a bigger threat to groundwater quality than point source contamination due to the difficulties of preventing and remediating diffuse source pollution. The focus in this thesis is on diffuse source of pesticide contamination at environmentally relevant low concentrations, therefore point sources, for instance landfills, are not discussed in detailed.

In conclusion, diffuse source contamination, in contrast to point source contamination, is characteristically more difficult to trace to a source, since it enters the environment from an extensive area, whereas point source contamination enters the environment from a localized site. Thus, it is more difficult to develop remediation solutions for diffuse sources of low concentrations of contaminants in groundwater.

3 Abstraction well fields

This chapter gives brief information on the abstraction well fields and groundwater vulnerability to contamination. One case study is presented with different pumping strategies based on modeling to show effects of pumping on the pollutant concentration and particularly oxygen concentration.

3.1 Groundwater abstraction

In many European countries, drinking water is derived from groundwater resources with a major percentage of abstraction (Figure 5) (IWA, 2010). In Denmark, groundwater is abstracted from well fields with a variety of aquifer types consisting of quaternary deposits overlying chalk, limestone, tertiary sand and clay deposits (Thomsen et al., 2000).

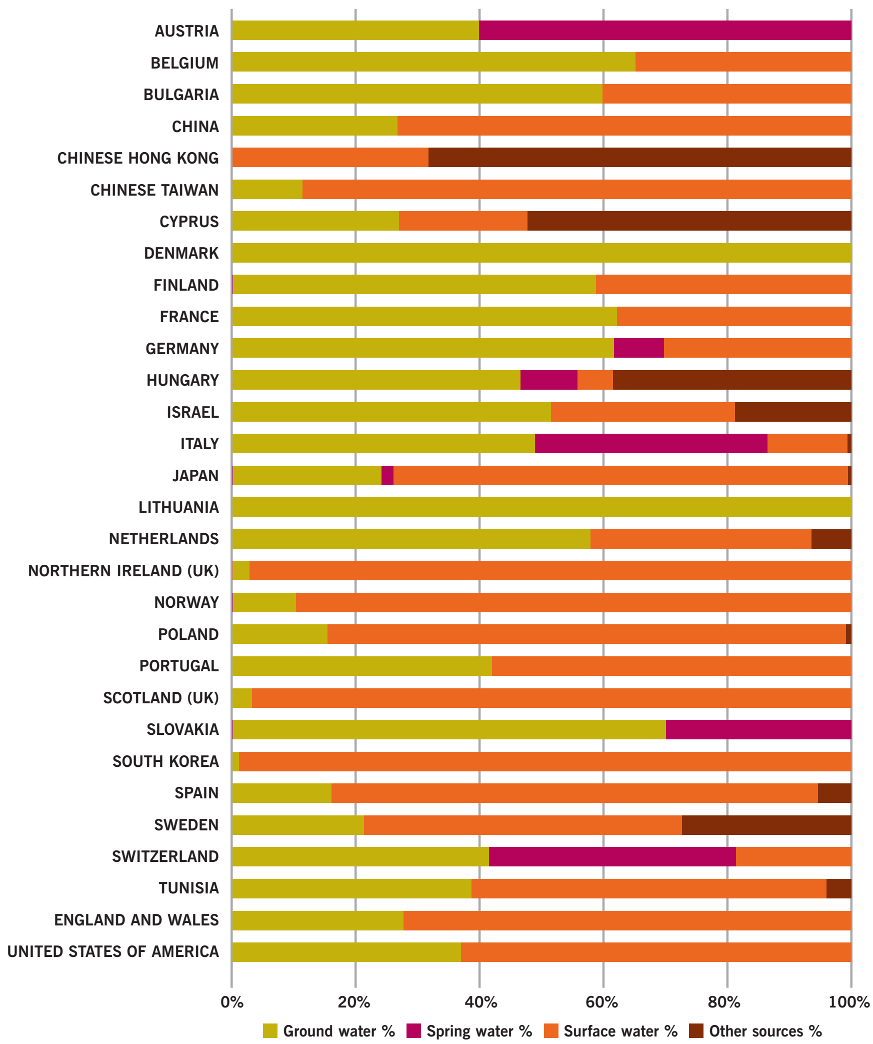


Figure 5. Abstraction of drinking water from groundwater, surface water, spring water and other sources in a number of European countries. From IWA 2010.

Assessment of groundwater vulnerability to contamination is a key concept that should be considered in sustainability of water supply wells. The locations of wells screens affect the fluid fluxes and the spreading of contaminants and are also important to assess the well's vulnerability (e.g., Weissmann et al. 2002). The abstraction well fields may be composed of a series of wells that located in a line perpendicular to the regional hydraulic gradient and to the flow direction. Such wells are quiet common (e.g., Larsen et al., 2003; Bekesi et al., 2012). If the well field consists of multiple wells, installed close to each other in line, *in situ* remediation of the well could be feasible in the case of only one well is contaminated (e.g. with pesticides). Depth of the wells is also important factors to assess the risk of drinking water well contamination. *In situ* remediation might be not possible in the well at deep depths, considering that pesticides are found at higher concentrations at shallow depths.

The significant relationship between groundwater age at a drinking water well and water quality may help to understand aquifer vulnerability to contamination (Molson and Frind, 2012). These authors reported that wells are highly vulnerable as young water reaches the well screens, whereas aquifers supplying mainly old water are less vulnerable to contamination. Another parameter to identify the wells vulnerable to contamination is emphasized on the effect of the distance between surfaces (well depth and thickness of the clay confining layer) and drinking water wells (Malaguerra et al., 2012). The wells close to surface are more vulnerable to contamination and clay layers provide less protection against the leaching of pesticides than sandy layers. The identification of environmental parameters for drinking water well contamination can help authorities to understand better how the water management of a well field can be improved with respect to the preventing contamination of wells. Nevertheless, good quality of drinking water in Danish water policy is defined as 'Groundwater which has only undergone a simple process at the waterworks' (Miljøministeriet, 2010).

3.2 Case study: Nybølle Øst abstraction well field

The Nybølle Øst abstraction well field is an area with special drinking water interests, with particular restrictions towards groundwater threatening activities and priority in relation to protection of groundwater for drinking purposes (Rambøl, 2005). The field is located east of Copenhagen in Denmark (Figure 6) and abstracts 900.000 $m^3 day^{-1}$ of water from 6 wells spanning a distance of 600 m paralleled to a stream (Jessen, 2001). Pesticides were used within the field area and detected in groundwater at low concentrations. As a result, pesticide contamination threats the abstraction wells and drinking water quality in this area.

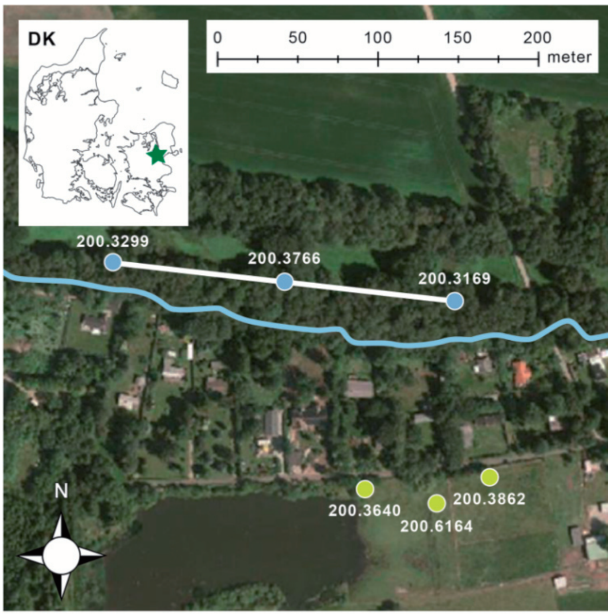


Figure 6. Nybølle Øst abstraction field located east of Copenhagen, Denmark. The drinking water abstraction wells (blue circles) are situated parallel to a stream (blue line) with spanning a distance of 100 m, whereas yellow circles are indication selected monitoring wells in this study.

The flow direction indicates that flow comes to the abstraction wells from both the eastern and the southern part of the area. The primary aquifer consists of Bryozo limestone and has a thickness of approximately 30 m. Above the limestone is a 10 m layer consisting of sand, gravel and clay deposits. The largest part of the water abstraction in the area is taken from the primary aquifer (Pre-Quaternary limestone) (Københavns Amt. Hovedrapport, 2005). The aquifer is unconfined with a recharge of 150 mm year^{-1} (Kürstein et al., 2009).

Phenoxy acids and several other pesticides were used within the Nybølle Øst area and pesticide contamination was detected at least once in 48 % of all analyzed wells in the period of 1990 - 2006. The most frequently detected compound was 2,6 dichlorobenzamid (BAM), followed by mecoprop, dichlorprop, 4-nitrophenol, bentazone and simazine in the area. Figure 7 shows the distribution of three selected pesticides per year (1992 - 2010) in 3 drinking water wells and 2 monitoring wells located in Nybølle. As can be seen from the Figure 3, findings of mecoprop and bentazone were stable over time where dichlorprop was more frequently detected in the period 1998 - 2008.

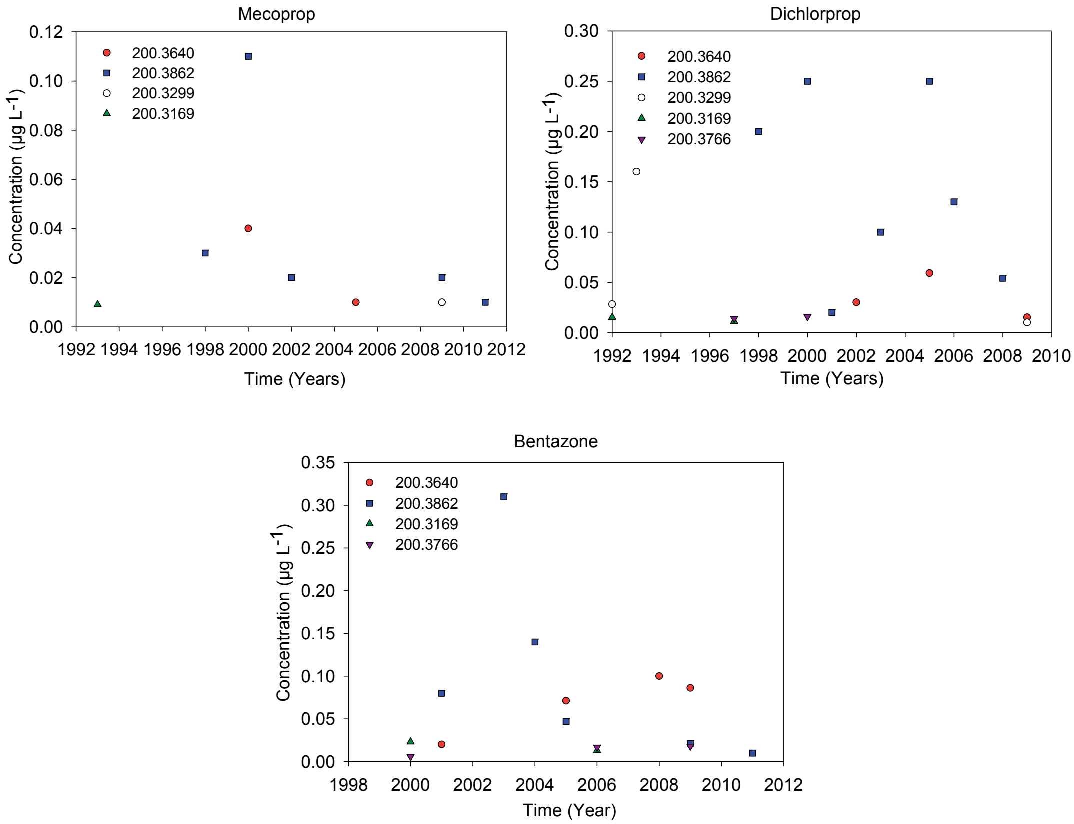


Figure 7. Distribution of three selected pesticides per year (1992-2010) in 5 investigated wells in Nybølle Øst. 200.3640 and 200.3862 are monitoring wells; 200.3299, 200.3169 and 200.3766 are drinking water wells. Data on the well is from Jupiter database (Jupiter, 2011).

The chalk aquifer at the Nybølle Øst abstraction area is anaerobic and is characterized by high levels of calcium and alkalinity. Significant concentrations of dissolved iron, dissolved manganese and ammonium in conjunction with methane indicate strongly reducing conditions (**Paper I**). The high dissolved iron concentration in the upper part of the chalk aquifer could be explained by reduction of iron (III) to iron (II). Low concentrations of sulphate in the same area could be a result of sulphate reduction.

3.3 Pumping strategies

The pesticide concentration reaching a drinking water well is a function of different parameters which will affect the transport and fate of pesticides, such as the pesticide characteristics in the aquifer, the location of the wells (Weissmann et al., 2002) and the hydrogeology of the aquifer (Jørgensen et al., 2004).

Different processes such as sorption and degradation depend on the aquifer redox conditions (Prommer et al., 2006) and affect the transport of pesticides from the surface into the aquifers (Højberg et al. 2005). An analytical model was developed by Beltam et al. (2008) to study the transport of pesticides from application to water supply well. The authors present that the pesticide characteristic travel times and their half-lives depend on the water wells. Pumping history has an important impact on these factors. Higher pumping rates that draw younger contaminated water from shallow aquifers and uncontaminated water from deep aquifers could result in decrease of pesticide concentrations (Aisopou et al. 2013).

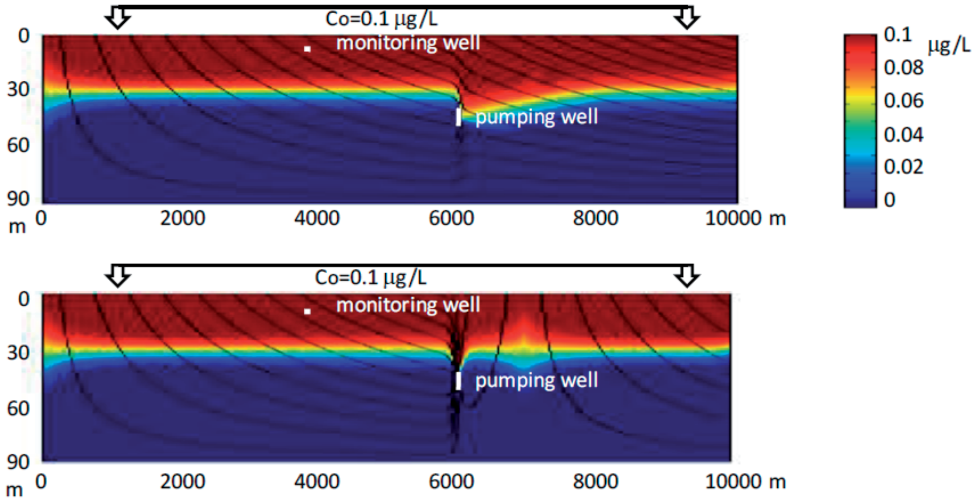


Figure 8. Bentazone concentration in the aquifer at low and high pumping rates (Q=20% of recharge and Q=70%of recharge), when a diffuse source is applied. From Tuxen et al. (2013).

Higher pumping rate (Q=70%) increases the capture zone of the drinking well and will draw contaminated water from the top of the borehole, which might result in a lower bentazone concentration in the well (Figure 8). In addition, younger contaminated water will be drawn from shallow aquifers and cleaner deep groundwater into the well with high pumping rate. The effect of pumping, including different parameters such as different pumping and recharge rates, geologies and different application histories of pesticides can provide information on the concentration of pesticides reaching a well. Zinn and Konikow (2007) showed significant effects of different pumping rates on the groundwater age which varies with depth (Engesgaard and Molson 1998; Shapiro et al., 1999; Castro and Goblet, 2005) (the time since the water first entered the aquifer) at a drinking water well by numerical modeling. The authors focused on the groundwater age changes in the aquifer for a single hydrogeological model excluding the effects of different geological settings on the pesticide concentration. These findings suggest that different pumping rates can affect the pesticide concentration and residence time of the groundwater. Aisopou et al. (2013) investigated the influence of pumping strategies and the hydrogeology of the aquifer on the concentration of two pesticides reaching a drinking water well, mecoprop and bentazone, using numerical simulations, for Nybølle Øst abstraction field. Variability in pumping rates resulted in lower concentration of mecoprop and bentazone at the well (Aisopou et al., 2013). Jepsen (2012) showed increased oxygen concentration to 1 mg L^{-1} with the highest pumping rate ($30m^2$/week), whereas low pumping rate ($7m^2$/week) increased the oxygen level to 0.57 mg L^{-1} by a two-dimensional model, for the same abstraction field (Figure 9). High pumping will change the flow pattern and water will be directly drawn from oxygen rich upper boundary resulting in decrease of bentazone concentration.

In conclusion, different pumping rates considering various parameters such as different geologies, different pesticides properties, their application history and input source type (i.e. point source and diffuse source) could affect pesticide concentration in wells and improve protection of drinking water wells. The conceptual models of pumping effect on pesticide concentrations developed in these studies (Aisopou et al., 2012; Jepsen, 2012) could support our hypothesis which suggests that enhancing oxygen content in the aquifers may create favorable conditions for biodegradation of pesticides and remediate the contaminated well before it reaches to the waterworks.

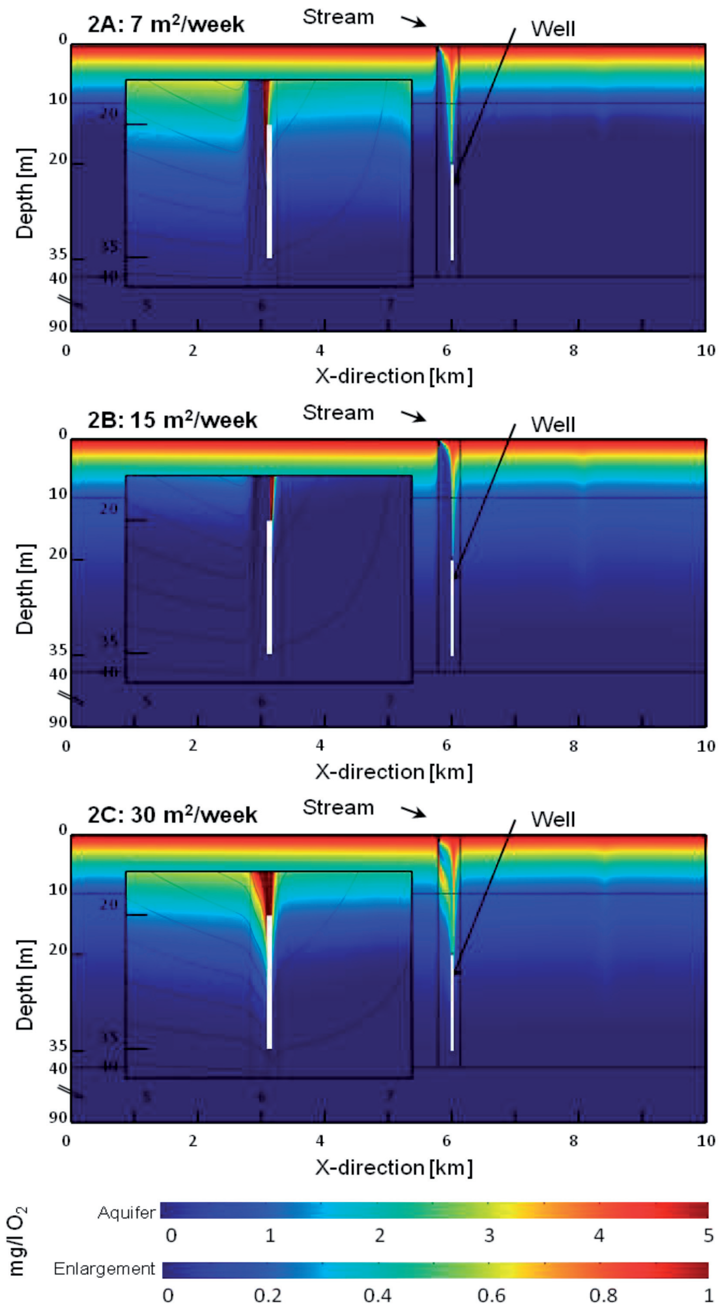


Figure 9. Oxygen concentration in the aquifer at different pumping rates (2A, 2B, 2C). From Jepsen (2012).

4 Degradation potential for the selected herbicides in aquifers

This chapter gives brief information on the usage of selected herbicides and addresses the degradation that is one of the most important processes determining the fate of herbicides in aquifers. An overview of the microbial degradation of each of the three selected herbicides reported in topsoils and aquifer materials under different redox conditions is provided below.

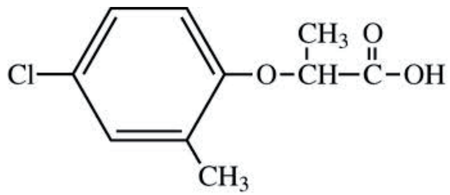
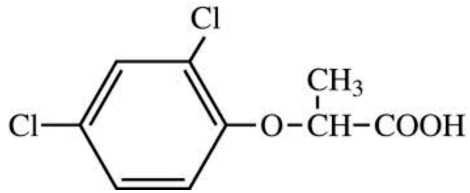
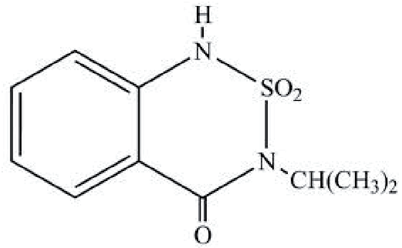
4.1 Phenoxy alkanoic acids

Mecoprop ((RS)-2-(2-methyl-4-chlorophenoxy) propionic acid) and dichlorprop ((RS)-2-(2, 4-dichlorophenoxy) propionic acid) belong to the group of phenoxy alkanoic acids that also includes widely used compounds such as 4-chloro-2-methylphenoxy acetic acid (MCPA), and 2,4-D. They are used as selective, hormone-type herbicides and are applied in agricultural practices to control growth of broad-leaved weeds as well as for horticultural and domestic purposes (Buss et al., 2006; Roberts et al., 1998) (Table 1). They have been among the 20 most sold active compounds in Denmark since 1959 with a total consumption of 9,038 tons for mecoprop and 29,017 tons for dichlorprop (Brüsch et al., 2007). Mecoprop and dichlorprop are among the substances considered as priority hazardous substance in the European Union (EC, 2008).

Degradation of phenoxy alkanoic acids was observed in top soils (Smith, 1989) with a half-life of less than 30 days (Bælum et al., 2008; Gonod et al., 2006), also in wetland areas (Larsen and Aamand, 2001) and marine sediments (Boyle et al., 1999). Biodegradation of these compounds was reported in laboratory experiments and in field injection experiments under aerobic conditions (Larsen et al., 2000; Tuxen et al., 2000; Broholm et al., 2001; Sørensen et al., 2006; Toräng et al., 2003; Janniche et al., 2011, Johnson et al., 2000; Janniche et al., 2010; **Paper I**). In contrast, persistency of mecoprop and dichlorprop were observed in some of the aerobic laboratory experiments (Johnson et al., 2000; Pedersen, 2000). Up to 27% of the added mecoprop was mineralized at 9 mg L^{-1} oxygen concentration (1.4 mg O_2 g^{-1} dw sediment), whereas at 1 mg L^{-1} oxygen concentration (0.3 mg O_2 g^{-1} dw sediment) the mineralization was 8% within 200 days incubation at 10 ˚C (**Paper I**). In similar batch experiments, mecoprop (2.5 µg L^{-1}), incubated at a low oxygen concentration (<0.3 mg L^{-1}), decreased rapidly after addition of 4.5 mg L^{-1} oxygen concentration to anaerobic aquifer material from point sources (Tuxen et al., 2006) (Figure 10). A significant increase in mecoprop degradation at high initial pesticide concentrations (25-100 µg L^{-1}) compared to degradation at low concentrations (1-10 µg L^{-1}) was also shown by Toräng et al. (2003) in aerobic aquifer materials. Dichlorprop was mineralized up to 8% with oxygen concentration of 1.19 mg O_2 g^{-1} dw (corresponding to 9 mg L^{-1} O_2) (**Paper I**). The reason behind the observation of limited degradation in our study might be the difference in the pesticide concentrations used in reported studies. Indeed, initial dichlorprop concentrations used in the studies mentioned above were higher (25-45 µg L^{-1}) compared to the studies in **Paper I** (1 µg L^{-1}). That can be explained by the phenomenon so-called 'threshold concentrations'. Threshold concentration is defined as the concentration below which growth of specific degrader organisms may not be supported to provide energy to the cells due to slow metabolism of the compound (Button, 1985; Alexander et al. 1999). Thus, degradation of the compound may not be initiated due to the lack of the population of bacterial degraders (Roch and Alexander, 1997). Toräng et al. (2003) showed that biodegradation rates of mecoprop and 2,4-D accelerated gradually above a threshold value, while the degradation followed non-growth kinetics below a concentration of 1-10 µg L^{-1} in sediment and groundwater samples.

Anaerobic degradation of dichlorprop was studied in landfill leachate affected groundwater samples in batch experiments (**Paper III**) to support the observed transformation of dichlorprop to 4-CPP in a field investigation along the groundwater flow at an old landfill (Risby site) by tracking their isotope and enantiomer fractionation without supportive microcosm studies on isotope fractionation (Milosevic et al., 2013). Dichlorprop and 4-CPP were found to be recalcitrant in groundwater samples. Neither biological effect (mineralization) nor chemical effect (sorption, volatilization) were observed since CO_2 production and removal of dichlorprop were surprisingly stable during the experimental period. Furthermore, no degradation was also observed in abiotic control incubations. Insignificant decrease in dichlorprop concentration and increase in 4-CPP formation quantified by HPLC also support our findings that dichlorprop was not degradable in groundwater samples.

Table 1. Information on selected herbicides in this study (modified from Tomlin, 1997).

	Mecoprop	Dichlorprop	Bentazone
IUPAC name	(RS)-2-(4-chloro-2-methylphenoxy) propanoic acid	(R)-2-(2,4-dichlorophenoxy) propanoic acid	3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide
Formula	$C_{10}H_{11}ClO_3$	$C_9H_8Cl_2O_3$	$C_{10}H_{12}N_2O_3S$
Structure			
Molecular weight (g/mol)	214.6	235.1	240.3
Water solubility (mg/L)	880	350	570
Log K_{ow}	3.2	1.77	0.46
pK_a	3.78	3	3.3
Application	Post-emergence control of broad-leaved weeds in e.g. wheat and barley	Post-emergence control of broad-leaved weeds	Post-emergence selective herbicide used for weed control in agricultural lands, orchards and in soybeans
Use	Restricted use in Denmark	Restricted use in Denmark	Restricted use in Denmark

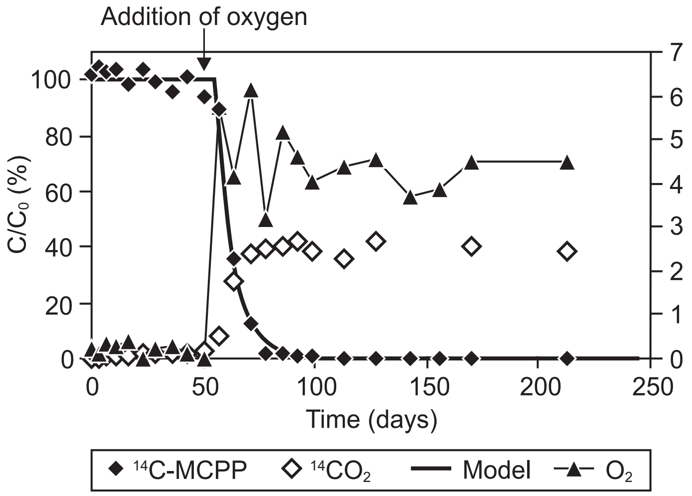


Figure 10. Degradation of ^{14}C-Mecoprop and production of $^{14}CO_2$ in a microcosm incubated at low oxygen concentration during the first 50 days and after oxygen addition (reproduced from Tuxen et al., 2006).

Furthermore, mecoprop and dichlorprop were also mineralized in aquifer samples amended with nitrate with up to 8% and 3% mineralization respectively in 300 days in this thesis (data not shown) (see section 4.1.2). Results indicated slow degradation potential with nitrate addition to anaerobic aquifer samples. However, nitrate added to microbiologically active microcosms was completely consumed compared to abiotic control incubations.

In summary, the investigations indicated a degradation potential for the two selected phenoxy alkanoic acids, mecoprop and dichlorprop, in groundwater environments under both aerobic and anaerobic conditions. Higher degradation was observed with oxygen addition than nitrate addition in our results.

4.2 Bentazone

Bentazone (3-isopropyl-1*H*-2, 1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide) is a post-emergence selective herbicide used for weed control in agricultural lands, orchards and in soybeans (Table 1). It is an active compound in Basagran 480 and other formulations (e.g. Zone 48 and troy 480) (Huber and Otto, 1994). Bentazone has been among the top five active compounds in agricultural practice in Europe (Nadin, 2009). It is still allowed for use in EU countries such as in Denmark with limited and regulated application and in Australia (IUPAC, 2010).

Several investigations have shown readily aerobic degradation of bentazone in topsoils (Huber and Otto, 1994; Leistra et al., 2001; Li et al., 2008; Rodriguez-Cruz et al., 2008; Larsbo et al., 2009). By contrast, van der Pas et al. (1998)

observed only slow aerobic degradation in aquifer sediment samples within 4 years of incubation. Similarly, bentazone was not degraded in aquifer sediment samples under aerobic (Albrechtsen et al., 2001; Tuxen et al., 2000; Broholm et al., 2001) and anaerobic conditions (Pedersen, 2000) within less than 1 year of incubation. Bentazone concentrations used in the studies mentioned above were higher (25-50 µg L^{-1}) than in our study (1 µg L^{-1}). The biostimulation of bentazone with oxygen addition was studied in laboratory batch experiments with anaerobic groundwater and anaerobic aquifer sediment at low concentrations typically found in the groundwater (**Paper I**). 20% of the added bentazone was mineralized in response to 1.48 mg O_2 g^{-1} dw sediment corresponding to aimed oxygen concentration 11 mg L^{-1} within 200 days (Figure 11). Substantial mineralization was also evident in samples amended with oxygen concentrations of 0.29 mg O_2 g^{-1} dw, reaching 7% mineralization.

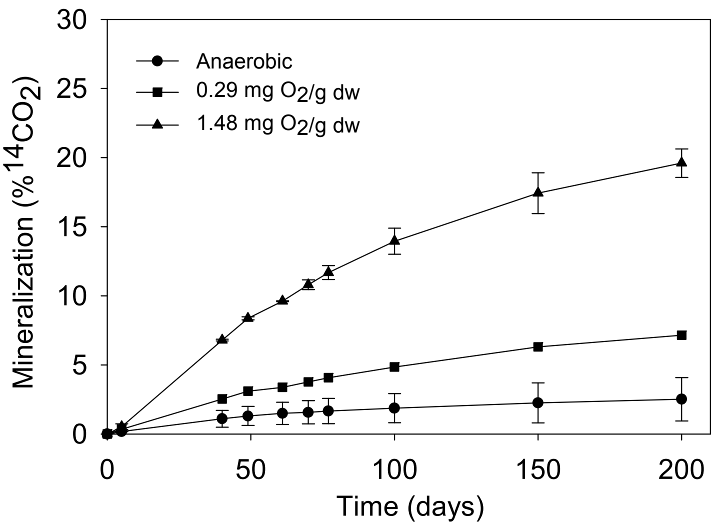


Figure 11. Mineralization of ^{14}C-labeled bentazone to $^{14}CO_2$ in microcosms amended with different oxygen concentrations. 0.29 mg O_2 g^{-1} dw corresponds to aimed oxygen concentration 1 mg L^{-1} and 1.48 mg O_2 g^{-1} dw corresponds to aimed oxygen concentration 11 mg L^{-1}. The bentazone concentration used in the experiment is 1 µg L^{-1}. Modified from **Paper I**.

Furthermore, bentazone mineralization was evident in sandy aquifer samples amended with nitrate, though slow and limited (4%), whereas no mineralization was observed in any of the sterilized control experiments (**Paper II**). Biodegradation kinetics of bentazone at low concentrations (1 to 100 µg L^{-1}) was studied in **Paper II**, by adding inocula capable of bentazone degradation transferred from aquifer material. Bentazone was mineralized (11-13%) at all tested concentrations during 200 days (Figure 12). Furthermore, up to 50% of the added bentazone incubated at highest concentration (100 µg L^{-1}) was removed in

100 days. Nevertheless, there was no clear correlation between increasing initial concentration and mineralization.

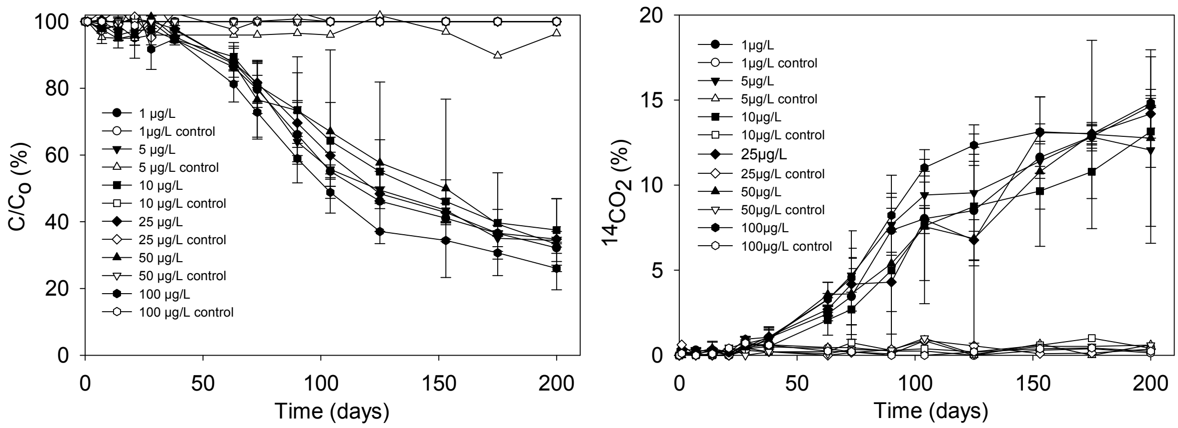


Figure 12. Degradation kinetics of bentazone were investigated over a wide range of bentazone concentrations from 1 to 100 µg L^{-1} with inoculum capable of degrading bentazone enriched from the aquifer material. Error bars represent standard deviations (n=2). Modified from **Paper II**.

In contrast to previous studies indicating slow or no mineralization under aerobic conditions in aquifer sediments (Tuxen et al., 2000; Broholm et al., 2001; Pedersen, 2000); we reported biodegradation and removal of bentazone with potential ability of the bacteria enriched from the aquifer material in aquifer sediments. In general, bentazone is reported to be nondegradable or very slowly degradable in groundwater. Prior to this study, no literature is available showing degradation of bentazone with aquifer sediment samples. This is the first time fast bentazone degradation was observed in aquifer material at low oxygen concentrations (2 mg L^{-1}) (**Paper I**).

5 Laboratory feasibility studies of bioremediation

This chapter gives an overview of biostimulation, bioaugmentation and natural attenuation strategies investigated in laboratory experiments. Prior to application of relevant bioremediation technologies in the real world aquifer systems, laboratory degradation experiments mimicking the natural conditions of groundwater environments are crucial in order to assess the degradation ability of indigenous microorganisms.

5.1 Biostimulation

Biological mineralization is the only possible process for the complete degradation of pesticides to CO_2, water and inorganic salts (Alexander, 1981). Biostimulation is one of the relevant approaches for enhancing the pesticide biodegradation. This involves addition of electron acceptors such as oxygen and nitrate, electron donors and essential nutrients to stimulate the growth of indigenous degradative microorganisms (Scow and Hicks, 2005). Reduction in lag phase and increase in degradation rate constants make biostimulation a significant remediation strategy in pesticide contaminated aquifers. In this thesis, effects of addition of two different electron acceptors (oxygen and nitrate) on biostimulation were emphasized. Table 2 gives an overview on the concept of electron acceptors and nutrient supplementation for enhanced degradation of herbicides brought by various research groups.

5.1.1 Oxygen-enhanced biostimulation

Many aromatic structured herbicides are easily degradable under aerobic rather than anaerobic conditions (Moorman, 1994). Natural attenuation of these aromatic compounds is strongly occurred in the presence of oxygen. Oxygen regulates functioning of certain degradative enzymes like oxygenases. Oxygenases initiate the transformation of aromatic organic compounds by incorporating oxygen into the pollutant (Weightman and Slater, 1988).

Introduction of oxygen into anaerobic sediments from phenoxy acid (PA) herbicides contaminated point sources has been shown to successfully enhance the aerobic biodegradation of PA by Tuxen et al. (2006) in laboratory experiments and for other organic compounds such as vinyl chloride (Gossett, 2010), MTBE (Landmeyer et al., 2001) and BTEX (Chapman et al., 1997). Tuxen et al. (2006) observed a significant increase in the mineralization degree of MCPP (30-50% $^{14}CO_2$ recovery at low oxygen concentrations and 50-70% $^{14}CO_2$ recovery at approximate 8 mg L^{-1} oxygen concentration) and decrease in the lag phase (from ~ 150 days to 5 to 25 days) with oxygen addition. In a column experiment with aquifer material from an atrazine contaminated area, oxygen addition decreased the atrazine concentrations with degradation rates of 380 and 240 µg L^{-1} d^{-1} (Patterson et al., 2002).

Laboratory experiments with anaerobic aquifer material and anaerobic groundwater were conducted to investigate the degradation potential of two phenoxy acids; mecoprop and dichlorprop and bentazone in response to addition of wide ranged oxygen concentrations (**Paper I**). Mineralization degree, particularly of mecoprop and bentazone were significantly increased by addition of oxygen. 14-27% of mecoprop, 3-9% of dichlorprop and 15-20% of bentazone were mineralized at high oxygen concentrations. Positive effects of oxygen addition on degradation even below 2 mg L^{-1} oxygen concentration were seen. Similar positive effects on mecoprop degradation were observed by Tuxen et al. (2006) in aquifer material from a point source contaminated site (Table 2).

There is still limited knowledge on herbicide degradation potential in low oxygenated systems. Increase in the degree of bentazone mineralization, 7% $^{14}CO_2$ recovery was observed by adding only 1.48 mg O_2 g^{-1} dw sediment corresponding to aimed oxygen concentration 1 mg L^{-1} (**Paper II**). The batch experiments demonstrated that bacteria in the aquifer material and groundwater were capable of degrading the three selected pesticides. Nevertheless, rapid oxygen consumption of sediment was observed below 2 mg L^{-1} oxygen concentration at an addition of 0.3 mg O_2 g^{-1} sediment (**Paper I**). Our results indicate that oxygen consumption increased with increasing amount of oxygen added to each microcosm. The rapid oxygen depletion in the microcosms may be explained by natural reductants such as organic matter, pyrite (FeS_2), ferrous iron (Fe^{2+}) or siderite ($FeCO_3$) that are present in aquifer sediments (Hartog et al., 2002; Heron and Christensen, 1995). Despite the rapid oxygen consumption, the addition of low amounts of oxygen is sufficient to sustain mineralization of herbicides (**Paper I**).

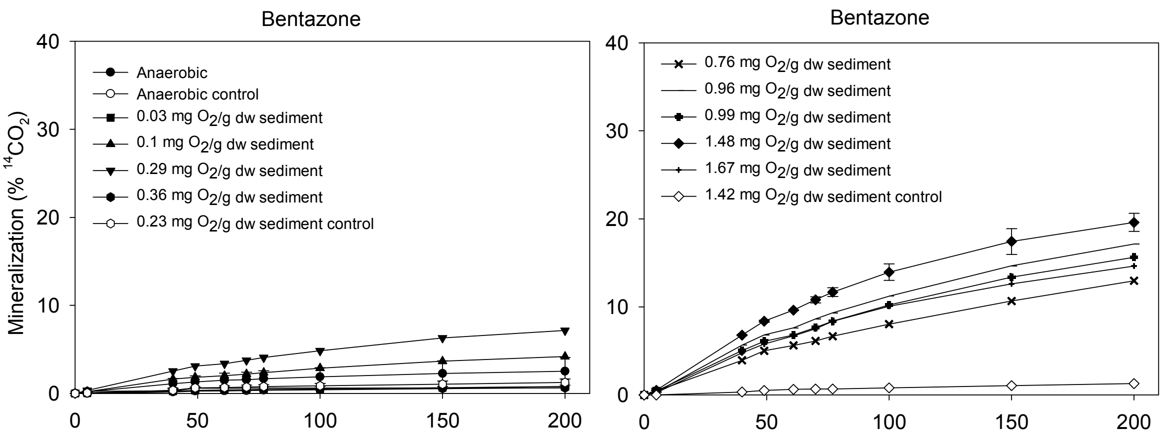


Figure 13. Mineralization of ^{14}C-labeled bentazone to $^{14}CO_2$ in microcosms amended with different oxygen concentrations. From **Paper I**.

In summary, biodegradation of the three herbicides was stimulated in anaerobic sediment materials by oxygen addition. Potential for enhancing especially the degradation of bentazone in aquifer materials was demonstrated for the first time in this study (Figure 13) (**Paper I**). These results suggest that enhancing the oxygen levels even to relatively low concentrations is a highly potential remediation technology for herbicides arising from diffuse source contamination.

Table 2. Overview of different electron acceptors or nutrient amendments used for stimulating the biodegradation of herbicides

Amendments	Target herbicides	Major finding	References
Oxygen	Mecoprop	Decrease of lag phases and increase in degree of mecoprop mineralization	Tuxen et al., 2006
Oxygen	Atrazine	Rapid decrease in atrazine concentration	Patterson et al., 2002
Oxygen	Mecoprop, dichlorprop and bentazone	Significant increase in mineralization at high and <2 mg L^{-1} oxygen concentration	Levi et al., 2013a
Nitrate&Citrate	Atrazine	Citrate served as an electron donor stimulated atrazine mineralization while nitrate served as an electron acceptor	Shapir et al., 1998
Nitrate	(R)-Mecoprop	Stimulated the anaerobic biodegradation after a lag period of about 20 days	Harrison et al., 2003
Nitrate	Bentazone	Achieved a slow mineralization of 5% in about 300 days	Levi et al., 2013b
Nitrate and phosphorus	Isoproturon	Degradation of isoproturon by converting the herbicide into a complex degradation product, 4, 4-diisopropylazobenzene	Perrin-Ganier et al., 2001
Phosphorus	Dichlobenil and atrazine	Complete degradation of dichlobenil in 60 hours and even higher degree of enhancement of atrazine degradation in 40 hours	Qiu et al., 2009
Organic and inorganic nutrients	Mecoprop and 2,4-D	Decrease of lag phase for mecoprop and positive effect on 2,4-D mineralization	de Lipthay et al., 2007
Nutrient-rich media	Mecoprop	Higher biodegradation and removal efficiency of mecoprop	Bestawy and Albrechtsen, 2007
Glucose	Atrazine	Enhanced mineralization of atrazine	Wagner and Chahal, 1996
Mannitol	Atrazine	Increased $^{14}CO_2$ production after 140 days	Assaf and Turco, 1994

5.1.2 Nitrate-enhanced biostimulation

Major focus in bioremediation technologies has been given to oxygen addition to stimulate biodegradation of contaminants in saturated zones. Nitrate is the second most common terminal electron acceptor for oxidation of organic compounds after all available oxygen is preferentially depleted by microorganisms in the environment (Lovley and Chapelle, 1995). Nitrate, which is very soluble in water, can be a good candidate to provide an alternative electron acceptor for biological activity and enhance the degradation rate of organic contaminants. Although less energy is gained from anaerobic respiration (-119$\Delta G^{\circ}(W)$) compared to aerobic respiration (-125$\Delta G^{\circ}(W)$), there are indigenous anaerobic bacteria in subsurface environments utilizing nitrate as an electron acceptor (Christensen et al., 2000). Biodegradation of organic compounds such as hydrocarbons occurred under denitrifying conditions has been presented by several studies (Hutchins et al., 1991; Kuhn et al., 1988).

The effect of nitrate addition to iron-reducing sediment samples on biodegradation of (R)-mecoprop and (S)-mecoprop has been investigated by Harrison et al. (2003). The authors conducted anaerobic microcosms using limestone aquifer material, where nitrate was added in the concentration of ~ 500 µg L^{-1} (Table 2). Anaerobic degradation of (R)-mecoprop was observed after a lag period of about 20 days, whereas nitrate addition had no effect on (S)-mecoprop degradation (Harrison et al., 2003). Positive effects of nitrate and citrate amendment on atrazine mineralization were also reported by Shapir et al. (1998).

In laboratory batch incubations with groundwater and sediment material amended with 11 mg L^{-1} NO_3-N concentration, only small fractions of two phenoxy alkanoic acids and bentazone were degraded, although potential degradation exceeded the radiochemical impurities level (**Paper II**). ~ 8% of the added mecoprop was mineralized, whereas only 3% and ~ 4.5% of dichlorprop and bentazone were mineralized, respectively, in about 300 days (Figure 14). In contrast to our study, Knauber et al. (2000) did not observe bentazone mineralization after addition of nitrate in anoxic soil samples.

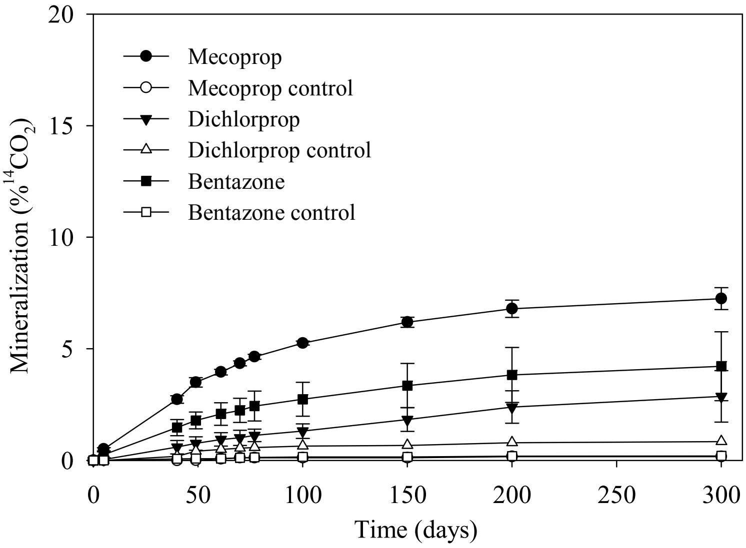


Figure 14. Mineralization of ^{14}C-labeled mecoprop, dichlorprop and bentazone to $^{14}CO_2$ by nitrate addition. The pesticide concentration used in the experiments is 1 µg L^{-1}. Modified from (**Paper II**).

In summary, the observations regarding limited effect of nitrate addition on degradation of two phenoxy alkanoic acids and bentazone in anaerobic aquifer materials suggest that all available nitrate might be consumed by the oxidation of other compounds in the sediment. However, the knowledge and development of nitrate enhancement of anaerobic pesticide degradation is still limited. Furthermore, a mixed oxygen/nitrate system may also suggest that the addition of nitrate would supplement the oxygen demand rather than replacing it, therefore allowing the pesticide to be biodegraded under microaerophilic conditions.

5.1.3 Nutrient-enhanced biostimulation

Essential nutrients such as carbon, nitrogen and phosphorus stimulate bacteria synthesizing the essential enzymes to break down the contaminants. The concentration of nutrients in soil is generally less than optimal levels for microbial activity (Manilal and Alexander, 1991; Morgan and Watkinson, 1989). Nutrient amendment in soils could provide a promising enhanced bioremediation of contaminants (Kanissery and Sims, 2011). One of first reports demonstrating acceleration of atrazine degradation in soil by inorganic nutrients addition has led the development of nutrient-enhanced degradation of contaminants (Hance, 1973). Positive effects of nutrient amendments on mecoprop mineralization were reported by Bestawy and Albrechtsen, (2007), where the authors showed that the mineralization degree of mecoprop and CO_2 production were higher in almost all nutrient-rich cultures.

Qiu et al. (2009) observed complete degradation of dichlobenil in 60 hours in phosphate amended soil extract compared to non-phosphate amended soil extract with 40% less degradation. Wagner and Chahal, (1998) reported enhanced mineralization of atrazine with the addition of glucose as a nutrient. Likewise, Assaf and Turco, (1994) observed an increase in $^{14}CO_2$ production as a result of enhanced atrazine mineralization with addition of mannitol as a carbon source after 140 days. Biostimulation potential of three PA herbicides (mecoprop, 2,4-D and 2,4,5-T) was investigated in soil and aquifer sediment focusing on the effect of addition of nutrients within a wide herbicide concentration range (de Lipthay et al., 2007). The authors showed that nutrient addition stimulated mineralization at higher herbicide concentrations, whereas no stimulation was possible with low herbicide concentrations.

In conclusion, biostimulation through the application of nutrients into the soil and aquifer sediment materials may accelerate the mineralization of pesticides and microbial activity and be a promising approach for removal of pesticides from contaminated groundwaters and soils as observed in all the cited literature reviews mentioned above.

5.2 Bioaugmentation

Bioaugmentation is defined as the technique for enhancement of the target contaminants by the introduction of specific microorganisms (indigenous or non-indigenous) to the contaminated matrix and is one of the most relevant bioremediation approaches (Fantroussi and Agathos, 2005). Bioaugmentation has been a subject in several literature reviews (Gentry et al., 2004). The use of microbial inocula isolated from aquifer material to stimulate atrazine degradation in aquifers has been shown in laboratory experiments (Shapir et al., 1998; Franzmann et al., 2000; Kristensen et al., 2001). Studies on bioaugmentation applications for remediating herbicides in aquifers are scarce; but remediation of dinoseb in soil was proven with this approach (Kaake et al., 1992). Despite the laboratory scale experiments, application of bioaugmentation approaches to a field site points out the issues in respect to its efficiency. In cases where inoculum enhanced the degradation and removal of the contaminant under controlled laboratory conditions may not give the similar efficiency under natural environmental conditions. This might be due to the fact that optimum conditions are not present for the microorganisms or competition occurs between inoculated and other microorganisms present in the field site.

5.3 Natural attenuation

Bioremediation via natural attenuation is naturally occurring microbial degradation process in groundwater of organic contaminants. Natural attenuation is defined as the reduction in mass or concentration of a contaminant through physical (dispersion, dilution, adsorption, and volatilization), chemical (ion exchange, abiotic transformation), and biological processes (aerobic and anaerobic biodegradation) without human intervention (Roling and van Verseveld, 2002). The natural attenuation of the phenoxy herbicides was investigated in groundwater by several researchers (Baun et al., 2003; Klecka et al., 2001; Tuxen et al., 2003; Zipper et al., 1998; Williams et al., 2001; Broholm et al., 2001; Janniche et al., 2010; Milosevic et al., 2013). Lyngkilde and Christensen (1992) observed removal of mecoprop under aerobic conditions near landfills. In several laboratory experiments (Tuxen et al., 2003; Zipper et al., 1998; Broholm et al., 2001; **Paper I**) with aquifer material and groundwater from different sites, aerobic degradation was observed indicating presence of bacteria capable of degrading phenoxy acids at the site.

6 Challenges in implementation of *in situ* bioremediation

Biostimulation of degradation of two phenoxy acids and bentazone by addition of oxygen was demonstrated in our laboratory experiments and it is suggested to be a promising remediation strategy in pesticide contaminated aquifers from a microbial point of view (**Paper I, II**). There are several aspects need to be taken in consideration for a better understanding of pesticide degradation in aquifers. The application of the results from controlled laboratory degradation experiments in the field is a significant challenge.

In this thesis, it is suggested that treatment of contaminated wells in an abstraction field is possible with pumping strategies before contaminated water reaches to the waterworks. This approach may significantly provide oxygenated water from upper part of the aquifer resulting increase in the oxygen content in an aquifer. Mixing different types of water between different water compartments in the aquifer may create aerobic conditions with high potential for biodegradation of pesticides. This approach was supported by model simulations showing different pumping strategies (pumping rates, location of wells and screens) that could result in enhanced oxygen concentrations in the lower part of the aquifer mixing with aerobic groundwater from the upper part of the aquifer (Jepsen et al. 2012; Aisopou et al., 2013). In order to test this approach, potential oxygen enhancement would be needed to be tested at the actual well fields with different pumping strategies suggested by these model scenarios. The possible challenges in implementation of biostimulation strategy in the field site are discussed in this chapter.

One of the main factors governing the contaminant transport in aquifers is permeability. Low permeability or high degree of heterogeneity may limit the ability to distribute the electron acceptors or nutrients effectively throughout the aquifer. The proper mixing between oxygen and the contaminant could be limited due to transport of oxygen into high permeability zones, since contaminants may be present in low-permeability zones or clayey layers (Tuxen, 2002). The dissolved oxygen supply could be also limited due to limited recharge which could bring oxygen-rich groundwater into the contaminant zone.

Enhanced aerobic degradation of pesticides relies on a sufficient supply of oxygen reaching the contaminants. Introduction of oxygen to anaerobic aquifer systems will raise oxygen competition between contaminants, organic matter and other reduces species. Demand of oxygen consumption will increase due to oxidation of organic matter and reduced inorganic species resulting in lower oxygen levels in order to enhance microbial degradation in aquifer environments. The amount of needed oxygen to initiate biodegradation could be calculated based on actual demand of oxygen by other compounds. We showed that degradation potential of pesticides is promising at relatively low oxygen concentrations (**Paper I**). Therefore, anaerobic groundwater and sediment samples are not necessarily highly oxygenated in order to create an optimum environment for indigenous microorganisms.

Another difficulty in introducing oxygen into the aquifers by pumping or injection is microbial Fe^{+2}oxidation in groundwater environments (Chapelle, 1993). Iron is soluble in the ferrous state (Fe^{+2}) and is oxidized in the presence of air to the highly insoluble ferric form (Fe^{+3}). All the iron will be in a soluble form in anaerobic groundwaters with low dissolved oxygen concentrations (Gray, 2008). Oxygen can enter aquifers from recharge water. When the groundwater is abstracted by pumping, Fe^{+2} is oxidized to Fe^{+3} in the wake of interaction of oxygen in the surface. The interaction of oxygen may raise the pH and causes iron in the groundwater to precipitate out as ferric hydroxide (Seifert and Engesgaard, 2012). Precipitation of iron builds up of a microbial slime comprised of iron bacteria on piped surfaces affecting the flow. Iron bacteria colonies can also grow in the wall of wells and cause clogging of intake screens (Seifert and Engesgaard, 2012).

The end product of nitrate reduction, N_2 is poorly water soluble contrary to water soluble nitrate. Injection of nitrate to groundwater is of concern because of accumulation of N_2 gas bubbles. N_2 gas bubbles prevent water from the pore spaces and decrease the hydraulic activity (Cunningham et al., 2001). Since drinking water maximum contaminant level (MLC) for NO_3^- is 45 mg L^{-1} (10 mg L^{-1} NO_3-N) (Gray, 2008), its concentration in groundwater requires attention to prevent contamination. Cunningham et al. (2001) suggest combined injection of nitrate and sulfate to avoid practical limitations of injection of a single electron acceptor from an engineering perspective. The authors demonstrated that combined injection of nitrate and sulfate into the contaminated aquifer accelerates BTEX removal.

Inorganic nutrients such as nitrogen and phosphorus are the two most critical compounds to support cell growth and sustain biodegradation process. Therefore nutrients need to be available in sufficient quantities in the aquifers. Nutrient injection many not be necessary if the groundwater contains sufficient quantities of nutrients. In cases where nutrient injection is needed, addition of these compounds should be released slowly and monitored throughout the aquifer to prevent water quality degradation. Otherwise, excessive amount of nutrients may lower the pH in groundwater. Thus, nutrient-enhanced bioremediation approach, if needed, should be operated at near nutrient-limited conditions.

The greatest challenge in implementation of a suitable bioremediation strategy is bringing the knowledge of natural processes occurring at the site and achieving good results in the field as it is in the laboratory. Hence, the applicability and the limits of biostimulation could ideally be tested in a simulated aquifer condition which is close to field-scale, after initial laboratory microcosm experiments. Enhanced biostimulation with addition of electron acceptors is not an instantaneous process. It should also be noted that time required creating the appropriate environmental conditions and to grow microorganisms capable of degrading specific contaminants may take from several months to years. Degradation of pesticides as a function of laboratory experiments within typically up to hundreds days is not comparable with the longer groundwater residence time in aquifers depending on redox conditions, pesticide and aquifer characteristics.

7 Conclusions

Contamination of groundwater is an important issue in many countries, especially in cases where the drinking water supplies is based on groundwater. When the contamination arises from diffuse sources, large volumes of water are often contaminated at low pollutants concentrations and both aspects are challenges for drinking water supplies in terms of prevention and treatment. Furthermore, if only one well in an abstraction field is contaminated, this contaminated water may after abstraction be mixed with uncontaminated water from the other wells during transport to the waterworks resulting in excessive volumes of water to be treated. This thesis investigated an alternative remediation approach: aerobic biostimulation to treat pesticide contaminated groundwater in situ in the abstraction field before the water reaches to the waterworks. The aim of this study was to investigate whether increasing the oxygen concentrations in aquifers with pumping strategies around an abstraction well field would be an *in situ* remediation option. The ambition for future implementations is to use these approaches is to treat the groundwater for this specific contamination before it reaches to the waterworks.

This thesis provides an overview of occurrence and degradability of three herbicides: bentazone, mecoprop and dichlorprop in groundwater and aquifer sediments. Furthermore, novel findings for stimulated degradation of bentazone at low oxygen concentrations in aquifer sediments were also presented.

The major contributions resulting from these studies are summarized below.

- Aerobic degradation potential for bentazone and mecoprop was evidenced in groundwater samples from an abstraction well field with autoclaved chalk sediment. The bacteria originated from the groundwater were able to degrade the considered herbicides.
- Laboratory experiments demonstrated that oxygen addition to anaerobic aquifer sediment collected near an operating drinking water abstraction field stimulated biodegradation of the three selected herbicides (bentazone, mecoprop and dichlorprop) at environmentally relevant concentration (1 $\mu g\ L^{-1}$). Biostimulation was seen not only at high oxygen concentrations but also at substantially lower oxygen concentrations (2 $mg\ L^{-1}$). Due to observed degradation potential in our laboratory studies, biostimulation is considered to be a viable remediation approach for pesticides with enhancing oxygen concentrations in the aquifers around water abstraction well fields.

- A literature review has deepened our understanding of biodegradation processes regarding bentazone in topsoils and aquifer sediments. Bentazone was degradable in aquifer sediments under aerobic conditions in our laboratory studies. In the literature no studies have previously reported on degradation of bentazone in aquifers was found. Furthermore, bacteria with the capability to degrade bentazone over a wide range of concentrations were successfully transferred from the aquifer material. Increasing initial concentrations did not significantly affect bentazone degradation.
- Dichlorprop was recalcitrant in the groundwater samples from an old landfill under anaerobic conditions in laboratory studies, with groundwater samples from the field site where transformation of dichlorprop to 4-CPP was observed by different analytic methods in the field. The observed degradation potential in the field could not be transferred to the laboratory, possibly because of the complexity of the field site with varying redox conditions and residence times of contaminants.

The experiments and the literature reviewed suggest that biostimulation may be a bioremediation solution with enhancing the oxygen contents with pumping strategies in the aquifers and creating feasible conditions for degradation of contaminants. However, biostimulation might be limited due to several issues such as major oxygen consuming process, oxidation of reduced iron (Fe^{2+}) and manganese (Mn^{2+}) and organic matter in the sediment and bioclogging by the growth of iron bacteria. Detailed investigations of biostimulation of pesticides in aquifers at diffuse sources especially at low contaminant concentrations are scarce. The results of this thesis would contribute significantly to our understanding for the development of alternative bioremediation strategies to remove organic contaminants at low concentrations in or around drinking water abstraction fields and to protect drinking water wells.

8 References

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9 Papers

I. Levi, S., Hybel, A. M., Bjerg, P. L., Albrechtsen, H. J., 2013. Stimulation of aerobic degradation of bentazone, mecoprop and dichlorprop by oxygen addition to aquifer sediment. Submitted to Environmental Pollution.

II. Levi, S., Jepsen, T. S., Bjerg, P. L., Albrechtsen, H. J., 2013. Potential for aerobic biodegradation of bentazone in aquifer sediments. Submitted to Pest Management Science.

III. Levi, S., Qiu, S., Bjerg, P. L., Elsner, M., Albrechtsen, H. J., 2013. Anaerobic degradation of dichlorprop in landfill leachate affected groundwater based on laboratory batch experiments. Technical note.

In this online version of the thesis, the papers are not included but can be obtained from electronic article databases e.g. via www.orbit.dtu.dk or on request from:

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