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Strategy for Monitoring Organic Pollutants in Waste Water with Focus on Improved Sample Preparation

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2006

Akademisk avhandling som för avläggande av filosofie doktorsexamen kommer att offentligen försvaras på Kemicentrum, Sölvegatan 39, Lund, Hörsal B, fredagen den 29 september 2006, kl 13.15, med vederbörligt tillstånd av matematisk-naturvetenskapliga fakulteten vid Lunds Universitet. Fakultetsopponent är docent Kari Hartonen, Laboratory of Analytical Chemistry, Department of Chemistry, University of Helsinki, Finland.

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Strategy for Monitoring Organic Pollutants in Waste Water with Focus on Improved Sample Preparation

Abstract

Strategy and methodology is presented for the analysis of organic pollutants, with the purpose of evaluating treatment procedures for landfill leachate. Today, many investigations of treatment procedures are focusing on the measurement of water quality parameters such as chemical and biochemical oxygen demand, (COD and BOD), and total organic carbon, (TOC) when assessing the organic compounds in waste waters. These parameters give an unclear picture of the actual organic constituents. A developed analytical protocol, the Laqua protocol, covers several classes of organic contaminants, including both polar and non-polar markers, as well as inorganic parameters. As markers for polar compounds some phenols are selected and for non-polar markers polychlorinated biphenyls (PCB) and polybrominated diphenylethers (PBDE) are used. Unidentified markers are also followed to back up trends. The monitoring of individual compounds gave valuable information in understanding the processes in the treatment procedures. A toxicity test suitable for leachate water based on the crustacean Artemia Salina was also developed and included in the evaluation protocol. Combined with a simple fractionation of the leachate water, this test gave valuable information about the origin of the toxicity, which mainly originated from ammonium. The protocol was implemented and tested on a pilot plant for different treatment procedures in Kristianstad, Sweden.

The bottle neck in the complicated analysis of organic pollutants is the expensive and resource demanding sample preparation step. In this thesis focus has been on developing automated, cost effective analytical procedures with sample preparation based on membrane technology. For PCBs, phthalates and organochlorine pesticides (OCP), automated procedures have been based on membrane-GC methodology, and for phenols an automated system, based on membrane-LC methodology, has been developed. A simple very efficient method based on disposable hollow fibre has been developed for the analysis of PBDE with GC-MS for final determination. All the developed methods dramatically decrease the time and effort spent on sample preparation, and demand only a very small fraction of organic solvents compared to conventional methods. The developed methods have very good performance, and as an example PCB extracted from 1 ml sample in 10 minutes gave detection limits of 2 - 3 ng/l, and relative standard deviations (RSD) at 0.1 µg/l of 1.6 - 5.0 % for all ten PCB congeners investigated.

Key words: Monitoring strategy, landfill leachate, organic pollutants, sample preparation, membrane extraction

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Strategy for Monitoring Organic Pollutants in Waste Water with Focus on Improved Sample Preparation

This thesis is a result within an on-going cooperation between Lund University and Kristianstad University. The project was supported by both universities.

The thesis is based on the following papers, referred to in the text by their roman numerals:

I. **Development and application of an analytical protocol for evaluation of treatment processes for landfill leachates.**
I. Development of an analytical protocol for handling organic compounds in leachate samples.
Bergström, S.; Svensson, B.-M.; Mårtensson, L.; Mathiasson, L.
In press, *Int. J. Environ. Anal. Chem.*, **2006**

II. **Development and application of an analytical protocol for evaluation of treatment processes for landfill leachates.**
II. Evaluation of leachate treatment efficiency of different steps in a constructed pilot plant.
Mårtensson, L.; Bergström, S.; Svensson, B.-M.; Mathiasson, L.
In press*, Int. J. Environ. Anal. Chem.*, **2006**

III. **Artemia Salina as test organism for assessment of acute toxicity of leachate water from landfills.**
Svensson, B.-M.; Mathiasson, L.; Mårtensson, L.; Bergström, S.
Environ. Monit. Assess., **2005**, *102*, 309-321

IV. **Miniaturized and automated sample pretreatment for determination of PCBs in environmental aqueous samples using an on-line microporous membrane liquid-liquid extraction-gas chromatography system.**
Barri, T.; Bergström, S.; Norberg, J.; Jönsson, J. Å.
Anal. Chem., **2004**, *76*, 1928-34

V. **Extracting Syringe for determination of organochlorine pesticides in leachate water and soil-water slurry: A novel technology for environmental analysis.**
Barri, T.; Bergström, S.; Hussen, A.; Norberg, J.; Jönsson, J. Å.
J. Chromatogr. A, **2006**, *1111*, 11–20

VI. **Determination of polybrominated diphenyl ethers at trace levels in environmental waters using hollow-fiber microporous membrane liquid-liquid extraction and gas chromatography-mass spectrometry.**
Fontanals, N.; Barri, T.; Bergström, S.; Jönsson, J. Å.
In press, *J. Chromatogr. A*, **2006**

VII. **Extracting Syringe for extraction of phthalate esters in environmental samples.**
Bergström, S.; Barri, T.; Jönsson, J. Å.; Mathiasson, L.
Manuscript*,* **2006**

CONTRIBUTION BY THE AUTHOR TO THE DIFFERENT PAPERS

Paper I. The author made a substantial part of the development of the LAQUA protocol and a major part of the evaluation strategy. The author further developed and automated the extraction procedure for the polar markers and supervised the development of the methodology for the non-polar markers. The author wrote the major part of the paper.

Paper II. The author was involved in the design of the pilot plant, and made a substantial part of the sampling strategy. The author performed the analysis of the organic markers and made the calculation for the evaluation of the different treatments for the complete analytical protocol. The author has substantially contributed to the manuscript.

Paper III. The author was involved in the experimental design and performance. The author was involved in the sampling and evaluation of the results. The author was closely involved in the scientific discussion during the preparation of the manuscript.

Paper IV – VI. In these papers, the author was involved in the experimental design, scientific discussions and in the performance of the experiments. The author has worked on improving and developing the extraction equipment and actively contributed to the writing of the manuscripts.

Paper VII. The author designed and performed most of the experimental work and wrote the major part of the manuscript.

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POPULÄRVETENSKAPLIG SAMMANFATTNING

Människan har alltid producerat avfall. I takt med att vårt samhälle blivit mer och mer komplicerat, har även mängden och komplexiteten av avfallet ökat. Från början samlades avfallet i så kallade kökkenmöddingar utanför bosättningarna. Än i dag är denna form av avfallshantering dominerande. I Europa produceras ca 3000 miljoner ton avfall årligen, varav 10 % är kommunalt avfall som till stor del består av hushållsavfall. Dessa mängder av avfall måste tas om hand. Runt om i Europa och även i Sverige ökar förbränningen av avfall till energiproduktion, men den största delen av avfallet deponeras fortfarande. Trots att europeiska unionen (EU) har gjort ansträngningar, i form av lagstiftning för återanvändning m.m. för att minska avfallsproduktionen, visar undersökningar på att den årliga produktionen av avfall fortsätter att öka. Detta leder till ökande problem med avfallshanteringen, framför allt i de mer tätbefolkade delarna av Europa. I EUs strategi för avfallshantering ingår krav på att optimala avfallshanteringsprocesser skall användas, samt krav på att avfallsövervakningen skall förbättras.

Den inledande forskningen i den här avhandlingen var en del av ett projekt, Laqua, för att främja utveckling av ekologiskt och ekonomiskt hållbara behandlingsmetoder för lakvatten från soptippar. Detta projekt var finansierat av EU-kommissionens program för samarbete inom östersjöregionen, SWEBALTCOP.

Lakvatten från soptippar bildas främst av nederbörd som faller på soptippen. I många äldre tippar kan även inträngning av grundvatten ske. Det vatten som kommer in i tippen tar med sig många ämnen som finns i soptippen, när det rinner ut. Dessa ämnen kan komma från sådant som deponerats, eller bildas under nedbrytningsprocesserna i tippen. Analyser av lakvatten har påvisat innehåll av stora mängder av ämnen med känd miljöpåverkan, och även stora mängder av salter och andra vanligt förekommande ämnen.

För att utvärdera lakvattenbehandlingsmetoder byggdes en försöksanläggning i Kristianstad. P.g.a. den ökande oron för organiska miljögifter, så som PCB och flamskyddsmedel, skulle behandlingsmetoderna utvärderas med fokus på sådana eller liknande miljögifter. Analyser av dessa ämnen är komplicerade och tidskrävande, vilket gör dem mycket dyra. Det är mycket resurskrävande att använda konventionella analysmetoder för att få tillräckligt med data för att kunna utvärdera effektiviteten av behandlingsmetoder på ett tillförlitligt sätt. Därför används vid många undersökningar idag ofta endast generella parametrar för att uppskatta innehållet av organiska ämnen. Dessa metoder ger ofta endast vag och oklar information om det egentliga innehållet i lakvattnet. Därför togs under avhandlingsarbetet ett utvärderingsprotokoll fram, och metoder utvecklades (artikel I) för att effektivt kunna bestämma (analysera) organiska miljögifter i lakvatten och andra

avloppsvatten. I detta protokoll ingår även analyser av flertalet andra parametrar, samt ett toxicitetstest för att få tillräcklig kunskap om lakvattnet och få förståelse om processerna som sker i dessa behandlingsmetoder. Artikel II beskriver försöksanläggningen och utvärderingen av den. Det visade sig att förbehandling med luftning och sedimentering är viktigt för att minska bl.a. metallinnehållet. De mer aggressiva behandlingsmetoderna, som oxidation med ozon eller Fentons reagens (tvåvärt järn och väteperoxid), var effektiva mot miljögifterna, men även filtermetoder baserade på torv och kolaska fungerar bra om de är rätt uppbyggda. I filtren ökade effektiviteten ytterligare där man kunde påvisa att bakterier börjat växa. Kunskapen från pilotanläggningen i Kristianstad, har kunnat användas vid utformandet av fullskaleanläggningar i Kalmar och Halmstad.

I och med svårigheten att analysera miljögifter är det lätt att missa några ämnen, som i olyckliga fall skulle kunna vara högtoxiska. Därför är det bra att ta med test, som på ett objektivt sätt kan mäta giftighet. Sådana test är olika typer av toxicitetstester. Lakvatten innehåller höga halter av salter, vilket i sig är giftigt för många av de organismer som vanligtvis används i dessa tester. För att hög salthalt inte skulle kunna dölja förekomster av andra toxiska ämnen, utvecklades ett toxicitetstest i artikel III baserat på det salttåliga kräftdjuret *Artemia Salina*. I artikel III, togs det även fram en enkel procedur för fraktionering av innehållet i vattnet, för att enklare kunna spåra vad i vattnet som är giftigt för organismen.

Att analysera miljögifter är komplicerat och man ser bara de ämnen som finns i det lilla ”analytiska fönster” som man öppnar med sin metod. Organiska miljögifter finns vanligtvis endast i mycket låga koncentrationer i vatten. Det är inte ovanligt att koncentrationer ligger under miljarddelar (1 ppb = 1 miljarddel = 1 microgram per liter vatten). Även med denna låga koncentration finns det många substanser med miljöpåverkande egenskaper. Därför behövs effektiva metoder för att kunna mäta låga koncentrationer och för att hitta intressanta ämnen bland alla andra störande ämnen, som ofta finns i betydligt högre koncentrationer i autentiska prover. För att få miljögifterna i mätbara halter, rena bort störande ämnen, och få provet i ett format som går att analysera i ett instrument använder man sig av provupparbetning.

Efter att provet är upparbetat används en del av det för slutlig bestämning med ett analysinstrument. För organiska ämnen är dessa instrument vanligtvis en gaskromatograf (GC) eller en vätskekromatograf (HPLC), där ämnen separeras på en kolonn efter deras specifika egenskaper. Man utnyttjar att ämnena fördelas olika mellan två faser, en rörlig fas som passerar genom kolonnen och en stationär fas. Beroende på hur mycket tid ämnena tillbringar i den rörliga fasen jämfört med den stationära fasen, tar det olika tid för dem att transporteras genom den kolonn där stationärfasen finns. När de kommer ut ur kolonnen utnyttjar man skillnader i fysikaliska och/eller kemiska egenskaper hos ämnena och den rörliga fasen, för att få kunna registrera en signal i en detektor. Denna signal ritas ut

mot tiden i vad som kallas ett kromatogram. Ju högre koncentration av ett ämne, desto större blir signalen från detektorn, och man får en topp i kromatogrammet. Toppens area eller höjd härleds sedan till koncentrationen på ämnet.

En undersökning bland laboratorier har påvisat att provupparbetningen tar hela 61 % av den totala tiden för analys av ett prov, medan själva slutanalysen endast tar 7 %. De metoder som används för provupparbetning idag består i regel av flera separata och manuella steg och de är mycket arbetsintensiva och tidskrävande. Dessutom förbrukas vid deras användning ofta relativt stora mängder av dyra lösningsmedel som är potentiellt farliga för hälsa och miljö. Dessutom är provupparbetningen och även laboranten själv en stor källa till fel vid analyser. Automatiserade metoder, eller metoder med få manuella steg, minskar inflytandet av dessa felkällor. För att komma tillrätta med dessa brister inriktades avhandlingsarbetet på att utveckla nya effektiva provupparbetningsmetoder.

De i huvudsak automatiserade metoderna för olika organiska miljögifter som utvecklats och använts i artikel I-II, IV-VII, har visat sig kunna mäta mycket låga koncentrationer på ett stabilt sätt, är avsevärt snabbare, och använder endast en bråkdel av mängden lösningsmedel, jämfört med de konventionella metoderna. Som exempel kan nämnas metoden för att analysera PCB som utvecklades i artikel IV, där man kunde mäta 0.002 – 0.003 mikrogram PCB per liter vatten efter endast 10 minuters extraktion, och med endast en bråkdel av lösningsmedelsförbrukningen jämfört med en konventionell metod. Att utföra samma extraktion med den konventionella metoden tar ungefär en halv dag. Resursbesparingen dessa nya metoder ger, gör att man enklare och oftare har möjlighet att inkludera dem i ett utvärderingsprogram för att få bättre och mer detaljerat underlag.

Till min familj

Anna

Sanna & Lina

ABBREVIATIONS

ANOVA	Analysis of Variance
AOX	Adsorbable Organic Halogens
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DAD	Diode Array Detector
DOC	Dissolved Organic Carbon
E	Extraction Efficiency
E_e	Enrichment Factor
ECD	Electron Capture Detector
EI	Electron Impact
EPA	Environmental Protection Agency
FID	Flame Ionisation Detector
GC	Gas Chromatography
HF	Hollow Fibre
HPLC	High Performance Liquid Chromatography
ICP-AES	Inductively Coupled Plasma - Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma - Mass Spectrometry
K_D	Partition coefficient
LC	Liquid Chromatography
LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
MDL	Method Detection Limit
MMLLE	Micro-Porous Membrane Liquid-Liquid Extraction
MS	Mass Spectrometry
MSW	Municipal Solid Waste
OCP	Organochlorine Pesticides
PAH	Polycyclic Aromatic Hydrocarbons
PBDE	Polybrominated Diphenyl Ethers
PCB	Polychlorinated Biphenyls
POPs	Persistent Organic Pollutants
PTFE	Polytetrafluoroethylene
RSD	Relative Standard Deviation
SBSE	Stir Bar Sorptive Extraction
SFE	Supercritical Fluid Extraction
SIM	Single Ion Monitoring
SLM	Supported Liquid Membrane
SPE	Solid Phase Extraction
SPME	Solid Phase Micro Extraction
TOC	Total Organic Carbon
VOC	Volatile Organic Compounds
β	Phase Ratio

TABLE OF CONTENTS

1. GENERAL INTRODUCTION

During the history of mankind, humans have always produced waste. And so far, along with the development of more and more complex society, the amounts and complexity of the waste produced have more or less constantly increased. Even in the early days the produced waste was often taken care of and placed in special kitchen middens. Even today this form of piling up of waste is still the dominating waste management strategy, even though it is classified as the lowest ranked in the waste disposal hierarchy. According to the European council directive 1999/31/EC [1] the deposition of waste on landfills should as far as possible be minimised in order to reduce the environmental impact.

The total production of waste in Europe is estimated to about 3000 million tones per year of which 10 % is municipal solid waste (MSW) produced mainly by the households [2]. About 1 % of the total waste production in Europe is classified as hazardous waste [2]. The fifth environment action programme [3] of the European Community had set as a target to stabilise the municipal waste generation in the European Union (EU) at the year 1985s level (300 kg/capita) by year 2000. This target has been significantly overdrawn in almost all countries. In the sixth environment action programme [4], which provides the strategic framework for the commission's environmental work during 2002 - 2012, no quantitative waste targets have been included. The data collected shows that the amounts of waste generated per capita still increases, thus also increasing the problem of waste disposal. The waste disposal problem is more pronounced in the more densely populated areas in central Europe.

Incineration of waste combined with energy production is in many cases a better alternative than landfills [5]. However, the public opinion might not always be in favour of this option. Additionally, since the incineration procedure also produces several known contaminants like dioxins and concentrates and releases heavy metals, expensive filters need to be installed. These, together with ash and in-combustible residues, which constitute about a quarter of the original weight of the waste, still need to be disposed on landfills.

Both disposal of waste on landfills and incineration procedures are well known to have negative environmental impact, and thus the European Union has set up a firm strategy for

waste management, where the key factor is the prevention of waste production, in order to reduce the environmental impact [6]. This should be done by awareness and responsibility in all stages of the society, from authorities to the producers and the consumers. The recycling and reuse of materials are important parts of this process. In this strategy, it is also stated that when waste anyway *is* produced, the optimum procedures for final disposal should be used and the monitoring should be improved.

Environmental contamination of groundwater and surface water from landfills is documented [7]. In Sweden studies have demonstrated ecological effects in lakes downstream of landfills, which are supposed to be related to the landfill activity in the vicinity [8, 9]. Taken into consideration that only in Sweden there is about 500 active landfills and about 6000 closed ones [10, 11], one can imagine a large environmental impact. Many old or closed landfills have no protective barrier towards the surrounding environment, except for the top cover. In many cases the ground water can penetrate the waste layer, giving potential for long range transport of potentially hazardous compounds.

Today there is generally good knowledge about the environmental impact of inorganic and water quality parameters, since well established and efficient analytical methods for these parameters have been available for a relative long period of time. The knowledge of the impact and composition of leachate regarding the organic pollutants is not as well developed. One major reason for this might be the complexity, and hence the cost, of the analytical procedures for this large group of contaminates. Further more, many of these compounds only exist at very low concentrations, but even though the concentrations of a compound might be low, the environmental impact can be high. Also the vast number of potentially hazardous, chemical substances present in the leachate makes the tracing of the villain of the piece hard. To address this problem there is a need for developing quick, reliable and cost effective methods for analysis or monitoring of organic pollutants.

1.1. Objectives

The main objective of this thesis is to simplify and improve the strategy for monitoring organic pollutants in environmental waters, such as leachate water from landfills where the complexity of the samples is very high. The strategy should be generally useful for characterisation of waste waters, but especially well aimed to follow trends and variations in efficiencies of different treatment procedures, to give reliable data regarding the total behaviour of the treatment procedure. Since cost effective and efficient methods already were available for water quality parameters and metals, the work in this thesis has especially been focused on the analysis of organic pollutants. Here the sample preparation usually is the bottle neck in both workload and expense.

In **paper I-III** the evaluation and monitoring strategy is developed and tested. The proposed strategy is applied on the evaluation of the efficiency of different treatment processes in a pilot plant for local treatment of leachate water from Härlöv landfill, the MSW deposit outside Kristianstad, Sweden.

Paper IV-VII are focused on developing fast and efficient sample preparation methods for different organic pollutants that might be expected in complex contaminated waters. The development of efficient sample preparation methods for these organic pollutants is essential, in order to facilitate characterisation and monitoring of the behaviour of these groups in our environment.

2. LANDFILL LEACHATE

Leachate water is formed when water percolates the waste in a landfill cell. The water can originate from rain, melting snow, inflow from groundwater or from the water content of the waste it self. Modern landfills should have liners to prevent leachate from reaching the surrounding groundwater, and to prevent groundwater from reaching the landfill. Modern landfills should also have a well designed leachate collection system and often also a system for collecting the gas formed in the landfill, which can be used e.g. as fuel for vehicles.

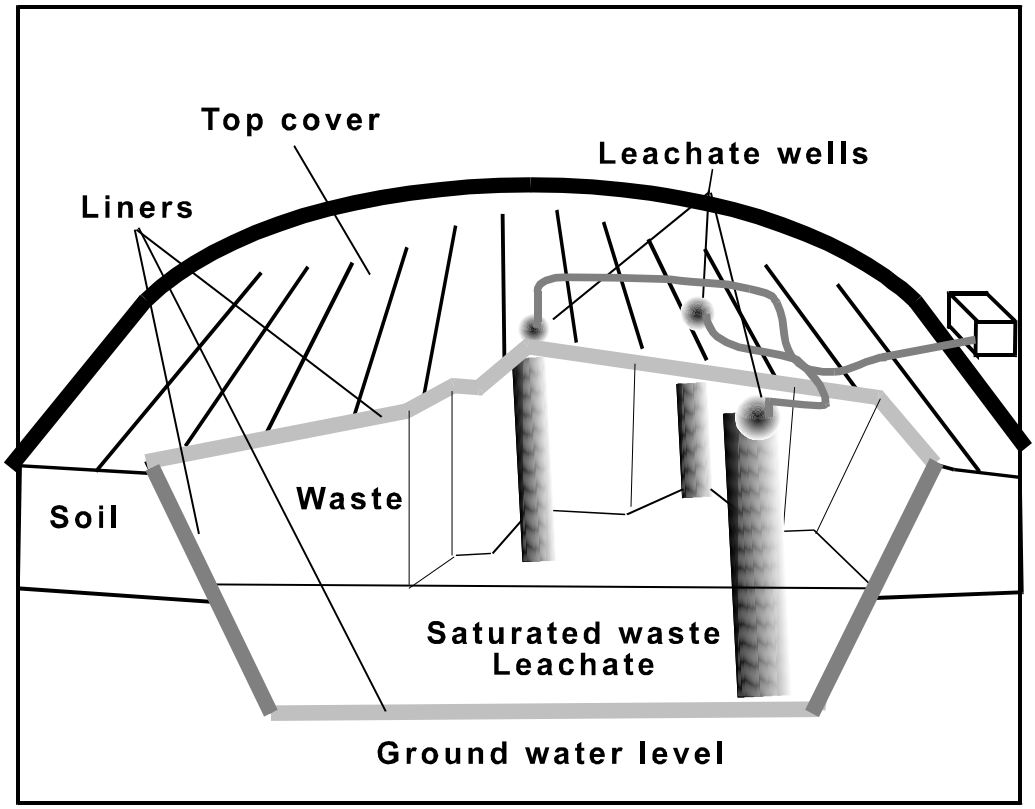

Figure 1. A covered landfill cell with wells for leachate collection.

One design (shown in Figure 1) of leachate drainage is to evenly place wells across the landfill area. In these wells, leachate is collected and intermittently pumped to the main leachate pipeline. The wells are often combined with drainage pipes across the landfill area. The intermittent pumping from different wells across the landfill can make the composition of the out-flowing leachate from the landfill vary greatly even within small time intervals, due to different waste composition and age in different parts of the landfill. This is a factor that needs to be accounted for, when sampling from landfills.

As the leachate percolates the waste, different groups of compounds are transported in the landfill, such as metals, organic and inorganic compounds that originates from the waste itself, biodegradation products or products from chemical reactions of existing compounds. Thus, the chemical composition of leachate is very complex and it is very much dependent on the type of waste deposited in the landfill, but also on the age of the landfill, the local climate and the design of the landfill.

High concentration of salts and metals together with a vast number of different organic compounds, e.g. polycyclic aromatic hydrocarbons (PAH), pesticides, polychlorinated biphenyls (PCB), polybrominated diphenyl ethers (PBDE), phthalic acid esters (phthalates), a variety of phenolic compounds, and many, many other compounds, have been reported [7, 12-25]. As an example it is worth mentioning that more than 400 organic pollutants where found in an investigation of leachate from 13 landfills for non-hazardous waste in the US [7].

Normal MSW deposited in the landfill generally contains more than 60 % organic matter, of which about two thirds are classified as biodegradable, e.g. food and garden waste, but also more moderate biodegradable products like paper, wood, textiles etc. The remaining third is classified as recalcitrant [26, 27]. Decomposition of the waste occurs through a combination of physical, chemical and most significant, biological processes, where the biological processes to a large extent control the chemical and physical ones.

2.1. Waste decomposition in landfills and Leachate Characteristics

The knowledge of waste decomposition in landfills arises from the control and monitoring of existing landfills and waste cell experiments. The decomposition in a landfill is expected to go through eight different defined phases. However, phase V – VIII are so far only theoretical and speculative, and not much data have so far proved their plausibility, due to the fact that data from existing landfills show that they still only have reached phase IV [24]. Below follows a short description of the different stages with focus on leachate production and composition.

2.1.1. Phase *I* – Aerobic

In the very beginning of the landfill life cycle there is still oxygen trapped in voids in the compacted waste. The oxygen is quickly consumed and carbon dioxide is produced. This first phase lasts only a few days, since no new oxygen is transported into the compacted waste, and only small amounts of leachate are produced, originating from the waste itself. This leachate is extracted when the waste is compacted or through precipitating water through channels in the waste. The chemical composition of the leachate very much reflects the waste deposited.

2.1.2. Phase *II* – Anaerobic Acidic

Once the oxygen is consumed, the interior of the landfill becomes anaerobic. Under these conditions fermentation processes start and much of the deposited waste is degraded. The dominating bacteria flora in the leachate is hydrolytic, fermentative and acetogenic and thus an accumulation of e.g. carboxylic acid will decrease the pH of the leachate. The acidic leachate formed during this phase is quite chemically aggressive and dissolves many components, hydrolysed materials etc. Due to this, the concentrations of several inorganic components, as well as of several organic compounds as easily degradable volatile fatty acids, are relatively high. High concentrations of small organic compounds are found in the leachate in phase II. It has been reported that more than 95% of the dissolved organic carbon (DOC) in leachate from a landfill in the anaerobic acidic phase consists of volatile fatty acids [28]. The biochemical oxygen demand (BOD) and chemical oxygen demand (COD) will be highest at the end of this phase, and the ratio BOD/COD is expected to be above 0.4. The onset of Phase II can last from one to more than nine years. The high load of organic compounds and the aggressive nature of the leachate make it desirable to control the landfills in a way that they, as soon as possible, progress to the next phase in their lifecycle.

2.1.3. Phase *III* – Initial Methanogenic

When the pH of the waste becomes sufficiently neutralised, the accumulation of carboxylic acids in phase II initiates the growth of methanogenic bacteria, which starts to consume the low molecular weight acids. The decomposition of cellulose and hemicellulose

also begins. As the carboxylic acids are consumed, the pH will increase and the BOD/COD ratio will decrease. As the pH increases, significant amounts of methane and carbon dioxide are formed, and many other low molecular weight organic components are produced and potentially emitted to the environment through gas and leachate [29].

2.1.4. Phase IV – Stable Methanogenic

As the landfill ages, it will come in to phase IV, the stable methanogenic phase, where the methane and carbon dioxide production will reach its maximum and then decline as the concentration of easily degradable organic compounds decreases. The hydrolysis of cellulose and hemicellulose will continue to supply the methanogenic bacteria with substrate, and a quite stable methane and carbon dioxide production can be observed for a very long time. As the carboxylic acids and other small organic compounds are consumed in about the rate they are produced, the level of BOD will be low compared to phase II, and the organic compounds present will be the more recalcitrant. The BOD/COD ratio will decrease to below 0.1, and thus the relative concentration of persistent organic pollutants (POPs) compared to easily degradable organic compounds will increase. The consumption of acids will turn the pH to neutral or slightly basic. The concentration of several inorganic compounds and metals in the leachate will decrease during the stable methanogenic phase. The higher pH will lead to precipitation of these constituents and the lower concentration of complexing organic compounds will keep them more stabilised and thus not as mobile as before. Low levels (<0.02 %) of the total amount of heavy metals deposited on landfills are leached during a time period of 30 years [24]. Sorption and precipitation are thought to be the main reason for this. It is well known that sulphide and carbonates, which are present in leachate, form precipitates with very low solubility with many metals. Some metals also form hydroxides with low solubility. Also soils and many organic matters present in the waste have significant sorbtive capacity, especially at the prevalent pH in this phase. The concentration of heavy metals in many methanogenic leachates is thus relatively low, and the concentration is even in many cases below the limits for US drinking water standard. However, the metal composition of the leachate needs to be monitored. Changes in the landfill may trigger release of bound metals.

2.1.5. Phase V-VIII

No present monitored landfill has yet come in to any of these phases, and their existence is based on theories. The different phases will not be presented in detail; however a short description and the theory of their onset will be presented.

As the degradable material in the waste minimises, the overpressure in the landfill caused by the production of methane will decline, air will start to intrude the waste in the landfill and the methane starts to oxidise. The oxygen will rapidly be consumed in the beginning of this process, which thus mainly will occur near the surface of the landfill. While the oxygen is consumed the nitrogen content will increase through out the waste. With time, oxygen penetrates further into the waste and some materials that have not been decomposed during the anaerobic conditions will start to oxidise under the more aerobic conditions. The formed carbon dioxide together with oxidation of reduced nitrogen, sulphur and iron will most likely decrease the pH of the leachate. The pH decrease will be buffered by the solubilisation of precipitated carbonates. This will release more and more previously precipitated metals to the leachate. With a lower pH, the solubilisation of several metals from the landfills will perhaps increase dramatically. However, calculations based on the alkalinity of the leachate, suggested that the buffer capacity in the landfill would be enough to keep alkaline conditions for more than the 2000 years Belevi and Baccini had as a time limit in their assessment [30]. This imposes a slow and diluted leaking of the metals.

The decomposition of organic compounds will leave only the most recalcitrant compounds in the residues. Most of the organic content in the waste will have left the landfill mainly through the decomposition into methane, carbon dioxide, or other organic compounds in the gas phase and as leachate. Many of the persistent organic compounds are however hardly sorbed to other materials such as disposed carbon of foam products, preventing them from leaching or decomposition. This might lead to an extended lifetime for many compounds. A total depletion of organic matter will take very long time, even more than half a million years under some conditions [31], which is far beyond the 30 years post closure monitoring time regulated by US EPA for MSW landfills [32].

2.1.6. Leachate – General Observations

Ammonia is formed during the decomposition of e.g. proteins. Throughout the observed phases the concentration of ammonia in the leachate is stable but rather high, due to that no reactions under anaerobic conditions transform it. Except for the continuous disposal of ammonia in the gas phase and in the leachate, it is not until the very end of the landfills lifecycle the levels of ammonia is expected to decrease through the aerobic transformation to nitrate. As we have demonstrated in paper III, and as reported by others, ammonia is often responsible for, not all, but a significant amount of the acute toxicity of leachate [33-37]. The removal of ammonia from the leachate, preferably by transformation, is thus a very important task before discharging the leachate. Aerated bio remediation procedures with this purpose are well established. As observed in paper II, and in other investigations, treatment procedures based on natural systems have also shown good ammonia removal efficiency [25, 38, 39].

The composition of leachate collected from existing landfills are to the largest extent typical for phase IV, the stable methanogenic phase, due to its long life time and the fact that the first three phases can be considered as transition phases with limited life time. The leachate from a landfill in operation can be a mixture of phase I-IV depending on the age of the different parts of the landfill and the leachate extraction system. As leachate from newer layers of waste percolates through the older waste layers, the composition can quickly change. E.g. if leachate from waste in the anaerobic acid phase, with high concentration of organic acids, percolates through an older layer with waste in the methanogenic phase, the acids are quickly consumed by the activity of the methanogenic bacteria, altering the pH, BOD/COD levels and ratio, etc.

Leachate often contains relatively high concentration of e.g. ferrous iron, and other reduced forms of inorganic compounds. If the leachate comes in contact with air, these compounds are quickly oxidised and precipitated. It is important to keep this in mind when sampling. Directly after sampling, the leachate from Härlöv Landfill was clear and had an olive oil-like greenish colour, but if not properly sealed, it quite quickly turned brown and immiscible, full of reddish brown hydrated ferric oxide precipitate.

$$4\ Fe^{2+} + O_2 + 10\ H_2O \rightarrow 4\ Fe(OH)_3 + 8\ H^+$$

However, this process is also a very important factor when considering treatment efficiencies, due to the possibility of co-precipitation of metals and other compounds. This has been demonstrated in the pilot plant in paper II and in full scale treatment systems [25, 38]. The precipitate is easily removed from the water body in calm ponds or in sedimentation tanks, as in the pilot plant in paper II.

2.2. Leachate composition

As described, the leachate from landfills reflects the composition of the waste deposited and the ageing processes in the landfill. Table 1 shows leachate characteristics for several landfills including those investigated in this thesis. As can be seen from the leachate data compiled by Kjeldsen et al. [24], the range of the concentration for the different parameters can vary several orders of magnitude.

The Swedish leachates investigated in this thesis are generally biased towards the lower concentrations in the ranges, except for chlorine, Cl^-, where they are in the midrange and dry substance (TS) where the level is high. The concentrations in the leachate from Siauliai, Lithuania are generally higher than in the Swedish leachates, and the concentration of chromium, Cr, chlorine, Cl^- and TS is even higher than any of the leachates in the compiled data. The well established tannery industry in Siauliai municipality is the likely reason for the high concentration of Cr in their leachate. The BOD/COD ratio and the pH indicate that the landfills investigated are in the stable methanogenic phase.

Table 1. Leachate composition of the leachates used in this thesis, and a compilation of leachate data from several leachates.

Parameter	Unit	Range Data compiled by Kjeldsen [24]	Härlöv landfill Sweden average 1993 - 2002	Halmstad Sweden average 2003 - 2006	Siauliai Lithuania
Mercury, Hg	µg l-1	0.05 - 160	<0.1	0.8	<0.1
Zinc, Zn	mg l-1	0.03 - 1000	0.06	0.06	170
Chromium, Cr	µg l-1	20 - 1500	15	8	2100
Nickel, Ni	µg l-1	13 - 1300	16	77	250
Copper, Cu	µg l-1	5 - 10000	20	190	43
Lead, Pb	µg l-1	1 - 5000	3.1*	8	<50
Cadmium, Cd	µg l-1	0.1 - 400	0.22*	0.7	<5
Iron, Fe	Mg l-1	3- 5500	5.9**	1.5	
Calcium, Ca	mg l-1	10 - 7200	368	30	81
Arsenic, As	µg l-1	10 - 1000	5.9*	4.6	<50
Phenol, total (phenol index)	µg l-1		57		57
PCBs	ng l-1		13*		
pH		4.5 - 9	7.2	8.1	8.3
Conductivity, 25°C	mS m-1	230 - 3500	722	470	1500
Suspended solids	mg l-1		144		54
Dry substance, TS	g l-1	2 - 6	5.1		10
Chemical oxygen demand COD_{Cr}	mg l-1	140 - 152000	661		1500
Biochemical oxygen demand BOD_7	mg l-1	20 - 57000***	27		13
BOD/COD ratio		0.02 - 0.8	0.04		0.01
Total organic carbon TOC	mg l-1	30 - 29000	128*		
Nitrite-nitrogen, NO2-N	mg l-1		0.036	0.2	0.68
Nitrate-nitrogen, NO3-N	mg l-1		0.42	3.8	11
Ammonium-nitrogen, NH4-N	mg l-1	50 - 2200	248	103	630
Nitrogen, total-N	mg l-1	14 - 2500	274	138	670
Phosphorus, total-P	mg l-1	0.1 - 23	1.3		4.2
Boron, B	mg l-1		1.7		4.2
Chlorine, Cl-	mg l-1	150 - 4500	1552	1190****	4600
AOX	µg l-1	30 - 27000	327		2260
Hydrogenbicarbonate	mg l-1	610 - 7320			

* Average from raw leachate used in an eight weeks pilot plant study

** Average spring 2002

*** BOD_5

**** Single value March 2003

3. PRESENT METHODS FOR ASSESSING ORGANIC POLLUTANTS

Assessing the environmental impact of human activity is of great importance. Knowledge is needed about both the affecting systems as well as the affected ones. If suspicion about possible environmental impact arises, good and efficient tools are essential for monitoring in order to asses the impact, follow the progress, or just to be assured that everything is fine. The analysis of the chemical composition of environmental water is a large field in analytical chemistry. Today several efficient methods exist, which are used for monitoring and determination of general water quality parameters and inorganic composition.

Regarding metals, technology like inductively coupled plasma with either atomic emission spectrometry (ICP-AES), or mass spectrometry (ICP-MS) are widely used and give very precise and accurate measures even at trace concentrations. For organic compounds, where the analytical work is complicated and time consuming (expensive), summary and general parameters, being easily measured, are often used for assessing the environmental impact. This is not always a good strategy, since little information of the actual composition of the samples is obtained. Different toxicity measurements and electrochemical sensors are developed in order to make a chemical risk evaluation of the water, often with the same diffuse response as for the general parameters. Nevertheless, for screening and supervision of known waters these methods can be very useful and important.

In order to understand mechanisms and to be able to more accurately follow the actual course of events in e.g. a treatment system, more detailed and accurate information about the properties of the organic content is needed. That is not to say that all compounds always need a clear and positive identification and 100 % accurate quantification. When applicable, this approach is of course preferable, but unfortunately the costs involved would be unbearable. In many cases, as when monitoring the changes during treatment procedures, it can be sufficient to monitor a few identified marker substances, with different physiochemical properties, relying on difference measurements and semi-quantification based on some standards, followed by an adequate statistical treatment. A strategy for monitoring and evaluating the efficiency of different treatment procedures for local treatment of leachate, with focus on organic pollutants is presented below. Preceding that, a brief introduction to some of the

parameters that often are used to monitor and characterise the organic compounds in leachate is given.

3.1. General and summary organic parameters

As mentioned in the previous section, there have been several investigations exemplified by references [7, 12-24], that have characterised landfill leachate with respect to their content of identified organic pollutants. This is an important and challenging task that requires a lot of effort and knowledge about expected groups of contaminants. However due to its laborious nature and thus the time consumed and the costs involved, much of the data related to leachate characterisation is based on general and summary parameters, which, to some extent, can be related to organic contents in the leachate. These include biochemical oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC) and adsorbable organically bound halogens (AOX). Below follows a short description of these methods and what they measure [40-42].

3.1.1. Biochemical Oxygen Demand – BOD

BOD is very often used in order to monitor the efficiency of waste water treatment. BOD is a measure of the relative oxygen requirement by the water during a specified incubation period. The amount of oxygen utilised depends on the biological degradation of organic material, but also includes the amounts used for the spontaneous oxidation of e.g. sulphides and ferrous iron, which generally occur at high concentrations in leachate water.

The sample is diluted with aerated buffered water containing nutrients and, if necessary, seeded with micro-organisms. The dissolved oxygen in the sample is measured initially and then an airtight bottle is filled with the sample by overflowing. The bottle is closed and left for incubation at 20° C for 5 ($BOD_{5)}$ or 7 (BOD_7) days. After the incubation time, the remaining dissolved oxygen is measured and the BOD is reported as

$$BOD_5\left(mg/l\right) = \frac{D_1 - D_2}{P}$$

where D_1 is the dissolved oxygen in mg/l before incubation and D_2 is the dissolved oxygen in mg/l after the incubation. P is the volumetric fraction of the sample bottle used.

The samples should be incubated in darkness to prevent the possibility of photosynthetic produced oxygen. The samples should be analysed as quickly as possible after the sampling to get as unbiased result as possible. Most of the data in the literature are based on five days incubation (BOD_5), but e.g. in Sweden BOD_7 is more often reported, because it is more efficient in work planning for the laboratories.

3.1.2. Chemical Oxygen Demand – COD

COD is considered as the amount of oxygen equivalent needed to oxidise the organic matter in the sample by chemical methods. This parameter is also frequently used when monitoring water quality. It is much faster to measure than BOD and can empirically be related to BOD or TOC. The value is given in mg O_2/l.

An excess of a strong oxidation agent is added to the sample, and after reacting with the compounds in the sample, the amount of un-reacted reagent is measured and re-calculated as oxygen equivalents. In the most commonly used method, COD_{Cr}, potassium dichromate, $K_2Cr_2O_7$, is added to the strongly acidified sample and the sample is refluxed for 2 h in the presence of mercuric sulphate, $HgSO_4$. Oxidation of most organic compounds is 95 – 100 % of the theoretical values. However pyridine and aromatic compounds are only moderately oxidised. Oxidation of inorganic compounds such as ferrous iron, sulphides, and nitrite can also contribute to the COD value. The methodology for removing interfering hydrogen sulphide, HS, and sulphur dioxide, SO_2, is to purge with a stream of air through the acidified sample. This will unfortunately also remove some of the volatile organic compounds (VOC). Also, during the reflux of the sample, the VOC will, to a large extent, be in the headspace which minimises the contact time with the oxidant, and thus lowers the fraction of oxidation. To decrease this effect a catalyst, Ag_2SO_4, is added to the sample, but this catalyst form precipitates in the presence of halides, such as Cl^-, Br^-, and I^- and thus $HgSO_4$ is added as a complexing agent. Nevertheless, the procedure is not recommended for saline samples containing more than 2000 mg/l Cl^-, which is not an un-common concentration for leachate samples, where also high concentration of ferrous iron and sulphides are commonly reported.

This, together with the fact that the organic fraction in the leachate consists, to a large part, of small volatile compounds, increase the uncertainty of what COD really measures in leachate samples. Due to the consumption of mercury it has been recommended by Swedish EPA to phase out COD_{Cr} in leachate characterisation [19].

For relatively pure water, a weaker oxidation agent such as potassium permanganate, $KMnO_4$, can be used, and COD_{Mn} values are then reported. The oxidation efficiency for COD_{Mn} is often only about 40 %.

The high amounts of reduced inorganic compounds in leachate make it hard to judge which part of both BOD and COD that is really originating from the organic content.

3.1.3. Total Organic Carbon – TOC

The amount of carbon that originates from covalently bound organic compounds in the sample is measured as total organic carbon (TOC) in mg/l. TOC is a more direct expression than BOD and COD, related to the amount of organic compounds found in the sample, and the information obtained differs in character. TOC is the remaining fraction of the amount of total carbon content, (TC), in the sample, when the inorganic carbon, (IC), mostly carbonates, have been subtracted from the TC value. The part of TOC that is dissolved is called dissolved organic carbon (DOC) and is defined as the fraction of TOC that passes a 0.45 μm pore-size filter. The different fractions of the TC are presented in Table 2.

In order to measure TOC the organic molecules have to be broken down to single carbon units and converted to single molecular form, such as carbon dioxide or methane, which can be measured quantitatively by e.g. infrared spectroscopy, titration, or by using a thermal conductivity detector (TCD) or a flame ionisation detector (FID). Different approaches for the breakdown of organic compounds exist. One can utilise UV radiation or chemical oxidants, or, as in the most frequently used method, heat and oxygen. In the latter case, a small portion of the sample is injected into a heated reaction chamber packed with an oxidative catalyst. The organic carbon is oxidised to H_2O and CO_2. The CO_2 is transported by a carrier gas to an analyzing chamber, where it is analysed by a non-dispersive infrared analyser.

Table 2. The different fractions of the total carbon content in a sample.

Content	**Fraction name**	
All carbon in the sample	Total Carbon	TC
Carbon from inorganic species e.g. carbonates and dissolved CO_2	Inorganic Carbon	IC
Carbon that originates from covalently bound organic compounds	Total Organic Carbon	TOC
Fraction of TOC that passes a 0.45 µm filter	Dissolved Organic Carbon	DOC
Fraction of TOC that is retained by a 0.45 µm filter	Nondissolved Organic Carbon	NDOC
Fraction of TOC that is purged away from the sample by a gas stream. Mainly originating from VOC	Purgable Organic Carbon	POC
Fraction of TOC that is not purged away from the sample by a gas stream	Nonpurgable Organic Carbon	NPOC

In most waters, the IC fraction (carbonates and CO_2) are many times larger than the TOC fraction, and since the methodology used for TOC determination also measures the CO_2 in the sample, formed by heating the carbonates, the IC must be removed in order to determine the TOC. This is generally done by acidifying the sample and purge away the formed CO_2 with a stream of gas. However this procedure will also remove a large part of the VOC, and thus the measured and reported TOC will in many cases instead be NPOC. For groundwater and many surface waters the VOC levels are low and their contribution to TOC is negligible, thus justifying this source of error. However, for leachate the very high fraction of VOC will give a far greater error in the reported TOC. Also high concentration of salts, mainly sodium chloride, may interfere with the analysis. Nevertheless TOC is the most objective method concerning the organic content in comparison to the oxygen demand methods and is gaining in favour for characterisation of waters [43].

3.1.4. Adsorbable Organic Halogens – AOX

Many organic compounds with known environmental impact contain halogenated groups. Therefore the measure of adsorbable organic halogens (AOX), in µg/l - mg/l, might give a quick assessment of the contamination in a water sample.

The sample is acidified with nitric acid and the organic compounds are adsorbed on activated carbon, either by shaking or on a column with the adsorbent. The inorganic halides are competitively constricted by nitrate. The carbon is then combusted with oxygen and the formed hydrogen halides are captured in an electrolyte solution and their concentrations can be determined e.g. by titration. High level of Cl^- in the sample can interfere with the result and give an overestimation of the organic halogens present in the sample. This must be considered when interpreting the result. Poor correlation of measured AOX with identified known halogenated pollutants in leachate water have been observed [24].

3.1.5. Phenol index - Sum of Phenols

The summary method for determining phenols in waste water measures the distillable phenols that react with 4-aminoantipyrine (4-amino-1,5-dimethyl-2-phenyl-3-pyrazolone). Clean up from interfering organic and inorganic compounds are made by distillation under acidic conditions. If the distillate is turbid, extraction to chloroform and back extraction to sodium hydroxide solution, followed by re-distillation, might be needed. The distillate is then reacted with 4-aminoantipyrine in the presence of potassium hexacyanoferrate(III). The formed reddish brown compound is measured with a spectrophotometer and quantified against phenol. For determining low concentrations of phenols (< 1 mg/l), the distillate needs to be extracted by chloroform before the spectrophotometric determination.

4-aminoantipyrine reacts poorly with several para-substituted phenols unless the substituted groups are strongly polar. It is stated that 4-aminoantipyren does not react with neither 2,4-dimethylphenol nor p-cresol [44], which are found in the leachate in paper I-II. By far p-cresol had the highest concentration of any of the identified phenols in the raw leachate. The concentration difference towards the second most abundant phenol (4-chloro-3-

methyl phenol) was about 5 times. An investigation by IVL Swedish Environmental Research Institute Ltd. also showed the highest level for p-cresol, and it was suggested that the origin was from the degradation of the amino acid tyrosine, since the consumption profile for phenols does not match the findings [45]. Thus, using phenol index for risk evaluation of landfill leachate seems questionable. A far better approach should be an identification and determination of individual phenols by HPLC or GC methodology, as in paper I and II.

3.2. Toxicity - Bioassays

The tradition of evaluating different waste water effluent by some of the above described water quality parameters have more and more been accompanied by different toxicity assays in order to increase the ability to assess the environmental impact of different effluents [42, 46-51]. Chemical and physical tests can often not alone assess the potential effects on aquatic biota, especially not since the number of chemical substances that are present in leachate waters is very high. Trying to determine all of them, which may even be impossible, would demand enormous resources. Hence, the use of quick tests for screening of adverse effects is necessary, even though the reason for the effects not always is discovered. To protect aquatic life, US EPA has issued regulation and standardised methods to assess whole effluent toxicity (WET) which incorporates measurements of acute toxicity as well as of short term chronic effects of effluents that are regulated to be monitored due to their potential environmental impact [52].

A wide range of bioassays have been developed in order to asses the toxicity for aquatic organisms. The bioassays can be based on fish, invertebrates, micro-organisms, plants, or other bio-indicators. These bio-indicators are then exposed to the water of interest. Depending on the purpose of the test and the indicators, different response can be measured. Toxicity tests are also classified according to the duration: short term test for acute toxicity, intermediate and/or long term test for assessing more chronic or reproductive toxicity in a life-cycle perspective of the bio-indicator. For acute toxicity a defined effect is measured after a limited time frame, normally after 24, 48 or 96 hours. For e.g. invertebrates, as in paper III, the measured effect can be mortality or, the more easily determined, immobility. The result is then generally reported as EC_{50}; the concentration of the water, mixed into a standard reference solution, which produce an effect on 50 % of the total population [53]. As presented

in paper II, the results can also be re-calculated to either lowest-observed-effect concentration, (LOEC), the lowest toxicant concentration where a statistical significant effect is observed compared to the control sample, or no-observed-effect concentration, (NOEC), the highest toxicant concentration where no statistical significant effect is observed. LOEC and NOEC are usually reported for long term toxicity in order to estimate "safe" effluent discharge rates.

For assessing the toxicity of leachate water from landfills, several different bio-indicators from different trophic levels in the ecosystem have been used, e.g. fishes, crustaceans, plants, algae and bacteria [8, 19, 21, 33, 50, 51, 54-60]. A commonly used test method is Microtox® where a luminescent bacteria (*Vibrio fischeri*) is used, and the inhibition of luminescence is measured for different concentrations of leachate mixtures [61]. This gives a rapid toxicity assessment, normally within 30 minutes. Another commonly used toxicity test is based on crustaceans and the most frequently used are the water fleas *Ceriodaphnia dubia* [62] and *Daphnia Magna* [63], which are hatched and exposed to different concentrations of leachate for 48 hours. The dead or immobilised crustaceans are then counted after 24 or 48 hours. *Ceriodaphnia dubia* is also frequently used for assessing chronic toxicity [64].

A problem when assessing both acute and chronic toxicity is the risk that common water quality parameters, such as pH, alkalinity, salinity etc, can mask the effects from xenobiotic organic compounds of more environmental concern [65]. Since the salinity in leachate water is generally quite high, it will influence the toxicity for fresh water organisms such as *Ceriodaphnia dubia* and *Daphnia Magna*. Therefore a toxicity test using the salt durable crustacean *Artemia Salina* was developed in paper III, and tested for assessing leachate toxicity. The purpose was to develop a toxicity method that was easy to use, did not require any specific costly instrumentation, and that gave reliable and reproducible results. Hence the method should be a good option for screening of toxicity of different leachates or similar effluents. The developed method was tested for different leachates and then incorporated in the analytical protocol developed in paper I, and thereafter implemented for the evaluation of different treatment procedures for local treatment of leachate, as described in paper II.

In order to identify the main origin of the leachate toxicity, a fractionation was made with columns containing ion-exchange resins and activated carbon respectively. When

passing the ion-exchange columns, which removed e.g. ammonium and metals, the toxicity of the leachate disappeared. Tests showed that the toxicity of heavy metals, towards *Artemia Salina* was low, but ammonia showed higher toxicity. When the leachate had percolated through the activated carbon column, thus removing mainly the organic content and keeping the ammonia level constant, the toxicity also decreased markedly, but was still relatively high. This shows that the main toxicity comes from the ammonia. This was also supported by the findings in the study of the treatment pilot plant, where the treatment procedures decreasing the ammonia concentration also gave the best detoxification for *Artemia Salina*. The correlation between ammonia and toxicity is also supported by the literature, as mentioned previously in section 2.1.6. However, the activated carbon column still removed a significant amount of the toxicity which shows that there are other factors which also are toxic in the organic fraction, and synergistic effects can not be ruled out.

In a monitoring strategy, toxicity tests should be included to prevent the risk of missing harmful compounds in the analytical window. The use of a simple fractionation of the leachate in paper III gave significant amounts of extra information that was useful in assessing the environmental impact of different constituents in the leachate. This approach will help to point out the direction when choosing a proper treatment or polishing step. It is desirable that fractionation steps more generally become included as a part of the toxicity test procedures in the future. However, one should also be aware of the risk that water with no, or low, observed acute toxicity still might contain sub-lethal levels of toxicants that can accumulate [66], e.g. in biota or sediments, and thus eventually reach toxic levels.

3.3. Biosensors

The use of different biosensors in environmental monitoring is a growing field and several devices are now commercially available [67]. Biosensor technology is based on a sensing biological element connected to a transducer that converts the biological response to a measurable physical signal [68]. The biological recognition can consist of enzymes, antibodies, cell receptors, tissue etc. and the signal to be transduced can be e.g. electrochemical, mechanic, optical, magnetic or thermal depending on the biological response.

The response of biosensors can be tuned to either measure single compounds or groups of compounds like phenols [69] or PCBs [70]. It can also be correlated to water quality parameters such as BOD [71, 72] or toxicity [49, 73, 74]. The generally small nature of the actual sensing part and their often rapid responses facilitates the development of biosensor arrays, where different sensors are combined. The varying responses can be treated statistically to find trends and correlations to e.g. different water quality parameters or to the waste water quality [75].

Disposable screen-printed carbon electrodes (SPCE), where the different layers that comprise the biosensor are printed or sprayed on an insulating substrate, with accompanying simple and easy to use instrumentation, are now more and more developed [76]. The possibility of mass production and simple handling assures an increasing market for this type of analysis in monitoring of different waters.

The potential to correlate the response of a biosensor to global water quality parameters is definitely a growing field for biosensors. However, today biosensors are more suited for monitoring of well defined waters and known treatment procedures, in a more process controlling way, in order to alarm or indicate deviations from the normal system. To be able to better understand the processes in the treatment steps, and to get a more accurate evaluation, more detailed information, given by classical chemical separation methods, is needed. Generally, elaborate calibration procedures are needed for biosensors. These procedures often involve a chemical characterisation of the effluent. The use of biosensors, for screening in search of polluted waters, is also an interesting approach, but has so far not been applicable to any great extent.

3.4. Chromatographic methods

Besides the more general and summary methods for assessing the fate of organic compounds in complicated systems, there is always the option of trying to separate and isolate the substances in groups or as individual components with respect to their physicochemical properties. When monitoring organic pollutants for evaluation of treatment systems, as in this thesis, this approach gives more detailed information and thus facilitates the understanding of the processes involved in the treatment.

3.4.1. Chromatographic instruments

At the end of the 19th and beginning of the 20th century, the Russian botanic Mikhail Tswett investigated the adsorption properties of chlorophyll for a large variety (more than hundred) of different substances and the corresponding solubility in different solvents inter alia in order to separate different chlorophylls and carotin. He discovered that when "filtering" chlorophyll dissolved in petroleum ether through a narrow glass tube packed with calcium carbonate ($CaCO_2$), the chlorophylls separated and formed coloured bands in the column. He called this a chromatographic method (from Latin; colour writing), and the result a chromatogram, and he assumed that the same rules should be valid for any sort of coloured or colourless chemical compounds [77]. 1941 A. J. P. Martin and R. L. M. Synge presented a paper on chromatography based on partitioning between two liquid phases, and transferred the theory of compound distribution from distillation to a chromatographic separation theory [78]. They tested their system for quantitative analysis of amino acids, and they also postulated that very refined separation of volatile substances should be possible by flowing gas over a gel impregnated with a non-volatile solvent, thus introducing gas chromatography (GC) as a concept. Martin and Synge also foresaw that decreasing the stationary phase particle size would increase the chromatographic efficiency. This is a foundation of modern high performance liquid chromatography (HPLC).

The separation in chromatography is based on the partitioning of compounds between two phases, a stationary phase and a mobile phase. In HPLC, the stationary phase is generally positioned on the surface of e.g. silica particles packed in a column, and modern GC utilises a thin film coating the inner walls of a long narrow glass column (normally between 15 - 60 m x ID 0.10 – 0.53 mm). The sample is mostly introduced as a plug at one end of the column containing the stationary phase, and the mobile phase is then used to transport the plug of sample through the column. The mobile phase can be a liquid, as in liquid chromatography (LC), a gas as in GC or a supercritical fluid as in supercritical fluid chromatography (SFC) [79]. The time the compound spends in the mobile phase is controlled by the partitioning of the compounds between the two phases. Thus, the partitioning of the compounds controls the time it takes for a certain compound to transport through the column, measured as retention time, t_R. In an ideal case, the distribution of the compounds in the column follows a normal, Gaussian, distribution curve. At the end of the column different detectors can be used,

depending on the technique and the analytes of interest, to record the elution as peaks in a plotted chromatogram. Quantification of the analytes is normally done by measuring the area under the peak (by integration) or measuring the peak height and then calculating the concentration or amounts of the analytes by calibration curves obtained by injecting known standards.

In normal phase LC, the analytes are eluted by an organic solvent as done by Tswett in the beginning of chromatography. Nowadays HPLC is mainly run in reverse phase mode, i.e. with a polar aqueous based mobile phase and a hydrophobic stationary phase, often $C_8 - C_{18}$ hydrocarbon chains bound to silica particles packed in a stainless steel column. The elution strength, and thus the separation, is controlled by changing the composition of the mobile phase by addition of an organic modifier, such as methanol or acetonitrile. In a GC run, the elution is controlled by changing the partitioning of the analytes to the stationary phase by changing the temperature in the GC oven where the column is placed. In both GC and HPLC, changes of the elution ability are normally done during the run by changing the temperature or the mobile phase composition by time. This gives a possibility to elute a large variety of compounds with different properties within reasonable time. The concept and principles of chromatography is since long well established and will not be further discussed here. Thorough descriptions about the chromatographic principles and techniques used in this thesis are given e.g. by references [80-82] (LC) and [83, 84] (GC).

3.4.2. Detectors

Different detectors have different selectivity towards different compounds or functional groups. A large variety of detectors exist for both LC and GC, utilising different chemical or physical properties of the analytes in order to produce a measurable and quantitative signal. Below follows a short description of the detectors used in this thesis. As can be seen, some selective information about different compounds can be obtained by their detector response.

The flame ionization detector (FID) used in paper VII is a very widely used general purpose GC detector. The column effluent is mixed with hydrogen, and the mixture burns from a narrow jet tip. When hydrocarbons enter the flame, they are ionized as they are combusted and a current can be measured between the grounded jet and a collector

surrounding the flame. The current is roughly proportional to the number of reduced carbon atoms in the flame. The addition of functional groups as halogens, alcohols, and amines gives fewer ions, and thus lower current, i.e. lower detector response [85]. However, with a large amount of carbon atoms in the compound compared to hydroxy groups, amine functions, or halogens, the detector response varies only slightly between the molecules. FID can thus be used in a semi-quantitative way to get good information of the total content of volatile and semi-volatile components in a sample, without the need to determine each component individually.

The GC detector used in paper I, IV, and V, the micro electron capture detector, μ-ECD, is based on a radioactive β-emitter as ^{63}Ni. The gas effluent from the column passes the emitter and radiating electrons ionises the mobile phase gas and produce a burst of electrons. As no other compounds are present, a constant current between two electrodes can be measured. When compounds that contain electronegative groups, which tend to capture electrons, elute from the column, the current decreases [85]. It is most common to pulse the potential and instead measure the frequency needed to keep a constant current, in order to dramatically increase the linear range of the detector. The ECD is very sensitive towards compounds that contain e.g. halogens, but not at all towards hydrocarbons without electronegative groups. The ECD is one of the most sensitive detectors available for GC analysis [86].

One of the most commonly used detectors in HPLC is the spectrofotometric UV-vis detector. The column effluent with the separated analytes flows through an optical cell, where the absorbance is measured for a specified wavelength. A detector based on the same principle is, the more and more commonly used, diode array detector (DAD), where the light passes a grating or a prism where the different wavelengths are separated and projected on to an array of diodes, which continuously can record a whole UV-vis spectrum [85]. A DAD detector was used in paper I and II.

Mass spectrometry (MS) is nowadays widely used as a detector after chromatographic separation. The basic concept of mass spectrometry is the separation of components, that have been charged, with respect to their mass-to-charge ratio, (m/z). MS can be used for both

qualitative and quantitative analysis. A MS detector is built up by the following basic components [85, 87]:

- Inlet – where the column effluent enters the high vacuum chamber of the detector.
- Ion source – where the analytes are ionised.
- Mass analyser – where the components are separated according to m/z.
- Detector – where the components are detected after m/z separation.
- Vacuum pumps, foreline and high vacuum – is needed to keep high vacuum in the ion source and mass analyser, in order to keep an interference free ionisation, separation and analysis.

In GC it is possible to directly introduce the analytes into the ion source, as they elute from the capillary column. To do so, the carrier gas flow must be set low enough to keep the desired vacuum [88]. Regarding LC, it is more problematic since the effluent is a liquid that produces large amounts of gas as it evaporates in the vacuum. Several different approaches to address this problem are available [89, 90], but will not be addressed here since LC-MS has not been used in this thesis.

The most frequently used ionisation method in GC-MS is electron impact, EI, where the analytes introduced into the ion source are bombarded with electrons at 70 eV [88]. The electrons knock away other electrons from the analytes, and these are fragmented into neutral and positive fragments. Each compound can be fragmented several times, and the positive fragments are accelerated by a potential over a repeller, through a set of lenses into the mass analyser, where the mass analyser parameters are set so that only a certain m/z value can pass each defined time. The fragments that pass the mass analyser are detected by the detector, which can be an electron multiplier. By scanning from low to high m/z a mass spectra is obtained, showing the fragments that are formed from molecules that constitute a chromatographic peak. The data is compiled as a chromatogram, usually showing the total ion current (TIC), where it is possible to get a mass spectrum from each data point in the total ion chromatogram. In this case, the MS detector will render a powerful three dimensional data set that can be used for identification of known and even unknown analytes in each chromatographic run. In the latter case, comparison with fragments obtained from a library or known standard is needed.

Several different types of mass analysers exist, such as magnetic sector-, ion-trap-, time-of-flight-, fourier transform ion cyclotron resonance- and quadrupole mass analyser [85, 87, 89-91]. They will not be reviewed in detail herein, except for a brief description of the quadrupole mass analyser, which has been used in paper I, II, V and VI. The basic quadrupole consists of four parallel rods where potential and radio frequency are applied. This generates movement among the ionised fragments entering the analyser at one end of the rods, accelerated by the ion source. Depending on the m/z, the fragments respond differently to the influence of the systematically changing field strength, mainly due to the inertial mass. At a given time, only a specified m/z can pass the mass analyser.

Since molecules fragment into very specific patterns reflecting their structure, several libraries based on 70 eV EI mass spectra are available to help identifying unknown compounds [92, 93]. When screening for unknown compounds as in complex environmental samples, a GC-MS is a good and well-tried option. Unfortunately it more or less limits the results to molecules ranging from small to medium size, which are volatile to semi-volatile and include non-polar to moderately polar compounds. For stronger polar volatile or semi-volatile compounds, the GC performance is often too bad, and a modification of the structures of such target analytes is needed before their determination is possible. A full m/z spectra takes time to perform, since the instrumentation needs to scan through the different masses. The larger the scan range the lower the sensitivity obtained, since less time is spent on measuring each fragment, i.e. less amount of each fragment will enter the electron multiplier detector. A normal GC-MS detector in scan mode is thus a quite insensitive instrument, which unfortunately is complicating the identification of unknown organic compounds present at low, but not safe, concentrations in e.g. landfill leachate. Trying to address this problem by increasing the time spent on each scan will quite rapidly render the chromatogram useless, since too few data points will be collected. The peak width of an analyte will be close to, or less than, the time for a single scan. Several data points are needed in order to nicely and quantitatively reproducibly plot a chromatographic peak. Having to large scan time can also make the mass spectra inaccurate since the analyte might elute away, or new analyte elute in, before the scan is finished. There is also the risk of co-eluting peaks that can complicate the identification, if the chromatographic separation is insufficient.

In order to increase the sensitivity of the MS detector, a limited number of m/z can be selected to be monitored for different given time windows of the chromatographic run. This is called to run the MS in single ion monitoring (SIM) mode. By selecting three highly abundant ions in the spectra for each analyte of interest, and setting the instrument to scan only these as the specified analyte elutes from the column, it is possible to increase the sensitivity several orders of magnitude. The relation between the selected m/z should be compared to the relation in a standard spectra, and it should be assured that they have constantly the same relation as in the spectra (within ±10 – 20%, depending on relative abundance), in order to have a good identification, and secure that no interfering compounds co-elute [94, 95]. A MS detector in SIM mode is very selective (you only see what you want to see, more or less) and sensitive. In paper I, II and VI, GC-MS in SIM mode is used to secure very sensitive and interference free determination of POPs, whereas in paper V scan mode was used for positive identification of the abundant analytes. SIM mode can only be used for already known compounds, where defined methods and standards are used. It is a powerful tool for determination and identification of a limited number of known analytes in a sample.

The use of a high resolution mass spectrometer (HR-MS) can dramatically increase the sensitivity, since it is capable of only seeing the very exact specific masses and isotopes of a fragment, which reduces the noise, thus reducing the limit of detection (LOD). A HR-MS instrument widely used is the triple sector, electrostatic-magnetic-electrostatic-sector, MS. Analysis at very low levels of compounds, like extremely toxic dioxins, requires the use of HR-MS to achieve the necessary LODs. Instrumentation like this are very sophisticated and expensive, they require trained personnel and have large maintenance requirements [96], which unfortunately limits their use. Another drawback of HR-MS instruments is their relative slow scanning (about 3 scans/s), which also somewhat limits their use for scanning of unknown compounds. Thus they are mainly used for determination of known pollutants.

3.4.3. Sample preparation

An essential aspect when analysing organic pollutants in complex matrixes is the sample preparation. To be able to analyse the samples on a conventional instrument, GC or HPLC, the samples need to be prepared, for instance by removing interferences, pre-concentrating the analytes, and to assure that they are in a medium suitable for the chosen final analysis. In

order to identify unknown pollutants, effective sample preparation methods with high pre-concentration are often necessary. However, one should be aware of, that already the selection of sample preparation method gives a clear discrimination of the analytes expected to be found in the final analysis. Sample preparation for analysis of organic compounds will be dealt with in detail in section 5.

4. MONITORING STRATEGY FOR ORGANIC POLLUTANTS

Environmental monitoring is a continuously growing field in analytical chemistry [97]. The strategies for the analytical work vary depending on the purpose of the environmental monitoring. In general environmental monitoring can be divided into the following categories:

- Quality and emission control programs. – Monitoring of known parameters in known media to ensure that regulations and guidelines are followed and to assess the extent of known emissions.
- Pollutants fate assessment. – Tracing known pollutants from known sources to determine the fate and transport of pollutants in the environment.
- Pollutant source identification. – Monitoring of pollutants in order to identify the source of emission.
- Environmental screening. – Screening of the environment for known or new suspected pollutants in order to evaluate their occurrence.
- Estimation of environmental impact. – Screening of known pollutants or known sources in order to find correlation to environmental responses.
- Evaluation of treatment procedures – Investigating treatment procedures in order to evaluate their effectiveness towards pollutants.

Quality and emission control can be done when known emissions and known procedures are monitored, as in sewage treatment plants or factory effluents. This is usually performed using existing standard methods, and evaluated by direct comparison to historical data and regulations. Pollutants fate and source assessments are also generally investigated using existing methods with grab sampling in a limited geographical region, whereas the environmental screening can cover national scale or even be worldwide. For the estimation of environmental impact the research on passive or/and equilibrium sampling devices is an interesting and growing field [98-100].

Regarding the evaluation of treatment procedures, it is important to follow markers in order to obtain understanding of the treatment mechanisms and their influence on treatment efficiency. As was shown in paper II, concerning phenolic compounds in the biological

remediation, it is not always sufficient to only monitor groups of compounds, since valuable information can be missed, see below in section 4.1.5. The use of conventional standard methods for analysing organic pollutants for investigation of a range of different treatment procedures requires huge resources. The purpose of this thesis has been aimed to set up an efficient, cost effective strategy and methodology for such monitoring.

When monitoring polluted waters and especially when evaluating treatment efficiency, it is of great importance to have a clear strategy, which includes measurements of a variety of parameters of environmental concern. As mentioned, it is also important to select parameters that can give information and understanding of the processes that controls the efficiency of the treatment. Until today, most treatment processes are, with respect to organic pollutants, only evaluated by general parameters as COD and BOD. As described above, this hardly provides any information of the organic constituents of the monitored water, and gives small contributions to our understanding of the removal processes in the treatments procedures.

4.1. Analytical protocol for leachate treatment evaluation

An objective with the project was to, within reasonable cost, provide more detailed information regarding organic pollutants in leachate water. With this in mind an evaluation protocol was developed in paper I and implemented in paper II. The evaluation protocol was named the Laqua protocol after the name of the project it was developed within, a project for development of ecological and financial sustainable treatment methods for local treatment of leachate from waste deposits, which was financed by SWEBALTCOP, a European Commission programme for Baltic region cooperation. The protocol is presented in Figure 2 and in paper I, and will be discussed here with focus on landfill leachate treatment. However, it should be pointed out that this protocol is a dynamic tool which can be adjusted to include interesting and important parameters, or to exclude parameters that yield little information, in order to suite other types of waste waters or investigations.

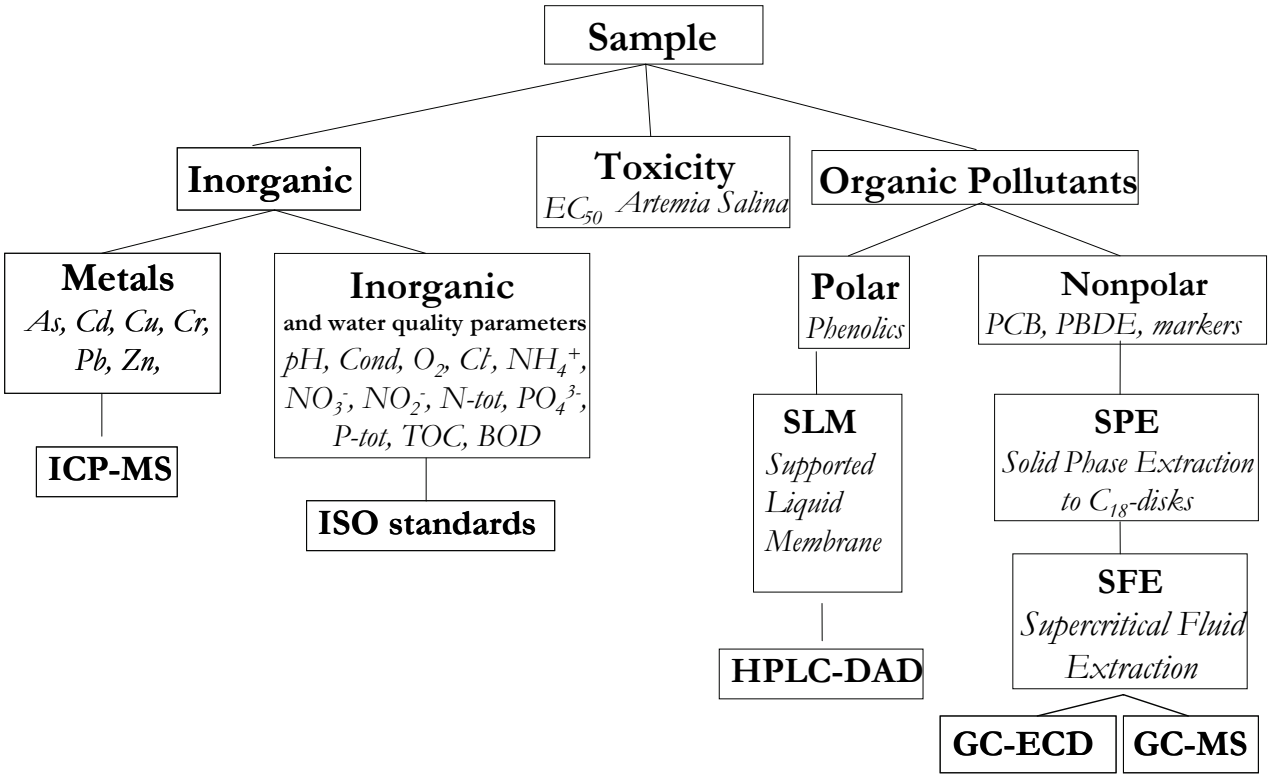

Figure 2. The Laqua protocol; an analytical protocol for evaluation of treatment procedures with focus on organic pollutants.

4.1.1. Sampling

In order to get representative samples they should be taken by time integrated sampling methods, e.g. as described in paper I, where intermittent pumping to temporary collection vessels was used. Aliquots from these were taken on a daily basis and stored in a refrigerator before analysis. Integrated sampling is necessary in order to compensate for the possible variability of the inflow to a treatment plant by time, depending on several factors like the construction of the leachate drainage system and precipitation. The diverse hold-up times for different treatment systems could also greatly influence the sample composition if only grab sampling were taken.

As seen in Figure 2, the samples collected were divided into three main categories: inorganic and water quality parameters, toxicity, and organic pollutants. Each sub-sample was

analysed on a weekly basis. For a long term monitoring process, this time for evaluating the system might be increased to a considerably longer time period. However, the actual sampling period should not be prolonged significantly, since the analysed samples should be reasonably fresh. An option could be to deep-freeze the intermittently collected sample portions temporary, and thaw and mix them before taking the integrated sample.

4.1.2. Inorganic and water quality parameters

Even though the focus of the evaluation of the treatment efficiency in paper I and II was on organic parameters, it is still absolutely necessary to monitor general inorganic and water quality parameters to get a good basis for any action program. The measurement of all nitrogen parameters have been shown to be very important in understanding the treatment procedures. As been shown in paper II, increasing efficiency towards removal of organic pollutants, in bio-remediation systems and in natural filter procedures, is obtained as the extent of de-nitrification increases. The de-nitrification is an indication of biological activity. This has also been confirmed in a full-scale treatment plant [39]. The increase of biological activity can be monitored by a decrease of ammonia, NH_4^+, and an increase of nitrate, NO_3^-.

Also general parameters as BOD and TOC are included among the water quality parameters. As described above, they are more general water quality parameters, than indicators of what is the real fate of the organic compounds entering a treatment process. The nature of leachate water makes it hard to assess what the BOD really measures. For treatment procedures based on natural materials, such as peat, TOC can even increase by the release of humic substances or other organic macro molecules of little environmental concern. BOD and TOC should however be included for a general characterisation of the water and for comparison with historical data. COD is intentionally left out due to environmental concern. The chloride content of leachate water would require the use of large amounts of $HgSO_4$ for removal of interferences in COD determination. The unspecific analytical response also fortifies this [97].

Due to their known environmental impact and occurrence, metals always need to be monitored. The selection of which metals to monitor should be based on historical information, metals of expected interest, or on previous screenings of the effluent entering the

treatment plant. With modern analytical techniques, as ICP-AES or ICP-MS, it is possible to measure a larger number of different metals simultaneously, without a noteworthy increase in cost or time compared to previous methods, which measure the metals one by one. The metals included should also be able to reflect any contamination that could occur from the different treatment procedures. For example, the increasing levels of arsenic at the outlet of the treatment plant (in paper II) from the carbon containing ash were expected. However, also contamination of cupper from the ozone treatment step, and zink from the bio-remediation step was discovered. These experiences point out the necessity to carefully consider the choice of materials when constructing treatment equipment. The materials used for tanks, vessels, and valves should be as inert as possible with consideration to the economy. For long term monitoring, metals are also important to include for other reasons. Events such as pH drop in a landfill might trigger the release of bound or precipitated metals.

4.1.3. Toxicity

Toxicity is an important parameter to measure, even though the interpretation of the results can be hard. Toxicity measurements act as an extra safety valve, not omitting any potentially harmful compounds in the chemical characterisation of the leachate. In Figure 2, an easy-to-use acute toxicity test, with the saline durable crustacean *Artemia Salina,* is included. This test was developed in paper III. The major reason for the toxicity of investigated landfill leachates has turned out to be ammonia. This was found out by the simple fractionation procedure described in paper III. Such fractionation of an original sample can assist when tracing the origin of the toxicity. Nevertheless, for future studies it would be recommended to also include longer term based toxicity tests, in order to assess potential chronic or endocrine toxicity. Chronic toxicity can be measured by growth, reproduction, hatchability and survival of aquatic organisms exposed to a series of diluted leachate for time periods lasting up to 7 days [64]. The use of *Artemia Salina* for chronic testing could also be investigated further. A good approach to assess endocrine disruption is by monitoring the vitellogenin gene expression in male fishes [101] [102]. These type assessments require more complicated long term tests, and are therefore not easily implemented in a continuous monitoring program. However, they should be included to evaluate any adverse effects that might evolve from longer exposure to a certain leachate. The time period between measurements should depend on the activities on the landfill producing the leachate.

Normally the variation in species and concentrations with time is slow, which should mean that one measurement per year would be sufficient.

4.1.4. Non-polar organic compounds

Most of the known POPs are non-polar organic compounds. This group is of great interest to monitor in any type of waste water treatment plant. Due to their hydrophobic character, they can be expected to be present only at low concentrations in the water phase. Even with low concentrations of these compounds, the environmental impact can be of major concern, especially in a long time perspective, due to their possibility to bioaccumulate. Suspended particles can also dramatically increase the presence of non-polar compounds in a treatment plant. Due to the expected low concentrations (ng/l - µg/l range), complicated and time consuming sample preparation steps are needed in order to determine these compounds. When using conventional methods, like liquid-liquid extraction, large amounts of expensive high quality solvents are needed. These factors make the analysis of non-polar markers very expensive, which dramatically decreases the possibility to follow the distribution of such compounds in aqua spheres. However in many cases, especially when evaluating treatment efficiency, it is sufficient to utilise difference measurements and make a semi quantification of target substances, based on a few standards or surrogate standards, which substantially reduces the costs.

In paper I and II, classical non-polar POPs as PCBs were selected for monitoring. Also PBDEs were found in the leachate. Since PBDEs have recently turned out to be of great environmental concern [103, 104], they were also selected for monitoring. Different groups of substances can be selected for monitoring, as PAHs, phthalates or pesticides depending on the expected composition of the water, and by judgement based on preliminary studies.

Even monitoring of unidentified markers found in the chromatograms can give extra information about the treatment system, and should not be neglected. This is true for many natural treatment systems, where a large part of the treatment is based merely on physiochemical properties, as in different kinds of filter systems. One can be sure that the compounds, eluting from a GC system in the same time range as the selected markers, have similar physicochemical properties as these. Both the sample preparation procedure and the

chromatographic process separate compounds by their physicochemical properties. The use of a selective detector, as the ECD, further increases the total selectivity. One can, as mentioned above, assume a similar behaviour in a filter based treatment system as well. When the concentrations of the identified and quantified compounds, as PCBs, are very low, the analytical uncertainty increases and the levels might even approach the background levels, where it is hard to establish statistical significant differences between the treatment procedures. Here the use of more abundant but unidentified peaks eluting in the same region as PCBs in the chromatogram, can help strengthen assumptions about treatment behaviour. Normalisation of the abundance with respect to peak area or peak height of the compounds towards their response in incoming water makes sure that trends visualised in the treatment systems are real. Still better reliability is obtained when this visualisation is based on the average of a group of compounds, and not on a single abundant compound. When monitoring treatment procedures based on treatments relying on the more chemical properties of the compounds, as ozone oxidation [105, 106], chemical oxidation by Fenton's reagent [107, 108], or bio remediation [109, 110], one should be more careful in formulating conclusions from data obtained from these unidentified compounds. Nevertheless, trends of the identified compounds can be backed up by such data, but care needs to be taken not to over-interpret the results.

4.1.5. Polar organic compounds

Polar organic compounds are an important group when monitoring organic pollutants in leachate water and different waste waters [111]. Conventional GC-MS analysis, which can cover the non-polar to semi-polar organic compounds, can often only account for a small fraction (< 1%) of the TOC in a sample. When including polar organic compounds, the larger part of TOC can be accounted in several cases [112]. Despite that, monitoring of polar organic compounds in leachate water and waste waters is not so common compared to measuring the non-polar fraction [24, 113]. In the suggested protocol, different phenols are monitored as markers for this group of contaminants due to their known presence in the leachate and known environmental impact [114, 115]. The results from the pilot plant study in paper I and II show that just monitoring the sum of phenols does not give the full insight in the behaviour of a treatment procedure. Monitoring individual compounds can give much more information of the actual processes that occur.

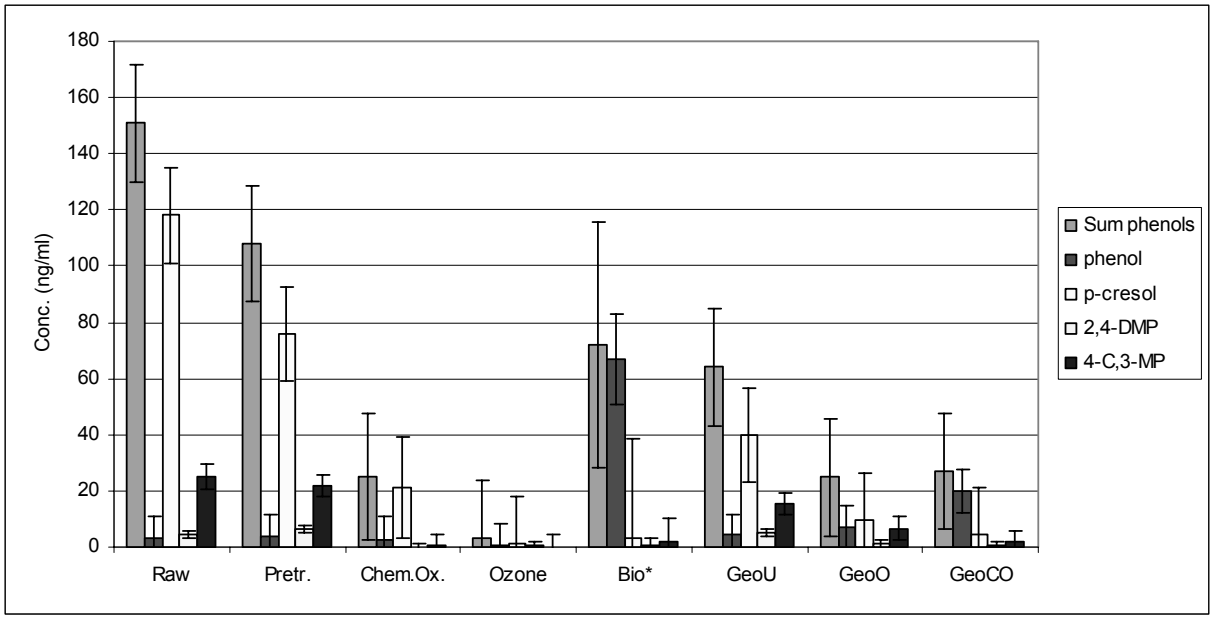

Figure 3. The monitored polar organic markers in a pilot plant study for local treatment of leachate water.

As can be seen for the bioremediation in Figure 3 (reprinted from figure 6B in paper I), it is clear that all phenols are efficiently removed except for phenol itself, which is dramatically increased. The pre-treated (aerated and settled) leachate, named Pretr. in the figure, is the inflowing leachate to all other treatments. The increase of phenol can be due to that other phenols and other aromatic compounds have degradation pathways through phenol or that some other contamination occurred. By looking at Figure 3 it is evident that the bioremediation is very efficient for removing most of the phenols, but an extra polishing step, or longer remediation time, is needed before discharging the effluent to a recipient. If only looking at the sum of phenols one should conclude that the bioremediation had no significant impact on phenols, which is absolutely wrong.

The setup of the automated complete analytical system (paper I), including sample preparation with supported liquid membrane (SLM) extraction coupled on-line to a HPLC with DAD, for direct determination of phenols, greatly simplified the monitoring process. The use of automated and semi-automated sample preparation steps, with low manual input and low consumable cost, is essential for efficient monitoring of organic markers.

4.1.6. Data handling

When monitoring several important parameters, as in the presented protocol, for evaluation of the efficiency of different treatment procedures, large amounts of data is generated that needs to be handled and presented in an easily overviewed way. Proper calculation needs to be done in order to draw statistically correct conclusions about the efficiencies. A good option for the evaluation is to use analysis of variance (ANOVA), which can separate and estimate the different causes of variation, as e.g. random errors or controlled factors. When comparing different treatment procedures, using the suggested protocol, the sources of variation of the analytical results are: the standard error of the analysis, the variation between different treatments, and the variation from different sampling dates. In this case it is suggested to use multifactor ANOVA [116, 117], which also was the approach used in paper I and II.

To greatly simplify the calculations and help visualise the results, it is highly recommended to use a statistical software. The response is set as the dependent variable, and date and treatment procedures, respectively, are selected as factors. When comparing different treatments relative to one original sample, as in the pilot plant study in paper I and II, it is recommended to use least significant difference (LSD) comparison, and when comparing all samples relative to each other, Bonferroni is a better choice [116]. The treatment procedures are then grouped according to significant differences. It is also easier to discover significant differences between the sampling dates using multifactor ANOVA. For the organic compounds in the pilot plant study, there was no significant difference between the sampling dates, which indicates that the selected method for taking time integrated samples gave representative results during the monitored test period.

5. SAMPLE PREPARATION FOR ORGANIC ANALYSIS

Analysing organic compounds generally requires some form of pre-treatment of the sample prior to introducing it to the final analytical instrument like a GC or an HPLC. This is usually referred to as sample preparation and may include steps like filtration, pH adjustment, acidic degradation, distillation, a variety of extraction procedures, analyte trapping, evaporation etc. Sometimes one step is sufficient, but more often a combination of several preparation steps is required. In environmental samples, the concentrations of the interesting organic compounds are usually very low (ng/l - µg/l range), and often concealed by a complex matrix. The purpose of sample preparation is then usually to comprise, as selectively as possible, clean-up and enrichment of the analytes, i.e. remove interferences and pre-concentrate the analytes into a phase suitable for the selected final analysis.

The complexity and large amount of work demanded, and hence the high cost for the analysis of discrete organic compounds in water samples, is a major reason for the general use of water quality parameters for assessing the organic content in many waters, as discussed in section 3. Many times it is also required to use large amounts of high quality, environmentally and occupationally hazardous and expensive solvents. It is therefore important to improve the analytical procedures for organic compounds, in order to increase their use and thus to get a more reliable and accurate picture when assessing the environmental impact of our modern life style.

Today, good, competent and reliable analytical instruments for final analysis that are stretching what is possible to measure with each defined technique exist. It is often the interferences in the sample that increase the noise in the detection, thereby setting the method detection limit (MDL). In an overview of sample preparation by Majors [118], it is estimated that 61 % of the time an analytical chemist spends on analysing a sample is on sample preparation, while only 6 % is spent on the final analysis, as shown in Figure 4. It is also estimated that the sample preparation together with the operator himself, contribute to 49 % (30 % and 19 %, respectively) of the total error in the analysis.

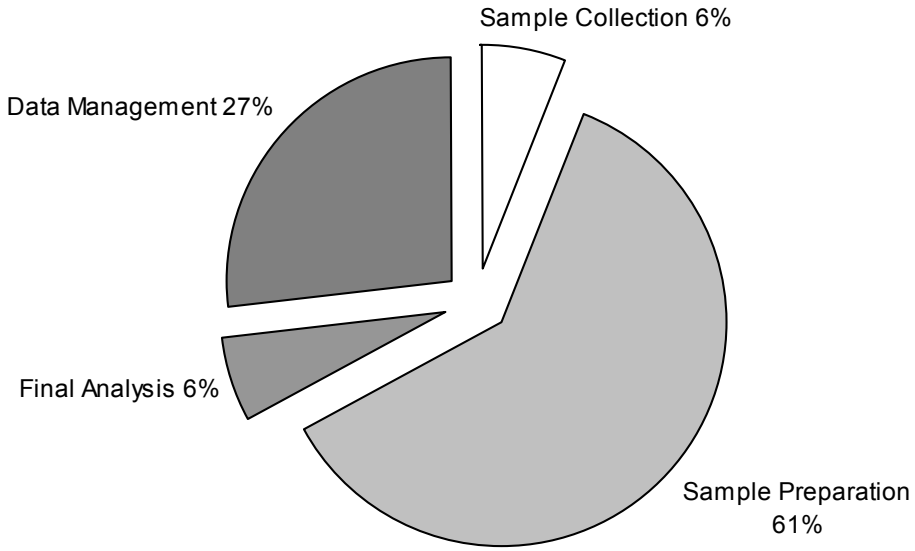

Figure 4. Distribution of the time an analytical chemist spends on analysing a sample [118].

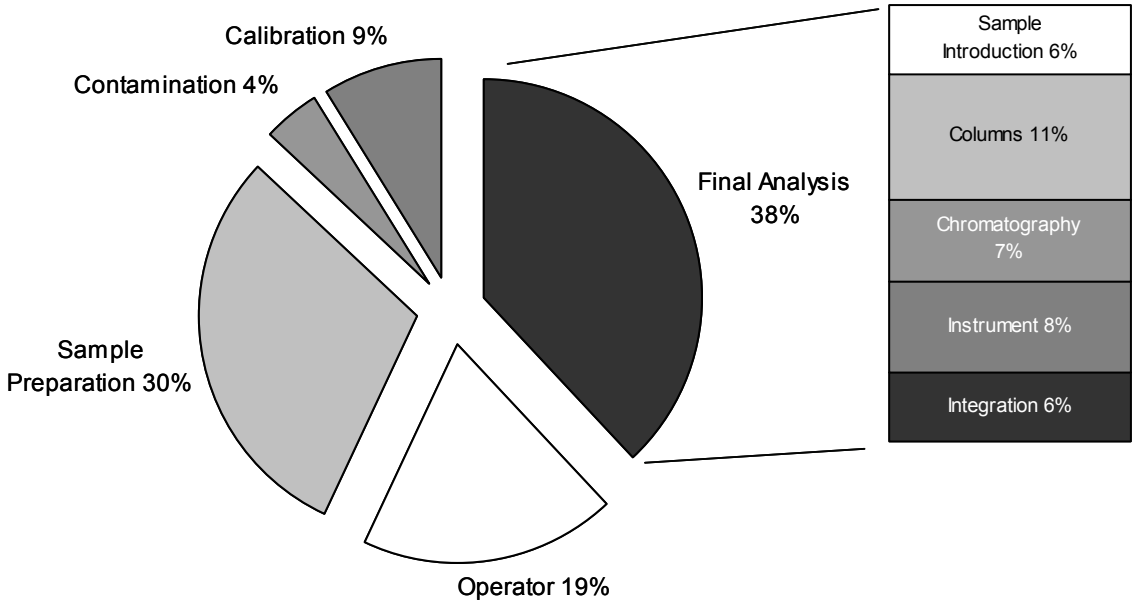

Figure 5. Distribution of cause of errors generated during analysis of a sample [118].

These figures alone show the necessity of improving the sample preparation in order to reduce the amount of manual input and the total time for an analysis, thus increasing the efficiency and reliability of the analysis. Development of accurate and reliable sample preparation methods for chromatographic analysis that offers good and selective pre-concentration within reasonable time and less manual input is thus an important field in analytical chemistry. Automation and on-line coupling to the analytical instrument, so that the

sample preparation can be done during the chromatographic run, would thus very much decrease the workload needed to measure organic pollutants in a complex environmental sample. This is of great importance for efficient monitoring in a future perspective. On-line coupled sample preparation methods for various organic pollutants are developed and used in paper I, II, IV, V and VII. A review of online coupling of sample preparation to chromatographic analysis was recently published by Hyötyläinen and Riekkola [119].

5.1. Liquid-Liquid Extraction (LLE) and Extraction Basics

LLE has historically been the most used extraction method for organic compounds. Still today, it is the most commonly used method in commercial and standardised laboratories, together with solid phase extraction [120]. It relies on the partitioning of the analytes between two, in each other insoluble, liquids. It is usually performed by pouring 0.25 - 1 l of aqueous sample and an organic solvent into a suitable separation funnel. The separation funnel is thoroughly shaken, and the solvents are thus mixed and the analytes distribute between the two phases. The solutions are set to rest so that the mixed phase is separated and then the organic solvent is collected. This procedure is often repeated three times for a quantitative transfer of the analytes from the aqueous phase. Most of the collected solvent is then evaporated in order to increase the concentration of the analytes distributed to the solvent. The evaporation can also comprise a solvent change [121] and after that sometimes also a cleanup step using gel-permeation is needed [122].

For most nonpolar organic solvents, the distribution coefficient K_D of an analyte is closely related to the very frequently tabulated partitioning coefficient of the analyte between octanol and water, K_{ow}. The logarithmic value of K_{ow}, log $K_{ow,}$ is usually used as a measure of the hydrophobicity of a compound. The LLE procedure can be tuned in several ways, e.g. by selection of solvent, by adjustment of pH, and by addition of ligands, ionpairing agents, or salts to increase K_D. This makes LLE a versatile tool for many different types of extractions.

The major drawbacks of LLE are the high amount of solvents used, the large amount of manual labour required, and the risk of emulsion formation, which complicates the necessary phase separation procedure. When sampling a large number of samples, batch-wise sample preparation can be performed with aid of mechanically shaking of the separation funnels.

Several apparatus for continuous flow LLE have also been developed [123-126] and some miniaturised versions based on the same basic liquid liquid partitioning will be described later.

A short description of the basic principles of extraction is given below [127-129]. The distribution of analytes between the two phases is controlled by their distribution coefficients, K_D, and this holds for all distribution extractions. In equilibrium, K_D can be expressed as

$$K_D = \frac{c_{org}}{c_{aq}} = \frac{m_{org} \cdot V_{aq}}{m_{aq} \cdot V_{org}} = \frac{m_{org}}{m_{aq}} \cdot \beta \qquad (5.1)$$

where c_{org} is the concentration in the organic phase, c_{aq} is the concentration in the aqeous phase, m_{org} is the mass of analyte in the organic phase, m_{aq} is the mass of analyte remaining in the aqueous phase, and V_{org} and V_{aq} are the volumes of the phases respectively. β is the phase ratio defined as V_{aq}/V_{org} .

A parameter commonly used as quality parameter in exhaustive extraction methods as LLE, is the extraction efficiency, E, which is the fraction of the amount of analyte recovered in the organic phase and the total amount of analyte in the system.

$$E = \frac{n_{org}}{n_{tot}} = \frac{m_{org}}{m_{tot}} \qquad (5.2)$$

$$m_{tot} = m_{org} + m_{aq}$$

The maximum extraction efficiency that can be obtained from a single extraction can be calculated from eq. 5.1 and 5.2 and rearranged to eq. 5.3 .

$$E = \frac{1}{\frac{\beta}{K_D} + 1} \qquad (5.3)$$

For an extraction with an E of 90 %, making a second extraction will extract one more fraction, thus the fraction extracted is E(1 - E), increasing the total E to 99 %. A third extraction will extract $E(1 - E)^2$ to a total E of 99.9% etc.

For extraction in e.g. a flow system, the efficiency can also be calculated by measuring the amount not extracted, i.e. the amount of analyte collected in the waste, n_w or m_w.

$$E' = \frac{(m_{tot} - m_w)}{m_{tot}} = 1 - \frac{m_w}{m_{tot}} \qquad (5.4)$$

For exhaustive extraction methods like LLE and solid phase extraction (SPE), E can also be expressed as recovery, R, the yield of the extraction.

For non-exhaustive dynamic extractions like solid phase micro extraction (SPME), or micro porous membrane liquid liquid extraction (MMLLE) described later, E gives no qualitative information of the extraction. To calculate the recovery in dynamic flow extractions, a different approach needs to be taken.

The recovery, R, for dynamic flow extraction can be defined as

$$R = \frac{E}{E'} \qquad (5.5)$$

For an extraction without losses, all the extracted amount, i.e. the amount removed from the sample, can be found in the organic phase, then $(m_{tot} - m_w) = m_{org}$, and thus the recovery will be 100 %. If analytes are adsorbed in the system, E < E' and R will be less than 100 % [129].

A different type of recovery that can be calculated and used for all types of dynamic extraction is the term apparent recovery, R' [130]. R' has also been referred to as relative recovery.

$$R' = \begin{cases} \frac{n_{obs}}{n_{\exp}} = \frac{m_{obs}}{m_{\exp}} \\ \frac{n_{obs}}{n_{ref}} = \frac{m_{obs}}{m_{ref}} \end{cases} \qquad (5.6)$$

i.e. comparing an observed amount, n_{obs} or m_{obs}, calculated from a calibration curve, towards an expected amount, n_{exp} or m_{exp} or a measured reference standard, n_{ref} or m_{ref}. This is useful e.g. when comparing matrix effects in dynamic extraction.

For extractions, another important quality factor is the enrichment factor, E_e, as defined in eq. 5.7.

$$E_e = \frac{c_{org}}{c_s} \qquad (5.7)$$

where c_{org} is the analyte concentration in the organic phase, and c_s is the original analyte concentration in the sample. As can be seen, the maximum enrichment obtained is the same as K_D. The enrichment factor, E_e, is also related to the extraction efficiency according to

$$E_e = \frac{E \cdot V_s}{V_{org}} = E \cdot \beta \qquad (5.8)$$

where V_s is the volume of the sample and V_{org} is the volume of the organic phase.

5.2. Solid Phase Extraction (SPE)

A more recent technology which compliments and often even substitutes LLE in the analytical laboratory is SPE, where the partitioning of the analytes occurs from the aqueous sample to a solid phase sorbent. The sorbent, often a C_{18} bounded-silica phase, can be contained in either disc format, as in paper I, or in columns. Prior to processing the sample, the sorbent is usually conditioned by rinsing with methanol and water, and then the sample is processed. If K_D towards the solid phase is large enough, the analytes are trapped, and rinsing steps can be performed before the analytes finally are eluted, by small portions of a suitable organic solvent. As in LLE, the volume of this solvent often needs to be reduced by evaporation for enrichment of the analytes, or totally evaporated for a solvent change, before a possible clean-up step and then eventually final analysis [131-133]. In this format, SPE still utilises not negligible amounts of solvents, and the procedure involves several manual steps. An alternative to organic solvent elution is to use supercritical carbon dioxide delivered by automated equipment. This approach was utilised in paper I, where a selective and automated supercritical fluid extraction (SFE) with carbon dioxide, CO_2, was performed to elute PCBs and PBDEs from filters and SPE discs used for extraction of leachate samples. A SPE column can also quite efficiently be connected on-line to HPLC equipment. A simple approach to this is to replace the sample loop with a SPE column [133, 134]. This method was used in paper I for pre-concentration of phenols before final analysis by HPLC.

Large effort on developing new sorbent materials has been made in the field of SPE. Nowadays many efficient sorbents are available [133, 135]. Most of them are based on polymers or, more or less, selective material bound to solids. Among the new sorbents there are also more refined ones as molecular imprinted polymer (MIP) [136]. Here a polymer is imprinted with a stencil molecule, the analyte itself or a molecule with very similar structure, which is then washed away. This leaves a very selective "pocket" for the analytes to be trapped in, and this gives a very selective extraction. MIP is so far mainly used for biological application, but there are some examples of environmental application and the numbers of investigations are increasing [137]. However, there are still some disadvantages with possible interference from remaining template molecules, and the deformation of the "pockets" if other media is used than the media the MIP was synthesised in. The current MIPs still suffer from low capacity, i.e. only small sample volumes can be extracted. The use of immunoaffinity sorbents is also increasing. The analytes are very selectively extracted by antibodies, which are covalently bound to packing material. Immunoaffinity sorbents have also been used for environmental applications [133, 135, 138] However, the expensive and time consuming manufacturing of antibodies and the restricted range of analytes, for which there are commercial available immunoaffinity sorbents, limit their use.

5.3. Solid Phase Micro Extraction (SPME)

The precursor to solid phase micro extraction was first reported in 1987 by Pawliszyn and Liu as a part of a tool for laser desorption GC injection by sample collection on an optical fibre [139]. It was later on developed as a tool for sample preparation [140], and during the 90's until today, SPME has developed into one of the most used solvent free extraction techniques.

A small fibre, usually coated with a sorbent, is used for partitioning of the analytes. SPME can be used for gas, liquid and solid sampling. When sampling solids, and in many liquid applications, the SPME fibre is put in the headspace of the sample (Figure 6).

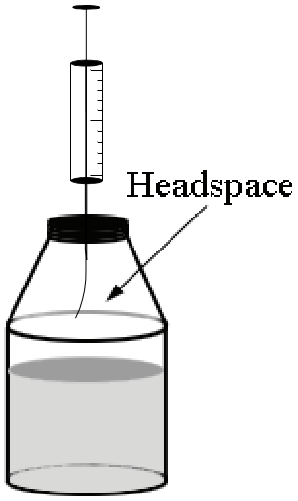

Figure 6. Head space SPME. The SPME fiber is exposed to the headspace for a defined time.

In liquid sampling, the SPME fibre can also be used directly in the liquid, but it is then much more sensitive to interferences and matrix effects. The fibre is allowed to be exposed to the sample for a defined time, between the surrounding medium and the solid phase on the fibre. Then the fibre is usually inserted in a hot GC injector, where the analytes are thermally desorbed. Several HPLC applications are also available [141], even though not as common as the GC applications. Due to the relatively slow sample transfer and small amounts of solid phase, SPME is used as a dynamic and not as an exhaustive extraction. By heating the sample, the mass transfer can be increased.

The small and versatile format of SPME and the development of more durable fibres has made it quite straightforward to automate, especially for GC analysis [142, 143]. On-fibre derivatisation is also possible by either direct derivatisation in the sample or by introducing the fibre to a derivatisation agent between the extraction and the GC injection [144]. The use of SPME in-field sampling and monitoring is also increasing [99, 145].

A major drawback of SPME is the low analyte capacity due to the thin sorbtive layer of the fibre. Another drawback of SPME is that the extraction can be matrix dependent and the fibres can, if sampling in a liquid, adsorb interfering particles and macro molecules. These interferences can be hard to get rid off, and they degrade the fibre efficiency. Carry over from previous samples can also occur if the desorption is insufficient. Furthermore, making proper calibration curves is a problem, especially if the matrix in the unknown samples varies much. As a result of the drawbacks discussed above, relative high relative standard deviations (RSD)

are common, but the simplicity and versatility of SPME makes it a very useful technique, especially for gas phase applications.

5.4. Stir Bar Sorptive Extraction (SBSE)

Stir bar sorbtive extraction (SBSE) [146] is another novel extraction technique, based on sorbtion and desorption of analytes, that is increasingly used in environmental applications [147]. A stir bar, a glass coated metallic rod, is coated with a layer of sorbent material, usually polydimethylsiloxane (PDMS), and placed in the liquid sample, which is mixed by the stir bar for a defined time while the analytes are partitioned between the sample solution and the sorbent. The stir bar is then often thermally desorbed and the analytes are cold trapped, e.g by a cryogenic cooled programmable temperature vaporising (PTV) GC injector before analysed by the GC. The stir bars can also be desorbed by a liquid, which can be injected to a suitable analytical instrument as GC, HPLC or capillary electrophoresis (CE). PDMS (Figure 7) is a well known non-polar polymeric liquid that is well established as a stationary phase in capillary columns for GC analysis [83]. The inert properties and the stability of PDMS have made it the most commonly used sorbtive phase for all sorbtive extraction techniques as SPME and SBSE. The PDMS is often cross-linked to increase the stability further, and prevent it from losing its shape. The extraction of polar compounds to PDMS is not very efficient due to its non-polar properties. Different more polar sorbents, as polybutylacrylate, are thus also used [128].

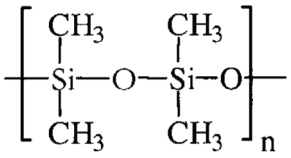

Figure 7. The structure of polydimethylsiloxane (PDMS)

As can be seen in eq. 5.3, the extraction efficiency is dependent on the phase ratio, β. The basic principles are the same for SBSE and SPME, but SBSE utilises 50 – 250 times more sorbent phase, which dramatically increases the analyte capacity and greatly increases the potential of higher extraction efficiency and higher sensitivity. The partition coefficient $K_{PDMS} \approx K_{ow}$ and can thus easily be found in the literature or calculated by different softwares.

It is then easy to calculate the theoretical maximum E, or E_e and compare it to the experimentally obtained values, and thus use it as a quality parameter for the extraction. Practically, optimisation of the SBSE extraction time is done by monitoring E for different extraction times until no more analytes are extracted.

The larger amount of sorbent in SBSE, requires usually about 5 – 15 min desorption at high temperature and gas flows between 10 – 50 ml/min, with special thermal desorption units, making this technique more complicated and time consuming compared to SPME. However, when using thermal desorption high sensitivity can be obtained, since all sorbed analytes are transferred to the GC. Liquid desorption dilutes the sample, but the use of large volume injection can to some extent counteract this. For dirty samples matrix effects have been reported. To compensate for this and other effects it is recommended to use internal standard addition of isotopic labelled standards, which off course increases the cost and may require more sophisticated instrumentation. The use of SBSE in environmental and biological applications has recently been reviewed by David et al. and Kawaguchi et al. [147, 148].

5.5. Supercritical Fluid Extraction (SFE)

Since supercritical fluid extraction (SFE) was used in paper I for elution of non-polar markers from SPE discs and filters, a short description will be presented here. In this paper, as in general, SFE is mainly used for extraction of solid samples.

When a gas is heated and pressurised until it reaches its thermodynamic critical temperature, T_c, and pressure, P_c, the phase boundary between the gas phase and the liquid phase will be smeared out as the density of the gas and the fluid becomes equal. A compound in this hybrid state between gas phase and liquid phase is known as a supercritical fluid, and it will have diffusion properties like a gas and solubility properties like a fluid, which is very favourable properties for efficient extraction. Carbon dioxide, CO_2, has relative low T_c and P_c, 31.1°C and 73.8 bar respectively (Figure 8), and is environmentally friendly and inexpensive. In supercritical state, carbon dioxide is non-polar and the density, and hence its solvent strength, is easily tuned by changing the temperature or pressure. These features make carbon dioxide the most frequently used compound in supercritical fluid extraction (SFE) [149].

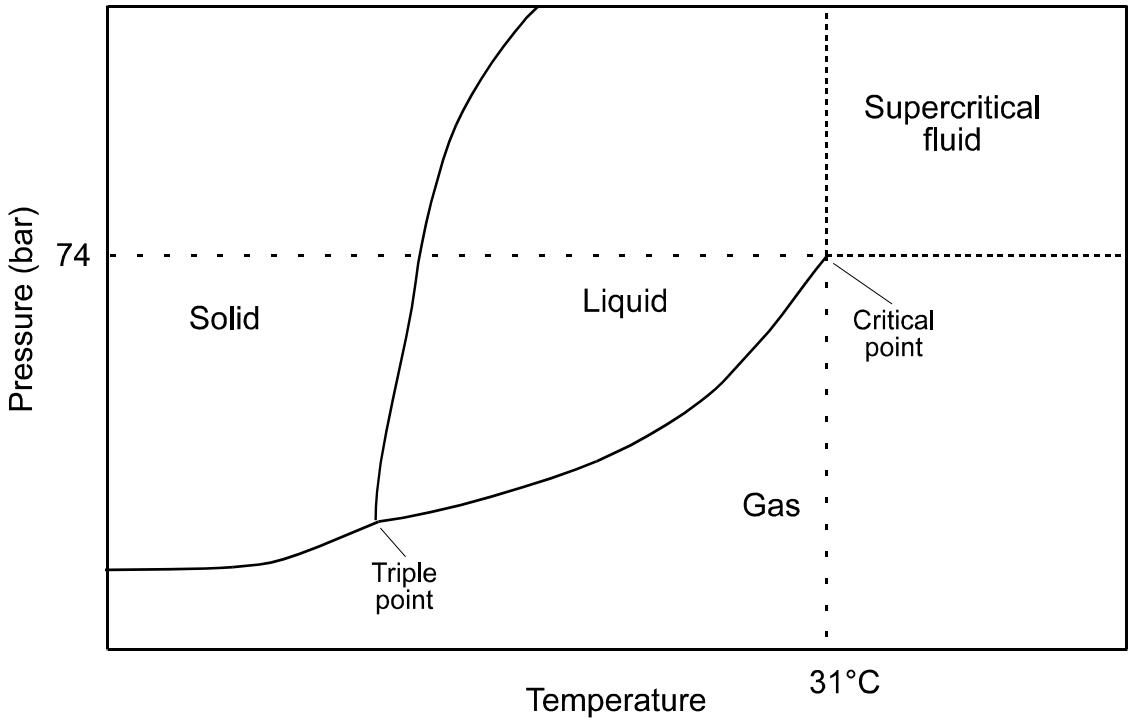

Figure 8. Schematic phase diagram for carbon dioxide, showing the critical point.

Since supercritical carbon dioxide is a non-polar solvent, it is sometimes necessary to add a polar modifier, e.g. methanol or acetone, to the extraction phase, in order to increase or tune its extractability. The extractability of an analyte depends on the properties of the compound and on the matrix.

In automated instruments, the sample is placed in thimbles and the extracting fluid is pumped in. The samples can be extracted at different conditions, usually between temperatures of 40 - 150°C and pressure of 150 - 450 bar. The extraction can be performed either in static or dynamic mode, i.e. with flowing or stagnant extraction fluid, or as a combination. The extraction fluid is then released through a restrictor, where the carbon dioxide is disposed as a gas. The analytes can thus be trapped either in a solvent, as in paper I and II, or by a solid trap. In the latter case they are then usually eluted by small volumes of a suitable solvent into a vial.

SFE yields an automated setup and low consumption of hazardous, expensive solvents. SFE gives a fast and efficient extraction of several classes of contaminants with as good as, or even better, efficiency than conventional methods, as Soxhlet extraction [150, 151]. When using the most common supercritical fluid carbon dioxide, which is gaseous after the

restrictor, SFE is relatively easily on-line coupled to GC [119, 152]. This shows that SFE has a very good potential for being a competitive extraction technique.

However, in the last years there has been a decrease in usage and published application concerning SFE [153]. Even though SFE has proven to be a very good technique, it has not fully fulfilled the expectations as a universal analytical extraction method for solid samples, as purported in the early 1990s. Some leading manufacturers have also ended their production of SFE equipment. Reasons for this development can be that the SFE is quite matrix dependent and thus not many of-the-shelf methods existed, in combination with the fact that method development is not as straight forward as with conventional methods. The instrumentation for SFE was often overcomplicated, resulting in expensive instruments and thus investment costs, which have been hard to justify among commercial laboratories [153-155]. Nevertheless, with the possibility to tune the extraction parameters, SFE has shown its potential in fundamental research like assessing the bioavailable fractions in sediments [156, 157].

The entry of automated instruments based on pressurised fluid extraction (PFE), also known as pressurised liquid extraction (PLE) or accelerated solvent extraction (ASE™) [158], which are extensions of existing solvent extraction methods, has also increased the competition for SFE. The solvent is heated and pressurised in order to increase the extraction performance. The resemblance to conventional methods makes the method development straight forward, and PLE was quickly adopted by US EPA for extraction of several organic pollutants in different solid matrixes [159]. Standardised SFE methods also exist but they are more analyte specific [160-162], and it took considerable longer time for them to be adopted by US EPA [154].

The recognition of the fact that the polarity of water decreases markedly when heated and pressurised, lead to further development of PLE to pressurised hot water extraction PHWE [163, 164]. This environmental friendly solvent has been used for extraction of several different pollutants. Recently it has also been adopted on-line coupled to GC analysis, where the water is extracted through MMLLE extraction of the water phase directly coupled to the GC with both flat-sheet and hollow-fibre membrane [165, 166]. Online coupling of MMLLE to GC will be discussed further in section 5.7. A review of extraction with heated water has

recently been published [167]. In paper V it is also shown that water, with addition of a small amount of a water soluble organic modifier, can efficiently be used for extraction of organochlorine pesticides (OCP) from soil, if followed by an efficient extraction method, such as MMLLE, for the aqueous extract.

5.6. Supported Liquid Membrane Extraction (SLM)

5.6.1. Principles

Supported liquid membrane extraction (SLM) is a three phase extraction, where a organic liquid membrane is positioned between two aqueous phases. The liquid membrane is kept in place and stabilised by a hydrophobic porous membrane, usually made of polytetrafluoroethylene (PTFE) or polypropylene (PP), where the organic solvent fills the pores. The conditions in the first aqueous phase, also called the donor phase, i.e. the original sample, is set so that the analytes are uncharged, e.g. by adjusting the pH, by ion-pairing, or by complex formation. In uncharged state the analytes partition into the organic phase, the organic liquid membrane. On the other side of the membrane the third phase, the aqueous acceptor phase, is positioned, where the conditions are set so that the analytes are e.g. charged and thus trapped, since the charge hinders the analytes from being back-extracted into the organic phase. The analytes can also be immunological trapped in the acceptor, as described in reference [168], by antibodies immobilised on magnetic particles, which resulted in very high sensitivity for the pesticide simazine. The mass transfer in SLM is driven by the concentration gradient that is kept high if the analytes are trapped in the acceptor, and thus removed from the organic phase. This approach was first presented for analytical purposes for extraction of amines by Audunsson [169], and has since then been utilised for many applications, concerning e.g organic pollutants, metals and drugs, in environmental, health and occupational, or biomedical analysis [170, 171]. SLM extraction in uncomplicated hollow fibre (HF) setups, called liquid-liquid-liquid micro-extraction (LLLME) by some authors, have been utilised for various applications [172, 173]. SLM-like extraction has also been performed in hollow fibre membranes without filling the pores of the membrane with an organic phase, thus letting the analytes diffuse to the acceptor through the air trapped in the pores. This technique, called liquid-gas-liquid micro-extraction (LGLME) by some authors,

was proven to work fine for phenols, i.e. more volatile compounds [174]. This approach was also tested by Audunsson for the extraction of amines in the first SLM application, but with lower extraction efficiency compared to the liquid membranes tested [169]. A similar approach was also used with gas stripping, in a porous flat membrane setup, for the analysis of benzene in urine at ppb level [175].

The extraction of acids and bases are the simplest case. In paper I and II, an automated SLM system was used for online HPLC analysis of phenols. Since phenols are weak acids, they will be uncharged as the sample is acidified for preservation. When acidified samples containing phenols are processed in the extraction unit, the uncharged phenols will be extracted into the organic phase. Then, as they come in contact with the basic acceptor, they will be charged and trapped in the acceptor. Compounds that are charged in the acidified sample will not be extracted at all, and permanent neutral compounds will partition between all phases and will thus not be enriched in the acceptor.

5.6.2. SLM Theory

The mass transfer in SLM has previously been described by Jönsson et al. [176] and the principles by Jönsson and Mathiasson [129]. The mass transfer rate is proportional to the concentration gradient, ΔC, over the membrane.

$$\Delta C = \alpha_s c_s - \alpha_A c_A \frac{K_A}{K_s} \qquad (5.9)$$

α_s and α_A are the fractions of the analytes in uncharged, thus extractable, form in the sample (donor) and acceptor respectively. c_s and c_A are the mean concentrations of analytes in the sample and acceptor phase respectively, and K_s and K_A are the partition coefficients for the analytes in sample and acceptor phase towards the organic phase. In most cases $K_s \approx K_A$ and thus eq. 5.9 simplifies to

$$\Delta C = \alpha_s c_s - \alpha_A c_A \qquad (5.10)$$

Mass transfer will occur as long as $\Delta C \neq 0$. To obtain an efficient extraction in a system with stagnant acceptor and continuously flowing sample, the conditions for the sample is usually selected so that α_s is as close to unity as possible, and α_A as small as possible. Considering eq. 5.7 and replacing c_{org} with c_A, it is evident that if the system is well designed it is possible

to obtain very large E_e. The maximum E_e that can be obtained is when ΔC reaches zero, i.e. $\alpha_A c_A = \alpha_s c_s$, which gives

$$E_{e(\max)} = \left(\frac{c_A}{c_s} \right)_{\max} = \frac{\alpha_s}{\alpha_A} \qquad (5.11)$$

As can be seen in eq. 5.11 the obtained E_e is not limited by K_D, as in LLE, MMLLE, SBSE etc.

For an acid or a base, where the fraction of the uncharged analyte, α, depends on the acid dissociation constant, K_a, and the pH of the solution, α can be derived to

$$\alpha = \frac{K_a}{K_a \left(1 + \frac{[H^+]}{K_a} \right)} = \frac{1}{1 + 10^{(pH - pK_a)}} \qquad (5.12)$$

For phenol $pK_a = 9.9$ extracted with the system used in paper I and II, where the sample has a pH of 2 and an acceptor with pH 14, one would get a theoretical $E_{e(max)}$ of about 13 000. This is practically unreachable when extracting real samples, since transport of acidic compounds eventually will decrease the pH of the acceptor, thus increasing α_A and lowering the $E_{e(max)}$. This pH drop was observed when processing large volumes of the leachate using SLM extraction in paper I and II. However, for the selected sample volume, no decrease of pH in the acceptor was observed. Also as ΔC decreases, the mass transport will slowly level out into a non-linear range. In sample preparation it is desirable to work in the linear range, where E, is independent of extraction time and volume. This is obtained if E_e is much lower than $E_{e(max)}$, and this should be obtained if pH in the acceptor is kept at at least 3.3 units above/below pK_a for acidic/basic compounds for a so called complete trapping [129].

On the contrary, an incomplete trapping can also be useful. Knowing the pH in the acceptor and pK_a for the analytes, and measuring the equilibrium concentration, it is possible to calculate the concentration of the freely extractable analytes in the sample from eq. 5.12. By then determining the total concentration of the analyte by an exhaustive extraction, it is possible to determine the freely dissolved and bound fractions of the analytes in the sample. This approach can be applied to environmental applications for both organic compounds [177] and metals [178], and in biomedical applications for determination of protein bindings of drugs [179]. The toxicity of a compound is controlled by its bioavailability, which generally is believed to be the freely dissolved fraction.

5.6.3. SLM Practice

In SLM, the rate limiting factor can be either the diffusion of the analyte in the donor to the membrane, or the diffusion of the analytes through the membrane, i.e. donor controlled or membrane controlled extraction. Donor controlled extraction generally gives a much higher mass transfer rate. Thus it is desirable to have an organic membrane solvent with good solubility of the analytes. If the mass transport in the membrane is slow, it is possible to add carrier to the solvent that helps transporting the analytes. As an example in paper I, undecane and di-n-hexylether with different concentrations of tri-n-octylphosphine oxide (TOPO) was investigated. TOPO was added to increase the polarity of the membrane. This made the more polar phenols more easily extracted but less polar phenols less extracted. This clearly shows the dependence of membrane composition in designing a SLM system. For overall better extraction, and especially concerning good membrane stability, pure d-n-hexylether was used.

When using SLM for monitoring purposes as in paper I and II, membrane stability is an important factor. Parameters affecting the membrane stability was reviewed and discussed by Norberg [180]. An important factor in having a stable membrane is the water solubility. When analysing several samples or large volumes even slight water solubility will eventually remove the liquid membrane from the support. This holds also for any carrier molecules dissolved in the membrane. If one is aware of the problem it is easily solved by replacing or doing re-impregnation of the membrane regularly. A good approach is to extract and monitor surrogate standards added to the samples to discover gradual or sudden changes.

Another factor that can limit the membrane stability is the trans-membrane pressure. Norberg [180] reports calculations that shows that some hundreds kPa could be tolerated, without pressing the liquid out of the membrane support. This pressure is normally not obtained in an open flow system. However, when loading the extract to a pre-column as in paper I and II, it can easily be reached. Thus it is important to include a valve so that the membrane unit can be “closed” before loading the pre-column. The pore size of the membrane is a factor that influences the membrane stability; the smaller the pores the more durable the membrane. On the other hand, using too small pores can cause clogging of the membrane, which was observed by Thordarson et al. [181].

Using a non-polar insoluble membrane and a proper designed system, very good stability is achieved. The automated SLM-HPLC-DAD system for analysis of phenols in leachate, used in paper I and II throughout the pilot plant investigation, and in other investigations since, was proven to be very robust. The membrane was stable for weeks, before an uncomplicated on-line re-impregnation was done. The repeatability of the system was also very good, even after re-impregnation, membrane changes, new pre-columns and other maintenance. The automated system showed good extraction performance and stability, which together with the fact that the extraction could be done during the chromatographic cycle time, implies that the system would be suitable for normal routine analysis.

5.7. Micro-porous Membrane Liquid-Liquid Extraction (MMLLE)

5.7.1. Principles

Micro-porous membrane liquid-liquid extraction (MMLLE) is based on the partitioning principles of classical LLE, but in MMLLE the organic and aqueous phases never mix. The organic phase is supported by a porous, normally hydrophobic, membrane and comes in contact with the aqueous sample through the pores of the membrane. The extraction relies on diffusion of the analytes from the sample into the organic solvent, usually referred to as the acceptor. Also in MMLLE the mass transfer is driven by ΔC, as in SLM. However, in MMLLE the value of E_e is limited by K_D as in normal LLE (eq. 5.7). A flowing acceptor, preferably in opposite direction compared to the sample, increases the mass transfer rate, since ΔC is kept large [124]. This would increase E; on the other hand it would also lead to a dilution of the sample, thus decreasing E_e, if no further pre-concentration steps are performed.

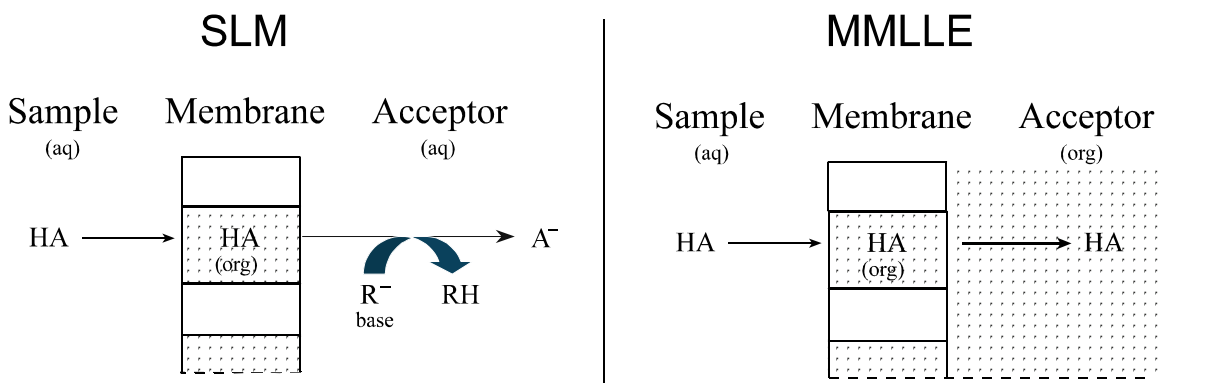

Figure 9. The basic principles for SLM extraction respectively MMLLE.

MMLLE can be performed in similar membrane units as used for SLM, utilising either flat-sheet or HF membranes [166, 182-184]. Figure 9 shows the basic priniples for SLM respectively MMLLE. HF-MMLLE has been utilised in both flow systems and as single disposable extracting devises. Single fibre HF-MMLLE is also called liquid phase micro extraction (LPME) or solvent bar micro extraction (SBME) by other researchers, and have been applied to several different environmental contaminants, as e.g. PAHs, OCP and PCBs [173, 185-187].

In paper VI, a simple low-cost extraction method was developed for the analysis of PBDEs based on cheap single-use HF membranes, embracing the organic solvent in the pores and the lumen. The HF membrane was heat sealed and impregnated with an extremely small amount of solvent, and then simply put in a stirred sample. After 60 minutes of extraction the analytes were enriched several thousand times with an E close to 100 % for most analytes, thus resulting in very low MDL with good linearity and reproducibility. The use of disposable fibres eliminates the risk of carryover between samples. The presented HF-MMLLE method is a very simple and low cost approach. The simplicity makes it fairly easy even for untrained laboratory personnel to perform trace level analysis. However, even though automation probably is not needed for a cost efficient analysis, these types of methods are hard to automate.

5.7.2. MMLLE-GC – The Extracting Syringe (ESy) concept

An extraction system based on a flow system is easily automated and coupled on-line to instrumentation for final analysis. As mentioned before, automation is preferable in order to reduce the manual input, and thus reduce workload and sources of error. This leads to improved productivity in the laboratories. Since the analytes are extracted into an organic solvent in MMLLE, the analytical procedure is quite readily automated and coupled on-line to both normal phase HPLC and GC, in different configurations [119, 182, 188]. In paper IV, V and VII, methods for automated MMLLE-GC extraction of different pollutants, PCB (paper IV), OCP (paper V), and phthalates (paper VII) were developed and tested on different environmental samples. The MMLLE extraction methods developed in these papers are based on the so called ESy, which is an acronym for extracting syringe.

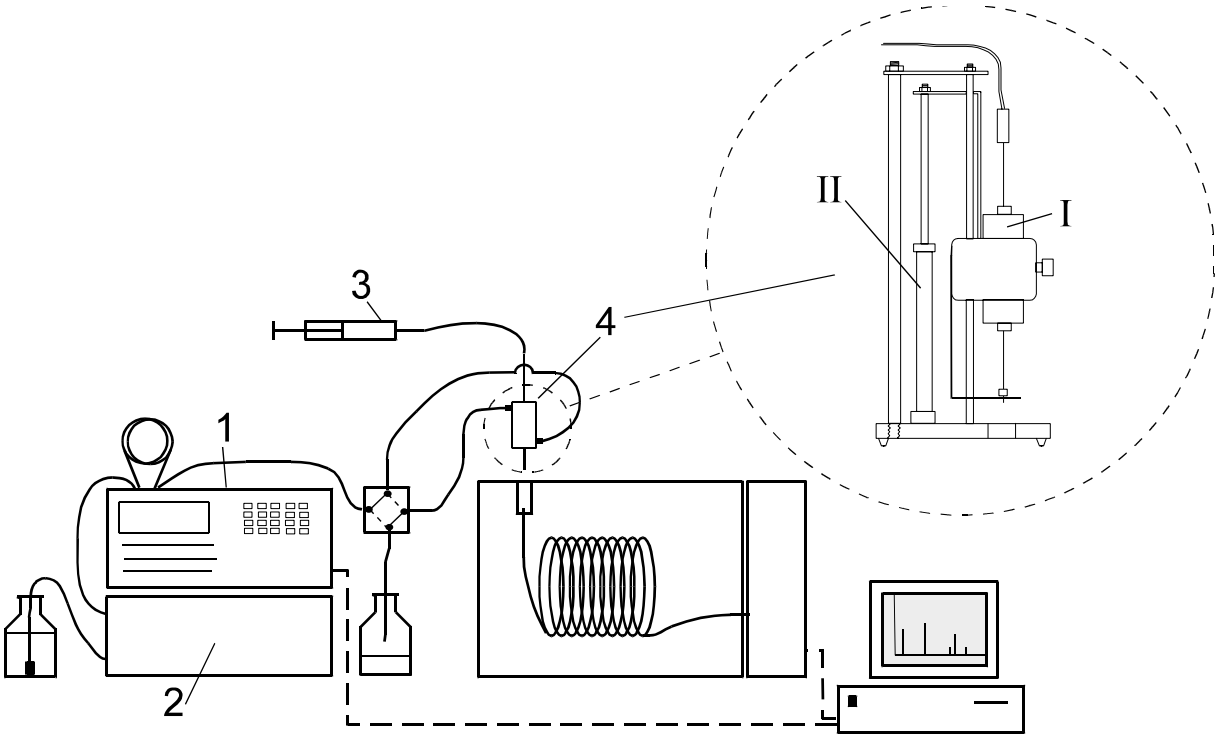

Figure 10. The first ESy prototype consisted of a HPLC autosampler (1), sample transfer pump (2), solvent syringe pump (3), and an extracting syringe, ESy (4). The ESy comprised a HF-MMLLE membrane unit (I) and a pneumatic piston (II).

The very first ESy prototype was developed as a diploma work [189], supervised by Norberg and Thordarson and the results obtained are published in reference [184]. Figure 10 shows the fully automated system, which was (roughly described) setup by a HPLC autosampler (1), a sample transfer pump (2), a small solvent syringe pump (3) and the ESy

(4). The HF-MMLLE extraction unit (I) in ESy has one end of the HF unit connected to a GC injection needle. After completing the extraction the ESy was brought down by the pneumatic piston (II), and the needle penetrated the septa, injecting the extract into the GC injector. For more details see references [184, 189].

The next prototype was developed by ESyTech AB, Lund (later Biotage AB, Uppsala). This is the prototype described and used in paper IV, V, and VII. In short, the system is built up from the following units, as can be seen in Figure 11, a sample pump (1), a solvent pump (2), a sampling unit (3), and the extraction unit (4). The extraction unit consists of a Card Guard™ which clamps the disposable extraction cards in position. In a few simple grips the extraction card is replaced if needed.

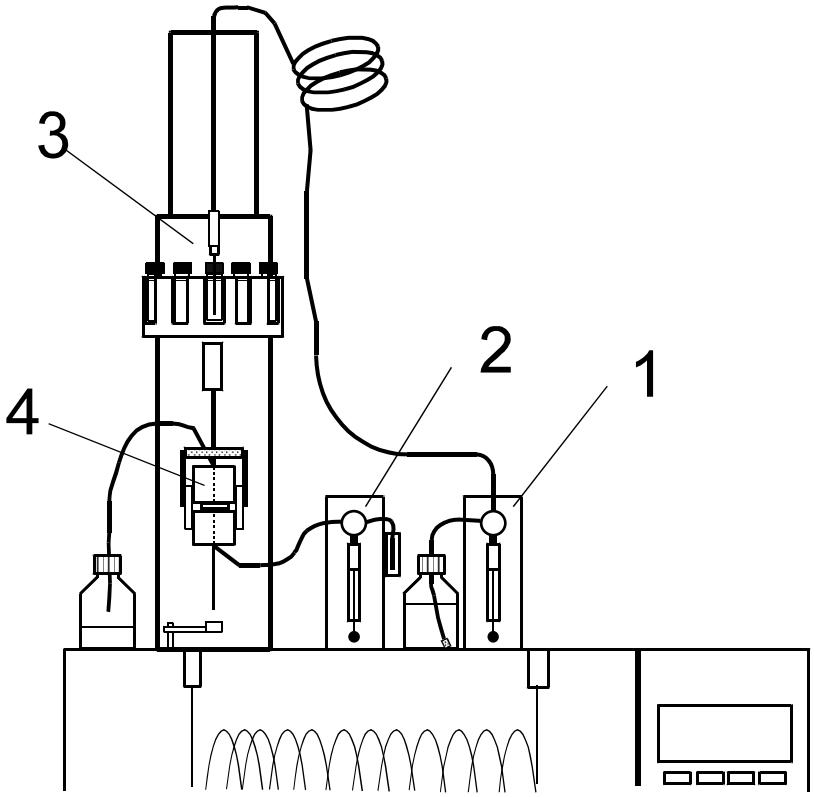

Figure 11. The ESy prototype used in paper IV, V and VII. The main components are sample pump (1), solvent pump (2), sampling device (3), and extraction unit (4).

5.7.3. Organic Modifier

A problem when designing systems, like ESy, and aiming at minimising dead volume, is that the surface to volume ratio also will be maximised, and thus the potential for analyte / surface interaction increases. The studies in paper IV, V and VII have shown that when analysing very hydrophobic compounds, it is necessary to add a fraction of organic modifier to the sample for an efficient extraction. This prevents adsorption of the analytes to the surfaces in the flow system. As modifier, a water soluble organic compound can be used, that should have only moderate solubility in the organic acceptor, e.g. methanol, acetonitrile or iso-propanol. The amount that needs to be added for maximum extraction depends on the hydrophobicity and adsorption charateristics for each analyte. Increasing the amount of organic modifier will also negatively influence K_D for each compound. Thus, the amount of organic modifier needs to be optimised for each group of analytes, and a compromise often needs to be made. Due to the large variation of the hydrophobicity for phthalates in paper VII, it was necessary to run two separate extractions for each sample, with 0 % or 50 % methanol, to cover the six investigated analytes. For OCP and PCB good compromises were found with the addition of 20 % acetonitrile for OCP and 40 % for PCB extraction.

5.7.4. Extraction Efficiency vs. Recovery

The ESy has proven to be very good for extraction of hydrophobic analytes and gives low MDL, short extraction times and almost negligible solvent consumption. The volume of the extraction card channels is 1.65 µl and normally a few ml of sample is extracted. E for an ESy extraction is usually only a few percent but the large phase ratio, β, ensures high E_e (eq. 5.8). The complete transfer of the extract to the GC ensures very good sensitivity, even though small volumes are extracted with low E, compared to e.g. LLE. It should be pointed out that in most routine analysis usually only a few (1-2) µl of a LLE extract is injected to the GC. That means that only a few thousandths of the extract is actually analysed. Comparing E for a flow system to E (i.e. R) for an exhaustive extraction is thus pointless. The real recovery, the amount that actually is recovered in the GC instrument is, as mentioned, only a fraction of what was partitioned into the organic phase. For the ESy it should usually be about 100 %. As

mentioned in section 5.1 the apparent or relative recovery, R', is a better way of describing the quality of a dynamic extraction.

5.7.5. Contamination

In LLE, it is quite common that three consecutive extractions of a single sample are performed, and the organic solvent from each extraction is collected in a single vessel. The solvent is then often evaporated down to about 1-2 ml. This means that any contamination in the solvent and from laboratory equipment also will be greatly enriched. Using ESy, no such enrichments needs to be done, since the complete extract is transferred. This means that the ESy is much less susceptible for contamination. Since only very small amounts of organic solvents are used, it is possible to buy solvents of highest quality to keep the noise in the final analysis at the lowest possible level.

5.7.6. Carry Over

Compared to the HF-MMLLE approach in paper VI, where disposable HF membranes were used, a flow system MMLLE always possesses greater risk of carry over between the samples, also measured as over all memory effect (OME) in percent. Initially this was also a problem with the ESy prototype. This turned out to depend on the use of polymeric (PTFE) tubing connecting different parts of the equipment. Not even a thorough washing of the tubing was sufficient, since analytes dissolved in the polymer slowly diffused into the liquid in the tubing. By removal of all PTFE tubing in contact with the sample, replacing it with stainless steel tubing, the carry over was greatly reduced.

Further improvement was accomplished by introducing an extensive washing procedure, with acetone and aqueous based washing fluid and some volumes of the organic solvent. Finally by coating the polypropylene extraction card with a gold film, the carry over became lower than the detectable level. The use of a multi-channel extraction cards would simplify the washing procedure, since a large part of the more retained analytes are likely to be found in the membrane, or even in the membrane support material, where the removal is controlled by the slower diffusion. With a multi channel card the membrane and extraction channels

would be replaced between each extraction. This shortens the washing procedure significantly, since harsher and quicker washing procedures of the tubing then could be utilised without a risk that unwanted solvents would be retained in the membrane, thus influencing the extraction performance.

The very good performance of the ESy showed in paper IV, V, and VII, shows that instrumentation like the ESy has very good potential to dramatically reduce the workload needed for trace analysis of organic compounds. This is also true for the automated SLM-HPLC-DAD system utilised in paper I and II. The more manual sample preparation developed in paper VI also dramatically simplifies trace analysis compared to conventional methods. All of the utilised extraction methods in this thesis consume only a fraction of the solvent needed in the LLE and SPE protocols. Implementing methods like these would even facilitate the logistics of sample handling to a great extent, since only a few ml of sample is needed compared to the sample volume of one litre often used in LLE and SPE to reach the sufficient enrichment.

6. CONCLUSIONS AND FUTURE PERSPECTIVES

Fortunately, there is today an awareness of that the waste produced in the modern society does not simply vanish, as it leaves our homes and work places. Historically, deposition of waste on landfills has been the dominating waste disposal strategy. The last decades, there has been an increasing concern about the pollution of the environment from human activity. The awareness of the large amounts of potentially hazardous compounds that have been, or currently are, deposited in the landfills, or are produced as the waste decomposes, has lead to a demand for monitoring and treatment of landfill emissions. One of the main gases generated, methane, is nowadays collected for energy production, and regulations have been formed by the authorities about leachate treatment before the leachate is allowed to be released back to the ecosystem.

The increasing demand for sustainable leachate treatment methods generates a demand for efficient evaluation of relevant parameters. In this thesis, a protocol and methodology for leachate analysis is presented, which covers the determination of important pollutants and considers the toxic impact of the outgoing treated leachate on the recipient. A very large expense in an evaluation protocol is often the analysis of insidious organic pollutants, for which summary parameters with dim response frequently are used. In this thesis, a strategy which covers both polar and non-polar organic compounds is presented. This strategy relies on automated or semi-automated analytical systems.

The bottle neck in the analysis of organic pollutants is often the sample preparation. Thus the development and implementation of efficient sample preparation methodology is needed, in order to better monitor our environment. The automated sample preparation methods developed in this thesis have been proven to give good and reliable results with little manual input from the operator (once the method and instrumentation is developed, that is). However, sometimes an uncomplicated straight forward extraction method that requires more manual input can give excellent performance (as shown in paper VI), without the need for complicated expensive instruments.

When running monitoring programs for an extended time, automated systems that can run day and night are by far the best choice, also reducing the sources of errors. Using

automated methods, the analysis of a complex environmental sample is accomplished in a very short time compared to conventional methods. In a future perspective, it should not be harder to perform organic trace analysis, than it is to load vials in an autosampler. The extractions should be possible to perform during the cycle time of the chromatographic equipment, thus preventing the sample preparation step to be the rate limiting factor. Nevertheless, the simplicity of recent manual methods, like the one developed in paper VI, will make them very suitable for smaller scale investigations and monitoring programmes, where the investment (in time and/or money) in automated systems is hard to justify. Also the fact that fairly untrained personnel easily can adapt to procedures like the one described in paper VI, speaks for a bright future for such miniaturised techniques, even if the degree of automation may be low.

All the methods developed in this thesis have also been aiming at reducing the use of potentially hazardous, both environmentally and occupationally, and expensive high quality solvents. The proposed methods demand only small fractions of the solvents used in conventional analytical methods.

Even if perfect black boxes existed where a list of each individual component and its amount came out in the rear end as the leachate sample was introduced in the front, the assessment of the impact towards biological systems would be hard, due to the complexity of the samples. Therefore toxicity assays on biological active species are very important. Today, no matter how well designed a monitoring program is, there is always a large risk of missing compounds of great environmental concern. The use of bioassays acts as a safety valve towards unknown contaminants. Further on, applying simple fractionation steps, as developed in paper III, helps in tracing the origin of the toxicity. This gives valuable information in designing treatment procedures, so that the environmental impact can be reduced.

A final but not least conclusion is: The best way to prevent pollution from waste is to prevent the waste from being produced.

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8. REFERENCES

[1] Council directive 1999/31/EC on the landfill of waste, *Off. J. Eur. Commun.*, **L 182**, 1 (1999).

[2] Europe's environment: the third assessment. Chapter 7: Waste generation and management **State of Environment report No 1/2003** *European Environment Agency (EEA)* (2003).

[3] The Fifth EC Environmental Action Programme, *Off. J. Eur. Commun.*, **C 138**, 5 (1993).

[4] Sixth EC Environment Action Programme, *Off. J. Eur. Commun.*, **L 242**, 1 (2002).

[5] J.-O. Sundqvist, J. Granath, and M. Reich Carlsson,
How should the household waste be taken care of? Evaluation of different treatment methods (In swedish), **B1462**, *IVL Swedish Environmental Research Intstitute Ltd., Stockholm* (2002).

[6] EU focus on waste management, *European Commission, Luxembourg: Office for Official Publications of the European Communities* (1999).

[7] Environmental Assessment for Final Effluent Limitations Guidelines and Standards for the landfills Point Source Category, **EPA-821-B99-006**, *US Environmental Protectioncy Agency* (2000).

[8] E. Noaksson, M. Linderoth, U. Tjärnlund, and L. Balk,
Toxicological effects and reproductive impairments in female perch (Perca fluviatilis) exposed to leachate from Swedish refuse dumps, *Aquatic Toxicology*, **75**, 162 (2005).

[9] E. Noaksson, U. Tjärnlund, T. Albertus, C. Bosveld, and L. Balk,
Evidence for Endocrine Disruption in Perch (Perca fluviatilis) and Roach (Rutilus rutilus) in a Remote Swedish Lake in the Vicinity of a Public Refuse Dump, *Toxicol. Appl. Pharmacol.*, **174**, 160 (2001).

[10] D. Bendz, T. Bramryd, J.-E. Meijer, and T. Ohlsson,
Landfilling of waste - trends strategies and sustainable development (In swedish), **AFR-Report 260**, *Swedish Environmental Protection Agency* (1999).

[11] Figures of waste (In swedish), **Report nr. 4875**, *Swedish Environmental Protectioncy Agency* (1998).

[12] N. Paxeus,
Organic compounds in municipal landfill leachates, *Water Sci. Technol.*, **42**, 323 (2000).

[13] M. Castillo and D. Barceló,
Characterization of organic pollutants in textile wastewaters and landfill leachate by using toxicity-based fractionation methods followed by liquid and gas chromatography coupled to mass spectrometric detection, *Anal. Chim. Acta*, **426**, 253 (2001).

[14] T. H. Christensen, P. Kjeldsen, P. L. Bjerg, D. L. Jensen, J. B. Chistensen, A. Baun, H.-J. Albrechtsen, and G. Heron,
Biogeochemistry of landfill leachate plumes, *Appl. Geochem.*, **16**, 659 (2001).

[15] M. Osako, Y.-J. Kim, and S.-i. Sakai,
Leaching of brominated flame retardants in leachate from landfills in Japan, *Chemosphere*, **57**, 1571 (2004).

[16] C. A. de Wit,
An overview of brominated flame retardants in the environment, *Chemosphere*, **46**, 583 (2002).

[17] C. Öman and P.-Å. Hynning,
Identification of organic compounds in municipal landfill leachates, *Environ. Pollut.*, **80**, 265 (1993).

[18] C. Öman, M. Malmberg, and C. Wolf-Watz,
Handbook for leachate judgement. Methodology for characterisation of leachate from landfills (In Swedish), **B-1354**, *IVL Swedish Environmental Research Intstitute Ltd., Stockholm* (2000).

[19] C. Öman, M. Malmberg, and C. Wolf-Watz,
Development of methods for characterisation of leachate from landfills - Final Report (In Swedish), **B-1353**, *IVL Swedish Environmental Research Intstitute Ltd., Stockholm* (2000).

[20] C. Öman and H. Rosqvist,
Transport fate of organic compounds with water through landfills, *Water Res.*, **33**, 2247 (1999).

[21] A. Baun, A. Ledin, L. A. Reitzel, P. L. Bjerg, and T. H. Christensen,
Xenobiotic organic compounds in leachates from ten Danish MSW landfills-chemical analysis and toxicity tests, *Water Res.*, **38**, 3845 (2004).

[22] H. Asakura, T. Matsuto, and N. Tanaka,
Behavior of endocrine-disrupting chemicals in leachate from MSW landfill sites in Japan, *Waste Manage.*, **24**, 613 (2004).

[23] B. Ozkaya,
Chlorophenols in leachates originating from different landfills and aerobic composting plants, *J. Hazard. Mater.*, **124**, 107 (2005).

[24] P. Kjeldsen, M. A. Barlaz, A. P. Rooker, A. Baun, A. Ledin, and T. H. Christensen,
Present and Long-Term Composition of MSW Landfill Leachate: A Review, *Crit. Rev. Env. Sci. Technol.*, **32**, 297 (2002).

[25] L. Thörneby, L. Mathiasson, L. Martensson, and W. Hogland,
The performance of a natural treatment system for landfill leachate with special emphasis on the fate of organic pollutants, *Waste Manage. Res.*, **24**, 183 (2006).

[26] P. A. Vesilind, W. Worrel, and D. R. Reinhart,
Solid Waste Engineering. Brooks/Cole. Thomson Learning Inc., (2002).

[27] A. D. Read, M. Hudgins, and P. Phillips,
Aerobic landfill test cells and their implications for sustainable waste disposal, *Geograph. J.*, **167**, 235 (2001).

[28] J. Harmsen,
Identification of organic compounds in leachate from a waste tip *Water Res.*, **17**, 699 (1983).

[29] B. Eklund, E. P. Anderson, and B. L. Walker,
Characterization of landfill gas composition at the fresh kills municipal solid-waste landfill, *Environ. Sci. Technol.*, **32**, 2233 (1998).

[30] H. Belevi and P. Baccini,
Long-term behavior of municipal solid waste landfills *Waste Manage. Res.*, **7**, 43 (1989).

[31] S. Bozkurt, L. Moreno, and I. Neretnieks,
Long-term fate of organics in waste deposits and its effect on metal release, *Sci. Total Environ.*, **228**, 135 (1999).

[32] US-EPA, Vol. 40CFR258.61, p. 425, 2005.

[33] B. Clément and G. Merlin,
The contribution of ammonia and alkalinity to landfill leachate toxicity to duckweed, *Sci. Total Environ.*, **170**, 71 (1995).

[34] B.-M. Svensson, L. Mathiasson, L. Mårtensson, and S. Bergström,
Artemia Salina as test organism for assessment of acute toicity of leachate water from landfills, *Environ. Monit. Assess.*, **102**, 309 (2005).

[35] K. C. Cheung, L. M. Chu, and M. H. Wong,
Toxic effect of landfill leachate on microalgae, *Water. Air. Soil Pollut.*, **69**, 337 (1993).

[36] B. Clement and G. Merlin,
The contribution of ammonia and alkalinity to landfill leachate toxicity to duckweed, *Sci. Total Environ.*, **170**, 71 (1995).

[37] B. Clement, C. Janssen, and A. Le Dû-Delepiere,
Estimation of the hazard of landfills through toxicity testing of leachates - 2. Comparison of physico-chemical characteristics oflandfill leachates with their toxicity determined with a battery of tests, *Chemosphere*, **35**, 2783 (1997).

[38] L. Mårtensson, S. Bergström, B.-M. Svensson, and L. Mathiasson,
Development and application of an analytical protocol for evaluation of treatment processes for landfill leachates II. Evaluation of leachate treatment efficiency of different steps in a constructed pilot plant., S*ubmitted, Int. J. Environ. Anal. Chem.,* (2006).

[39] P. Kängsepp and L. Mathiasson,
Industrial leachate treatment in full scale treatment plant., *Manuscript, In Preparation*, (2006).

[40] L. M. L. Nollet,
Handbook of Water Analysis. Marcel Dekker, Inc., New York, (2000).

[41] G. Rosén,
Naturvårdverkets metodhandbok - Vatten. Naturvårdsverket, Solna, (1993).

[42] A. E. Greenberg, L. S. Clesceri, and A. D. Eaton,
Standard Methods For the Examination of Water and Waste Water 18th Ed. Amer. Pub. Health Assoc., Washinton, DC, (1992).

[43] F. W. Fifield and P. J. Haines,
Environmental Analytical Chemistry. Blackwell Science Ltd., Oxford, UK, (2000).

[44] Determination of phenolic compounds in water, **SIS 02 81 28**, *Sveriges standardiserinskommision* (1976).

[45] M. Remberger, L. Kaj, A. Palm, J. Sternbeck, E. Kvernes, and E. Brorström-Lundén,
Screening tertiary butylphenols, methylphenols, and long-chain alkylphenols in the Swedish environment, **B1594**, *IVL Swedish Environmental Research Institute Ltd.* (2003).

[46] Technical Support Document for Water Quality-Based Toxics Control, **EPA-505/2-90-001**, *US Environmental Protection Agency* (1991).

[47] I. E. Tothill and A. P. F. Turner,
Developments in bioassay methods for toxicity testing in water treatment, *Trends Anal. Chem.*, **15**, 178 (1996).

[48] M. Farré, R. Brix, and D. Barceló,
Screening water for pollutants using biological techniques under European Union funding during the last 10 years, *Trends Anal. Chem*., **24**, 532 (2005).

[49] M. Farré and D. Barceló,
Toxicity testing of wastewater and sewage sludge by biosensors, bioassays and chemical analysis, *Trends Anal Chem*., **22**, 299 (2003).

[50] P. Kristensen,
Ecotoxicological characteristics of landfill leachate, *Danish water quality insitute, VKI* (1992).

[51] B. Clément, G. Persoone, C. Janssen, and A. Le Dû-Delepierre,
Estimation of the hazard of landfills through toxicity testing of leachates - I. Determination of leachate toxicity with a battery of acute tests, *Chemosphere*, **33**, 2303 (1996).

[52] Guidelines Establishing Test Procedures for the Analysis of Pollutants; Whole Effluent Toxicity Test Methods; Final Rule, **40 CFR Part 136**, *US Environmental Protection Agency* (2002).

[53] J. T. Litchfield and F. A. Wilcoxon,
A simplified method of evaluating dose-effect experiments, *J. Pharmacol.*, **96**, 99 (1949).

[54] M. L. Ward, G. Bitton, T. Townsend, and M. Booth,
Determining toxicity of leachates from Florida municipal solid waste landfills using a battery-of-tests approach, *Environ.Toxicol*., **17**, 258 (2002).

[55] S. M. Mackenzie, S. Waite, D. J. Metcalfe, and C. B. Joyce,
Landfill Leachate Ecotoxicity Experiments using Lemna minor, *Water, Air, and Soil Pollution: Focus*, **3**, 171 (2003).

[56] J. I. Seco, C. Fernández-Pereira, and J. Vale,
A study of the leachate toxicity of metal-containing solid wastes using Daphnia magna, *Ecotoxicol. Environ. Saf.*, **56**, 339 (2003).

[57] M. C. Bloor, C. J. Banks, and V. Krivtsov,
Acute and sublethal toxicity tests to monitor the impact of leachate on an aquatic environment, *Environ*. *Int*., **31**, 269 (2005).

[58] A. Magdaleno and E. De Rosa,
Chemical composition and toxicity of waste dump leachates using Selenastrum capricornutum Printz (Chlorococcales, Chlorophyta), *Environ*. *Toxicol*., **15**, 76 (2000).

[59] T. Assmuth and S. Penttila,
Characteristics, determinants and interpretations of acute lethality in daphnids exposed to complex waste leachates, *Aquatic Toxicol*. **31**, 125 (1995).

[60] G. E. Schrab, K. W. Brown, and K. C. Donnelly,
Acute and genetic toxicity of municipal landfill leachate, *Water Air Soil Pollut.*, **69**, 99 (1993).

[61] Water quality - Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test), **ISO 11348-1 to 3:1998**, *International Organization for Standardization (ISO)* (1998).

[62] Water quality - Determination of acute toxicity for the crustacean Ceriodaphnia dubia - Static method, **SS 028214**, *Swedish Standards Institute (SIS)* (1996).

[63] Water quality - Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) - Acute toxicity test, **ISO 6341:1996**, *International Organization for Standardization (ISO)* (1996).

[64] Water quality - Determination of chronic toxicity to Ceriodaphnia dubia, **ISO/CD 20665**, *International Organization for Standardization (ISO)* (2006).

[65] A. Baun, L. Kløft, P. L. Bjerg, and N. Nyholm,
Toxicity testing of organic chemicals in groundwater polluted with landfill leachate, *Environ*. *Toxicol*. *Chem*., **18**, 2046 (1999).

[66] A. L. Buikema Jr, B. R. Niederlehner, and J. Cairns Jr,
Biological monitoring part IV—Toxicity testing *Water Res.*, **16**, 239 (1982).

[67] S. Rodriguez-Mozaz, M. J. López de Alda, M.-P. Marco, and D. Barceló,
Biosensors for environmental monitoring: A global perspective, *Talanta*, **65**, 291 (2005).

[68] A. P. F. Turner, I. Karube, and G. S. Wilson,

Biosensors Fundamental and Applications. Oxford University Press, Oxford, (1987).

[69] C. Nistor, A. Rose, M. Farré, L. Stoica, U. Wollenberger, T. Ruzgas, D. Pfeiffer, D. Barceló, L. Gorton, and J. Emneus,
In-field monitoring of cleaning efficiency in waste water treatment plants using two phenol-sensitive biosensors, *Anal. Chim. Acta*, **456**, 3 (2002).

[70] M. Del Carlo, I. Lionti, M. Taccini, A. Cagnini, and M. Mascini,
Disposable screen-printed electrodes for the immunochemical detection of polychlorinated biphenyls, *Anal. Chim. Acta*, **342**, 189 (1997).

[71] J. Liu and B. Mattiasson,
Microbial BOD sensors for wastewater analysis, *Water Res.*, **36**, 3786 (2002).

[72] G.-J. Chee, Y. Nomura, and K. Ikebukuro,
Development of photocatalytic biosensor for the evaluation of biochemical oxygen demand, *Biosens. Bioelectron.*, **21**, 67 (2005).

[73] J. C. Philp, S. v. Balmand, and E. Hajto,
Whole cell immobilised biosensors for toxicity assessment of a wastewater treatment plant treating phenolics-containing waste, *Anal. Chim. Acta*, **487**, 61 (2003).

[74] M. Farré, O. Pasini, M. Carmen Alonso, M. Castillo, and D. Barceló,
Toxicity assessment of organic pollution in wastewaters using a bacterial biosensor, *Anal. Chim. Acta*, **426**, 155 (2001).

[75] E. Tønning, S. Sapelnikova, J. Christensen, C. Carlsson, M. Winther-Nielsen, E. Dock, R. Solna, P. Skladal, L. Nørgaard, T. Ruzgas, and J. Emnéus,
Chemometric exploration of an amperometric biosensor array for fast determination of wastewater quality, *Biosens. Bioelectron.*, **21**, 608 (2005).

[76] J. P. Hart, A. Crew, E. Crouch, K. C. Honeychurch, and R. M. Pemberton,
Some Recent Designs and Developments of Screen-Printed Carbon Electrochemical Sensors/Biosensors for Biomedical, Environmental, and Industrial Analyses, *Anal. Lett.*, **37**, 789 (2004).

[77] M. Tswett,
Physical-chemical studies on chlorophyll. Adsorptions., *Berichte der Deutschen Botanischen Gesellschaft* **24**, 316 (1906).

[78] A. J. P. Martin and R. L. M. Synge,
A new form of chromatogram employing two liquid phases. I. A theory of chromatography. II. Application to the microdetermination of the higher monoamino acids in proteins. , *Biochem. J.*, **35**, 1358 (1941).

[79] T. L. Chester, J. D. Pinkston, and D. E. Raynie,
Supercritical fluid chromatography and extraction, *Anal. Chem.*, **70**, 301R (1998).

[80] L. R. Snyder and J. J. Kirkland,
Introduction to Modern Liquid Chromatography, 2nd Ed. John Wiley & Sons, Inc. , New York, USA, (1979).

[81] J. G. Dorsey, W. T. Cooper, and B. A. Siles,
Liquid chromatography: Theory and methodology, *Anal. Chem.*, **70**, 591R (1998).

[82] W. R. LaCourse,
Column Liquid Chromatography: Equipment and Instrumentation, *Anal. Chem.*, **72**, 37R (2000).

[83] W. Jennings, E. Mittlefehldt, and P. Stremple,
Analytical Gas Chromatography, 2nd Ed. Academic Press, San Diego, USA, (1997).

[84] G. A. Eiceman, H. H. Hill, and J. Gardea-Torresdey,
Gas Chromatography, *Anal. Chem.*, **72**, 137R (2000).

[85] D. A. Skoog and J. J. Leary,

Principle of instrumental analysis, 4th Ed. Saunders College Publishing, Fort Worth, USA, (1992).

[86] Electron Capture Detector (ECD), Library4science.com, LLC http://www.chromatography-online.org/topics/electron/capture/detector.html, Last visited Aug. 2006.

[87] What is Mass Spectometry?, ASMS - American Society for Mass Spectrometry, http://www.asms.org/whatisms/index.html, Last visited Aug. 2006.

[88] M. McMaster and C. McMaster,
GC/MS A Practical User's Guide. Wiley-VCH, New York, USA, (1998).

[89] R. E. Ardrey,
Liquid Chromatography Mass Spectrometry: An Introduction John Wiley & Sons Ltd., West Sussex, UK, (2003).

[90] A. L. Burlingame, R. K. Boyd, and S. J. Gaskell,
Mass spectrometry, *Anal. Chem.*, **70**, 647R (1998).

[91] J. Abian,
The coupling of gas and liquid chromatography with mass spectrometry *J. Mass Spectrom.*, **34**, 157 (1999).

[92] *Wiley Registry of Mass Spectral Data, 8th Edition*. John Wiley & Sons, Inc., New York, USA, (2006).

[93] *NIST/EPA/NIH 05 Mass Spectral Library* U.S. Department of Commerce, (2005).

[94] Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, 2002/658/EC, *Off. J. Eur. Commun.*, **L221**, 8 (2002).

[95] B. L. Milman,
Identification of chemical compounds, *Trends Anal. Chem*., **24**, 493 (2005).

[96] E. Eljarrat and D. Barceló,
Congener-specific determination of dioxins and related compounds by gas chromatography coupled to LRMS, HRMS, MS/MS and TOFMS, *J. Mass Spectrom.*, **37**, 1105 (2002).

[97] J. Namieśnik,
Trends in Environmental Analytics and Monitoring, *Crit. Rev. Anal. Chem.*, **30**, 221 (2000).

[98] P. Mayer, J. Tolls, J. L. M. Hermens, and D. Mackay,
Equilibrium Sampling Devices, *Environ. Sci. Technol.*, **37**, 184A (2003).

[99] G. Ouyang and J. Pawliszyn,
Recent developments in SPME for on-site analysis and monitoring, *Trends Anal. Chem.*, **25**, 692 (2006).

[100] B. Vrana, G. A. Mills, I. J. Allan, E. Dominiak, K. Svensson, J. Knutsson, G. Morrison, and R. Greenwood,
Passive sampling techniques for monitoring pollutants in water, *Trends Anal. Chem.*, **24**, 845 (2005).

[101] C. Porte, G. Janer, and L. C. Lorusso,
Endocrine disruptors in marine organisms: Approaches and perspectives, *Comparative Biochemistry & Physiology Part C: Toxicology & Pharmacology*, **143**, 303 (2006).

[102] T. V. Reddy, J. Lazorchak, and D. Lattier,
Vitellogenin Gene Expression in Male Fathead Minnow as an Indicator of Exposure to Endocrine Disrupting Chemicals (EDCs) in an Aquatic Environment, US Environmental Protection Agency, http://www.epa.gov/eerd/VGQPCR.htm, Last visited Aug 2006.

[103] T. Hyötyläinen and K. Hartonen,

Determination of brominated fame retardants in environmental samples, *Trends Anal. Chem.*, **21**, 13 (2002).

[104] A. Covacia, S. Voorspoelsa, and J. de Boer,
Determination of brominated flame retardants, with emphasis on polybrominated diphenyl ethers (PBDEs) in environmental and human samples—a review, *Environ. Int.*, **29**, 735 (2003).

[105] Jerry J. Wu, W. Chih-Chao, M. Hong-Wen, and C. Chia-Chi,
Treatment of landfill leachate by ozone-based advanced oxidation processes, *Chemosphere*, **54**, 997 (2004).

[106] A. B. C. Alvares, C. Diaper, and S. A. Parsons,
Partial oxidation by ozone to remove recalcitrance from wastewaters - a review, *Environ. Technol.*, **22**, 409 (2001).

[107] A. Lopez, M. Pagano, A. Volpe, and A. Claudio Di Pinto,
Fenton's pre-treatment of mature landfill leachate, *Chemosphere*, **54**, 1005 (2004).

[108] E. Neyens and J. Baeyens,
A review of classic Fenton's peroxidation as an advanced oxidation technique, *J. Hazard. Mater.*, **98**, 33 (2003).

[109] C. G. Whiteley and D. J. Lee,
Enzyme technology and biological remediation, *Enzyme Microb. Technol.*, **38**, 291 (2006).

[110] U. Welander and T. Henrysson,
Degradation of organic compounds in a municipal landfill leachate treated in a suspended-carrier biofilm process, *Water Environ. Res.*, **70**, 1236 (1998).

[111] E. Benfenati, E. Porazzi, R. Bagnati, F. Forner, M. P. Martinez, G. Mariani, and R. Fanelli,
Organic tracers identification as a convenient strategy in industrial landfills monitoring, *Chemosphere*, **51**, 677 (2003).

[112] L. D. Betowski, D. S. Kendall, C. M. Pace, and J. R. Donnelly,
Characterization of Groundwater Samples from Superfund Sites by Gas Chromatography/Mass Spectrometry and Liquid Chromatography/Mass Spectrometry, *Environ. Sci. Technol.*, **30**, 3558 (1996).

[113] M. Castillo, M. C. Alonso, J. Riu, and D. Barceló,
Identification of Polar, Ionic, and Highly Water Soluble Organic Pollutants in Untreated Industrial Wastewaters, *Environ. Sci. Technol.*, **33**, 1300 (1999).

[114] D. Puig and D. Barceló,
Determination of phenolic compounds in water and waste water, *Trends Anal. Chem.*, **15**, 362 (1996).

[115] List of substances which could belong to List I of Council Directive 76/464/EEC European Commission, Environment Directorate-General http://ec.europa.eu/environment/water/water-dangersub/candidate_list_1.htm, Last visited Aug 2006.

[116] R. Romero, *Personal Communication*. Amería University, Almería, ES, (2005).

[117] J. C. Miller and J. N. Miller,
Statistics for Analytical Chemistry, 3rd Ed. Ellis Horwood Ltd., Chichester, UK, (1993).

[118] R. E. Majors,
An overview of sample preparation, *LC-GC*, **9**, 16 (1991).

[119] T. Hyötyläinen and M.-L. Riekkola,
Approaches for on-line coupling of extraction and chromatography, *Anal. Bioanal. Chem.*, **378**, 1962 (2004).

[120] R. E. Majors,
Trends in Sample Preparation, *LCGC North America*, **20**, 1098 (2002).
[121] EPA Method 3510C, Separatory funnel Liquid Liquid Extraction, *US Environmental Protection Agency* (1996).
[122] EPA Method 3640A, Gel-permeation cleanup, *US Environmental Protection Agency* (1994).
[123] B. Karlberg and S. Thelander,
Extraction based on the flow-injection principle : Part I. Description of the Extraction System, *Anal. Chim. Acta*, **98**, 1 (1978).
[124] M. Valcárcel and M. D. Luque de Castro,
Non-Chromatographic Continuous Separation Techniques. The Royal Society of Chemistry, Cambridge, UK, (1991).
[125] E. Fogelqvist, M. Krysell, and L. G. Danielsson,
On-line liquid-liquid extraction in a segmented flow directly coupled to on-column injection into a gas chromatograph, *Anal. Chem.*, **58**, 1516 (1986).
[126] EPA Method 3520C, Continuous Liquid-Liquid Extraction, *US Environmental Protection Agency* (1996).
[127] P. R. Loconto,
Trace Environmental Quantitative Analysis Principles, Techniques, and Applications. Marcel Dekker, Inc., New York, US, (2001).
[128] E. Baltussen, C. A. Cramers, and P. J. F. Sandra,
Sorptive sample preparation – a review, *Anal. Bioanal. Chem.*, **373**, 3 (2002).
[129] J. Å. Jönsson and L. Mathiasson,
Liquid membrane extraction in analytical sample preparation - I. Principles, *Trends Anal. Chem.*, **18**, 318 (1999).
[130] D. T. Burns, K. Danzer, and A. Townshend,
Use of the term "recovery" and "apparent recovery" in analytical procedures (IUPAC Recommendations 2002), *Pure Appl. Chem.*, **74**, 2201 (2002).
[131] EPA Method 3535A, Solid Phase Extraction, Rev. 1A, *US Environmental Protection Agency* (1998).
[132] R. Westbom, L. Thörneby, S. Zorita, L. Mathiasson, and E. Björklund,
Development of a solid-phase extraction method for the determination of polychlorinated biphenyls in water, *J. Chromatogr. A*, **1033**, 1 (2004).
[133] M.-C. Hennion,
Solid-phase extraction: method development, sorbents, and coupling with liquid chromatography, *J. Chromatogr. A*, **856**, 3 (1999).
[134] K. Pyrzynska and E. Pobozy,
On-Line Coupling of Solid Phase Extraction Sample Processing with High-Performance Liquid Chromatography, *Crit. Rev. Anal. Chem.*, **32**, 227 (2002).
[135] I. Ferrer and D. Barceló,
Validation of new solid-phase extraction materials for the selective enrichment of organic contaminants from environmental samples, *Trends Anal. Chem.*, **18**, 180 (1999).
[136] B. Sellergren,
Direct drug determination by selective sample enrichment on an imprinted polymer, *Anal. Chem.*, **66**, 1578 (1994).
[137] E. Caro, R. M. Marce, F. Borrull, P. A. G. Cormack, and D. C. Sherrington,
Application of molecularly imprinted polymers to solid-phase extraction of compounds from environmental and biological samples, *Trends Anal. Chem.*, **25**, 143 (2006).

[138] M.-C. Hennion and V. Pichon,
Immuno-based sample preparation for trace analysis, *J. Chromatogr. A*, **1000**, 29 (2003).
[139] J. Pawliszyn and S. Liu,
Sample introduction for capillary gas chromatography with laser desorption and optical fibers, *Anal. Chem.*, **59**, 1475 (1987).
[140] C. L. Arthur and J. Pawliszyn,
Solid phase microextraction with thermal desorption using fused silica optical fibers, *Anal. Chem.*, **62**, 2145 (1990).
[141] H. Kataoka,
Automated sample preparation using in-tube solid-phase microextraction and its application - a review, *Anal. Bioanal. Chem.*, **373**, 31 (2002).
[142] J. O'Reilly, Q. Wang, L. Setkova, J. P. Hutchinson, Y. Chen, H. L. Lord, C. M. Linton, and J. Pawliszyn,
Automation of solid-phase microextraction, *J. Sep. Sci.*, **28**, 2010 (2005).
[143] B. Zygmunt, A. Jastrzecbska, and J. Namiesnik,
Solid Phase Microextraction — A Convenient Tool for the Determination of Organic Pollutants in Environmental Matrices, *Crit. Rev. Anal. Chem.*, **31**, 1 (2001).
[144] E. E. Stashenko and J. R. Martínez,
Derivatization and solid-phase microextraction, *Trends Anal. Chem.*, **23**, 553 (2004).
[145] G. L. Hook, G. L. Kimm, T. Hall, and P. A. Smith,
Solid-phase microextraction (SPME) for rapid field sampling and analysis by gas chromatography-mass spectrometry (GC-MS), *Trends Anal. Chem.*, **21**, 534 (2002).
[146] E. Baltussen, P. Sandra, F. David, and C. Cramers,
Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: Theory and principles Presented at the 21st International Symposium on Capillary Chromatography and Electrophoresis, Park City, Utah, June 20-24, 1999., *Journal of Microcolumn Separations*, **11**, 737 (1999).
[147] M. Kawaguchi, R. Ito, K. Saito, and H. Nakazawa,
Novel stir bar sorptive extraction methods for environmental and biomedical analysis, *J. Pharm. Biomed. Anal.*, **40**, 500 (2006).
[148] F. David, B. Tienpont, and P. Sandra,
Stir-Bar Sorptive Extraction of Trace Organic Compounds from Aqueous Matrices, *LCGC North America*, **21**, 108 (2003).
[149] T. Clifford,
Fundamentals of Supercritical Fluids. Oxford University Press, Oxfork, UK, (1999).
[150] K. Hartonen, S. Bøwadt, H. P. Dybdahl, K. Nylund, S. Sporring, H. Lund, and F. Oreld,
Nordic laboratory intercomparison of supercritical fluid extraction for the determination of total petroleum hydrocarbon, polychlorinated biphenyls and polycyclic aromatic hydrocarbons in soil, *J. Chromatogr. A*, **958**, 239 (2002).
[151] S. Bøwadt, B. Johansson, S. Wunderli, M. Zennegg, L. F. de Alencastro, and D. Grandjean,
Independent Comparison of Soxhlet and Supercritical Fluid Extraction for the Determination of PCBs in an Industrial Soil, *Anal. Chem.*, **67**, 2424 (1995).
[152] S. B. Hawthorne and D. J. Miller,
Directly coupled supercritical fluid extraction—gas chromatographic analysis of polycyclic aromatic hydro-carbons and polychlorinated biphenyls from environmental solids, *J. Chromatogr. A*, **403**, 63 (1987).
[153] M. Zougagh, M. Valcárcel, and A. Ríos,

Supercritical fluid extraction: a critical review of its analytical usefulness, *Trends Anal. Chem.*, **23**, 399 (2004).

[154] R. E. Majors,
Modern Technique for the Extraction of Solid Materials - An Update, LCGC North America, http://www.lcgcmag.com/lcgc/article/articleDetail.jsp?id=358290, Last visited Aug 2006 (2006).

[155] R. M. Smith,
Supercritical fluids in separation science - the dreams, the reality and the future, *J. Chromatogr. A*, **856**, 83 (1999).

[156] T. Nilsson, S. Sporring, and E. Bjorklund,
Selective supercritical fluid extraction to estimate the fraction of PCB that is bioavailable to a benthic organism in a naturally contaminated sediment, *Chemosphere*, **53**, 1049 (2003).

[157] T. Nilsson,
Extraction of PCBs from Sediments: Towards Bioavailability Assessment Based on Supercritical Fluid Extraction. PhD Thesis, Department of Analytical Chemistry, Lund University, (2004).

[158] B. E. Richter, B. A. Jones, J. L. Ezzell, N. L. Porter, N. Avdalovic, and C. Pohl,
Accelerated Solvent Extraction: A Technique for Sample Preparation, *Anal. Chem.*, **68**, 1033 (1996).

[159] Method 3545A, Pressurized Fluid Extraction (PFE), Rev. 1A, *US Environmental Protection Agency* (2000).

[160] Method 3562, Supercritical Fluid Extraction of Polychlorinated Biphenyls (PCBs) and Organochlorine pesticides, *US Environmental Protection Agency* (1998).

[161] Method 3561, Supercritical Fluid Extraction of Polynuclear Aromatic Hydrocarbons, *US Environmental Protection Agency* (1996).

[162] Method 3560, Supercritical Fluid Extraction of Total Recverable Pertoleum Hydrocarbons, *US Environmental Protection Agency* (1996).

[163] Y. Yang and S. Bowadt,
Subcritical water extraction of polychlorinated biphenyls from soil and sediment, *Anal. Chem.*, **67**, 4571 (1995).

[164] K. Hartonen, K. Inkala, M. Kangas, and M. L. Riekkola,
Extraction of polychlorinated biphenyls with water under subcritical conditions, *J. Chromatogr. A*, **785**, 219 (1997).

[165] K. Kuosmanen, T. Hyotylainen, K. Hartonen, J. A. Jonsson, and M. L. Riekkola,
Analysis of PAH compounds in soil with on-line coupled pressurised hot water extraction-microporous membrane liquid-liquid extraction-gas chromatography, *Anal. Bioanal. Chem.*, **375**, 389 (2003).

[166] K. Kuosmanen, T. Hyötyläinen, K. Hartonen, and M.-L. Riekkola,
Analysis of polycyclic aromatic hydrocarbons in soil and sediment with on-line coupled pressurised hot water extraction, hollow fibre microporous membrane liquid–liquid extraction and gas chromatography, *Analyst*, **128**, 434 (2003).

[167] S. Morales-Munoz, J. L. Luque-García, and M. D. Luque de Castro,
Pure and modified water assisted by auxiliary energies: An environmental friendly extractant for sample preparation, *Anal. Chim. Acta*, **557**, 278 (2006).

[168] M. Tudorache, M. Co, H. Lifgren, and J. Emnéus,
Ultrasensitive Magnetic Particle-Based Immunosupported Liquid Membrane Assay, *Anal. Chem.*, **77**, 7156 (2005).

[169] G. Audunsson,

Aqueous/aqueous extraction by means of a liquid membrane for sample cleanup and preconcentration of amines in a flow system, *Anal. Chem.*, **58**, 2714 (1986).

[170] J. Å. Jönsson and L. Mathiasson,
Membrane-based techniques for sample enrichment, *J. Chromatogr. A*, **902**, 205 (2000).

[171] J. Å. Jönsson and L. Mathiasson,
Liquid membrane extraction in analytical sample preparation - II. Applications, *Trends Anal. Chem*., **18**, 325 (1999).

[172] S. Pedersen-Bjergaard and K. E. Rasmussen,
Liquid-Liquid-Liquid Microextaction for Sample Preparation of Biological Fluids Prior to Capillary Electrophoresis, *Anal. Chem.*, **71**, 2650 (1999).

[173] E. Psillakis and N. Kalogerakis,
Developments in liquid-phase microextraction, *Trends Anal. Chem.*, **22**, 565 (2003).

[174] J. Zhang, T. Su, and H. K. Lee,
Development and application of microporous hollow fiber protected liquid-phase microextraction via gaseous diffusion to the determination of phenols in water, *J. Chromatogr. A*, **1121**, 10 (2006).

[175] G. Ljungkvist, A. Azimia, and L. Mathiasson,
A combination of micro-porous membrane liquid–gas extraction and solid-phase trapping for ultra trace determination of benzene in urine, *J. Environ. Monit.*, **6**, 143 (2004).

[176] J. Å. Jönsson, P. Lövkvist, G. Audunsson, and G. Nilvé,
Mass transfer kinetics for analytical enrichment and sample preparation using supported liquid membranes in a flow system with stagnant acceptor liquid, *Anal. Chim. Acta*, **277**, 9 (1993).

[177] J.-f. Liu, J. Å. Jönsson, and P. Mayer,
Equilibrium Sampling through Membranes of Freely Dissolved Chlorophenols in Water Samples with Hollow Fiber Supported Liquid Membrane, *Anal. Chem.*, **77**, 4800 (2005).

[178] R. Romero, J.-f. Liu, P. Mayer, and J. Å. Jönsson,
Equilibrium Sampling through Membranes of Freely Dissolved Copper Concentrations with Selective Hollow Fiber Membranes and the Spectrophotometric Detection of a Metal Stripping Agent, *Anal. Chem.*, **77**, 7605 (2005).

[179] T. Trtic-Petrovic and J. Å. Jönsson,
Determination of drug-protein binding using supported liquid membrane extraction under equilibrium conditions, *J. Chromatogr. B.*, **814**, 375 (2005).

[180] J. Norberg,
Non-porous membrane extractions in contemporary analysis. PhD Thesis, Department of Analytical Chemistry, Lund University, (2000).

[181] E. Thordarson, S. Pálmarsdóttir, L. Mathiasson, and J. Å. Jönsson,
Sample Preparation Using a Miniaturized Supported Liquid Membrane Device Connected On-Line to Packed Capillary Liquid Chromatography, *Anal. Chem.*, **68**, 2559 (1996).

[182] Yin Shen, J. Å. Jönsson, and L. Mathiasson,
On-Line Microporous Membrane Liquid-Liquid Extraction for Sample Pretreatment Combined with Capillary Gas Chromatography Applied to Local Anaesthetics in Blood Plasma, *Anal. Chem.*, **70**, 946 (1998).

[183] M. Sandahl, L. Mathiasson, and J. Å. Jönsson,
Determination of thiophanate-methyl and its metabolites at trace level in spiked natural water using the supported liquid membrane extraction and the microporous

membrane liquid–liquid extraction techniques combined on-line with high-performance liquid chromatography *J. Chromatogr. A*, **893**, 123 (2000).

[184] J. Norberg and E. Thordarson,
Extracting syringe-connecting sample preparation and gas chromatography, *Analyst*, **125**, 673 (2000).

[185] L. Zhao and H. K. Lee,
Liquid-Phase Microextraction Combined with Hollow Fiber as a Sample Preparation Technique Prior to Gas Chromatography/Mass Spectrometry, *Anal. Chem.*, **74**, 2486 (2002).

[186] C. Basheer, H. K. Lee, and J. P. Obbard,
Determination of organochlorine pesticides in seawater using liquid-phase hollow fibre membrane microextraction and gas chromatography-mass spectrometry, *J. Chromatogr. A*, **968**, 191 (2002).

[187] C. Basheer, H. K. Lee, and J. P. Obbard,
Application of liquid-phase microextraction and gas chromatography-mass spectrometry for the determination of polychlorinated biphenyls in blood plasma, *J. Chromatogr. A*, **1022**, 161 (2004).

[188] J. Norberg, E. Thordarson, L. Mathiasson, and J. A. Jonsson,
Microporous membrane liquid-liquid extraction coupled on-line with normal-phase liquid chromatography for the determination of cationic surfactants in river and waste water, *J. Chromatogr. A*, **869**, 523 (2000).

[189] S. Bergström,
Continuous on-line micro-liquid-liquid extraction-gas chromatography for quick and sensitive determination of volatile organic compounds in blood plasma (In swedish). Lund University, Diploma work 200, Department of Analytical Chemistry, (1999).