Gdańsk University of Technology
Chemical Faculty
Department of Chemical Technology

PhD dissertation

Investigation on the behaviour of imidazolium ionic liquids in the environment

by

Marta Markiewicz (M.Sc.Eng.)

Advisor: Prof. dr hab. inż. Jan Hupka

Gdańsk 2012

Acknowledgements

TABLE OF CONTENTS

List of abbreviations

acetyl Co-A – acetyl coenzyme A
AFM – atomic force microscopy
CAC – critical aggregate concentration
CEC – cation exchange capacity
CMC – critical micelle concentration
CTAB – cetyltrimethylammonium bromide
CVP – colloid vibration potential
DOM – dissolved organic matter
DPC – dodecylpyridinium chloride
EC_{50} – half maximal effective concentration
EDL – electrical double layer
ESI-MS – electrospray ionisation mass spectrometry
FA – fulvic acids
GC/MS – gas chromatography coupled with mass spectrometry
HA – humic acid
HOC – hydrophobic organic compound
HPLC – high performance liquid chromatography
IHP – inner Helmholtz plane
IL/ILs – ionic liquid/ionic liquids
K_d – soil/water partition coefficient
LC/MS – liquid chromatography coupled with mass spectrometry
OC – organic carbon
OECD – Organisation for Economic Cooperation and Development
OHP – outer Helmholtz plane
PAH – poly-aromatic hydrocarbons
PDI – potential determining ions
PV – pore volume
QACs – quaternary ammonium compounds
SOM – soil organic matter
TOC – total organic carbon
VOCs – volatile organic compounds
WWTP – wastewater treatment plant
ζ – zeta potential

Ionic liquids nomenclature

Cl^- - chloride anion,
Br^- - bromide anion,
BF_4^- - tetrafluoroborate anion,
PF_6^- - hexafluorophosphate anion,
$N(CN)_2^-$ - dicyanidoamide anion,
$C(CN)_3^-$ - tricyanomethanide anion,
$[B(CN)_4]^-$ - tetracyanidoboranuide anion,
$[N(CF_3SO_2)_2]^-$ - bis(trifluoromethylsulfonyl)imide anion, TF_2N,
$[CF_3SO_3]^-$ - trifluoromethanesulfonate anion, TfO,
$[(C_2F_5)_3PF_3]^-$ - trifluorotris(pentafluoroethyl)phosphate anion,
$[C_8H_{17}OSO_3]^-$ - octylsulfate anion,

$[EMIM]^+$ – 1-ethyl-3-methylimidazolium cation,
$[BMIM]^+$ – 1-butyl-3-methylimidzolium cation,
$[HMIM]^+$ – 1-hexyl-3-methylimidazolium cation,
$[OMIM]^+$ – 1-methyl-3-octylimidzolium cation,

Abstract

Ionic liquids (ILs) are low melting salts having the potential to revolutionise chemical industry as they offer both economic and ecological advantages over so far applied technologies. Imidazolium ILs are among the most widely used in industrial processes which makes them potential aquatic and terrestrial contaminants. Certain environmental parameters (e.g. biodegradability, toxicity, partition coefficients) of ILs were investigated in laboratory conditions to some extent. This knowledge suggests that imidazolium ILs might indeed pose an ecological threat. So far little is known about ILs fate in environment and possible ways of reducing their concentration.

The aim of the present work is to gain a better understanding of processes affecting ILs in the environment and to fill the gap between knowledge built on the basis of standard laboratory tests and a need to evaluate the fate of xenobiotics in realistic conditions. From among all ILs one exemplary compound, 1-methyl-3-octylimidazolium chloride ([OMIM][Cl]), was selected as key structure for the current investigation. [OMIM][Cl] is a common representative of this substance class with high technological relevance, which comes along with the increased probability that [OMIM][Cl] as well as other ILs will be released into the biosphere.

Two different ways of entering the environment can be distinguished: (1) usually unintentional, permanent release of small amounts e.g. from products containing ILs, leaching out from spent products in landfills or breaking through wastewater treatment processes or (2) release of high concentrations due to spills or accidents. These two ways force two different approaches in hazard assessment and mitigation of negative impacts. In this regard biodegradation and sorption are the most important parameters for reducing adverse effects of ILs on the environment. Both processes were investigated with respect to their range and mechanisms whenever possible or necessary.

Dissertation begins with a general introduction (Chapter 1) followed by the description of aim and scope (Chapter 2). Since the experimental work covers a broad range of diverse topics it was divided into three main chapters, each of them having its own literature, experimental and results sections closed by a summary. The dissertation ends with another three chapters discussing practical meaning of results (Chapter 6), suggesting further research (Chapter 7) and concluding the whole work (Chapter 8).

In Chapter 3 the fate of [OMIM][Cl] in wastewater treatment process was investigated. Standard biodegradation and toxicity test were modified and expanded to account for conditions and processes which typically occur during wastewater treatment. The removal of [OMIM][Cl] from wastewaters by adsorption on biomass and biodegradation was examined across four orders of magnitude, biodegradability threshold and partition coefficients were determined. Based on this finding a patent application for a method and installation for removing xenobiotics from wastewaters was filed. Additionally, the toxicity of [OMIM][Cl] towards activated sewage sludge biocenosis was measured. To simulate real wastewater treatment plant (WWTP) conditions, microbial biocenosis was gradually exposed to increasing concentrations of [OMIM][Cl] to allow adaptation - gradually increasing rate of degradation was observed. In another experiment additional, easily available sources of carbon and nitrogen were supplemented together with [OMIM][Cl], reflecting a complex composition of sewage reaching WWTP. It was noted that the availability of other nutrient sources clearly hinders the biodegradation of [OMIM][Cl]. This finding questions the relevance and value of standard biodegradation test demanded by environmental legislation to evaluate the hazards of chemicals.

Chapter 4 pertains to the behaviour of [OMIM][Cl] in soils as one of endpoint sinks of xenobiotics. Adsorption to soil solids was investigated since it defines the bioavailable concentration of xenobiotic and influences its mobility. Batch and soil column tests were performed, as the result adsorption isotherms, breakthrough curves and partition coefficients were obtained. Additionally the biodegradability of [OMIM][Cl] using soil microbial community was tested. The rate of degradation was lower than in activated sewage sludge.

In Chapter 5 the influence of [OMIM][Cl] on soil was examined taking its two most distinctive structural features into account: surface activity and ionic nature. Via electroacoustic measurements it was discovered that [OMIM][Cl] interacts with minerals changing charge characteristics of the inorganic fraction of soil. Moreover, the IL solubilises soil organic matter and other hydrophobic compounds present in soil as proved on a group of 16 poly-aromatic hydrocarbons (PAH), causing secondary pollution.

As a result of assembling of all abovementioned results a set of guidelines that should be taken into account when estimating environmental fate of [OMIM][Cl] either from standard laboratory tests results or from modelling based on structural properties was created. Several of the conclusions presented within this dissertation are not compound specific and can be extended to other classes of ILs and xenobiotics in general.

Streszczenie

Ciecze jonowe są solami o niskiej temperaturze topnienia, które mogą zrewolucjonizować przemysł chemiczny ponieważ w wielu przypadkach oferują zarówno ekonomiczny jak i ekologiczny zysk w porównaniu z dotychczas stosowanymi technologiami. Imidazoliowe ciecze jonowe należą do najczęściej stosowanych co sprawia, że mogą stanowić źródło zanieczyszczenia środowiska glebowego i wodnego. Niektóre parametry środowiskowe (np. biodegradowalność, toksyczność, współczynnik podziału gleba/roztwór glebowy) cieczy jonowych zostały wyznaczone w warunkach laboratoryjnych. Zdobyte w ten sposób informacje sugerują, że imidazoliowe ciecze jonowe mogą stanowić zagrożenie ekologiczne. Trudno jednak ekstrapolować wyniki badań przeprowadzonych czasem w bardzo wyidealizowanych warunkach bezpośrednio do skomplikowanych systemów środowiska przyrodniczego. Z tego względu wciąż niewiele wiadomo na temat losu cieczy jonowych w środowisku oraz sposobów redukcji ich stężenia.

Istnieją dwa sposoby przedostawania się tych wiązków do środowiska: (1) ciągłe uwalnianie małych ilości np. z produktów je zawierających, ługowanie ze zużytych produktów w składowiskach odpadów czy przedostawanie się przez systemy oczyszczania ścieków lub (2) uwalnianie wysokich stężeń w wyniku rozlewów czy wypadków. Sposób, w który ciecze jonowe dostają się do środowiska wymusza inne podejście do oceny ryzyka oraz stosowanie innych metod neutralizacji. Spośród imidazoliowych cieczy jonowych wybrany został jeden przykładowy związek o kluczowej strukturze dla obecnych badań. Chlorek 1-metylo-3-oktyloimidazolu ([OMIM][Cl]) reprezentuje najistotniejsze właściwości tej grupy związków. [OMIM][Cl] charakteryzuje się dobrą biodegradowalnością, umiarkowaną toksycznością pozwalając na obserwacje zarówno pozytywnego jak i negatywnego wpływu zmieniających się waruków. Ponadto jest to pierwszy z serii homologów imidazoliowych wykazujący wyraźną aktywność powierzchniową, która powoduje, że związek ten wykazuje wiele ineresujących oddziaływań powierzchniowych.

W niniejszej pracy zostały wzięte pod uwagę dwa procesy mające najistotniejsze znaczenie dla łagodzenia negatywnego wpływu cieczy jonowych na środowisko: sorpcja i biodegradacja. Oba zjawiska zostały zbadane pod względem zakresu oraz mechanizmu jeżeli było to możliwe lub konieczne.

Dysertacja rozpoczyna się ogólnym wprowadzeniem teoretycznym (rozdział 1) oraz opisem celu i zakresu pracy (rozdział 2). Z uwagi na to, że część badawcza obejmuje szeroki zakres tematyczny została ona podzielona na trzy główne rozdziały, z których każdy posiada odrębny

przegląd literatury, opis metodyki badawczej i wyników zakończony podsumowaniem. Dysertacja została zakończona kolejnymi trzema rozdziałami opisującymi praktyczne znaczenie uzyskanych wyników (rozdział 6), kierunki dalszych badań (rozdział 7) oraz podsumowującymi całą pracę (rozdział 8).

W rozdziale 3 został opisany los imidazoliowych cieczy jonowych w procesie oczyszczania ścieków. Standardowe testy biodegradacj i toksyczności zostały zmodyfikowane lub rozszerzone tak aby uwzględniały warunki i procesy normalnie zachodzące w oczyszczalni ścieków. Możliwość usuwania cieczy jonowych ze ścieków poprzez adsorpcję na biomasie lub biodegradację, próg biodegradowalności oraz współczynnik podziału osad czynny/ciecz nadosadowa zostały zbadane dla stężeń obejmujących cztery rzędy wielkości. Ponadto wyznaczono toksyczność w stosounku do biocenozy osadu czynnego i obliczono wartość EC_{50}. W celu symulacji procesów zachodzących w oczyszczalni ścieków, organizmy osadu czynnego poddawane były działaniu stopniowo wzrastających ilości cieczy jonowej aby umożliwić im adaptację. Zaobserwowano stopniowio wzrastającą szybkość degradacji. W innym badaniu, dodatkowe źródła łatwoprzyswajalnego węgla i/lub azotu zostały dodane do medium chodowlanego razem z cieczą jonową, ponieważ są one zwykle obecne w ściekach trafiających do oczyszczalni. Zauważono, że dostępność alternatywnych substancji pokarmowych zaburza biodegdarację cieczy jonowych.

Rozdział 4 dotyczy zachowania się cieczy jonowych w środowisku glebowym. Adsorpcja na powierzchni frakcji stałej określa ilość ksenobiotyku, która pozostaje biodostępna oraz wpływa na mobilność. W celu określenia stopnia adsorpcji wykorzystano zarówno test kolumnowy jak i test okresowy, jako wynik otrzymano izostermy adsorpcji, krzywe przebicia ksenobiotyku przez złoże oraz współczynniki podziału gleba/roztwór glebowy. Ponadto zbadano biodegradację cieczy jonowych prowadzoną przy wykorzystaniu inokulum złożonego z organizmów glebowych metodą respirometrii manometrycznej (mineralizacja) oraz w przepływie przez kolumnę glebową i kolumnę glebową augmentowaną osadem czynnym. Tempo biodegradacji było niższe niż w przypadku organizmów osadu czynnego.

W rozdziale 5 przedyskutowano wpływ jaki ciecze jonowe mogą mieć na glebę biorąc pod uwagę dwie najbardziej charakterystyczne cechy ich struktury: aktywność powierzchniową i jonowy charakter. Odkryto, że ciecze jonowe oddziaływują z frakcją mineralną zmieniając jej ładunek. Ponadto ciecze jonowe solubilizują frakcję oraginczną gleby oraz inne związki organiczne, które się w niej znajdują, jak pokazano na przykładzie grupy 16 wielopierścieniowych związków aromatycznych, powodując wtórne skażenie gleby.

W wyniku kompilacji wszystkich wspomnianych wyników powstał zestaw wytycznych, które powinny być brane pod uwagę przy szacowaniu losów cieczy jonowych czy to na podstawie

standardowych wyników badań laboratoryjnych czy modelowania w oparciu o właściwości strukturalne. Wiele z wniosków przedstawionych w tej pracy nie jest specyficznych wyłącznie dla cieczy jonowych i może zostać rozszerzona na inne grupy ksenobiotyków lub może służyć jako ogólne wskaźniki do badania ksenobiotyków.

1. Introduction

1.1. Ionic liquids

Ionic liquids (ILs) are salts composed entirely of ions and having melting temperatures below 100°C. Three main building blocks can be distinguished in the chemical structure of an ionic liquid: head group (cationic core) together with substituents (side chains) forming a cation and an anion (Figure 1). Anions and cations building ILs can be both organic and/or inorganic however, most widely used and studied ones are those composed of bulky organic cation, usually substituted with alkyl chains, and inorganic/organic anion [1-3]. Since there are three structural elements building an IL, there is a great number of possible combinations of arranging those elements together to form an IL. The theoretical number of ionic liquids structures has virtually no limits. Earle and Seddon estimated the number to be 1 billion [2].
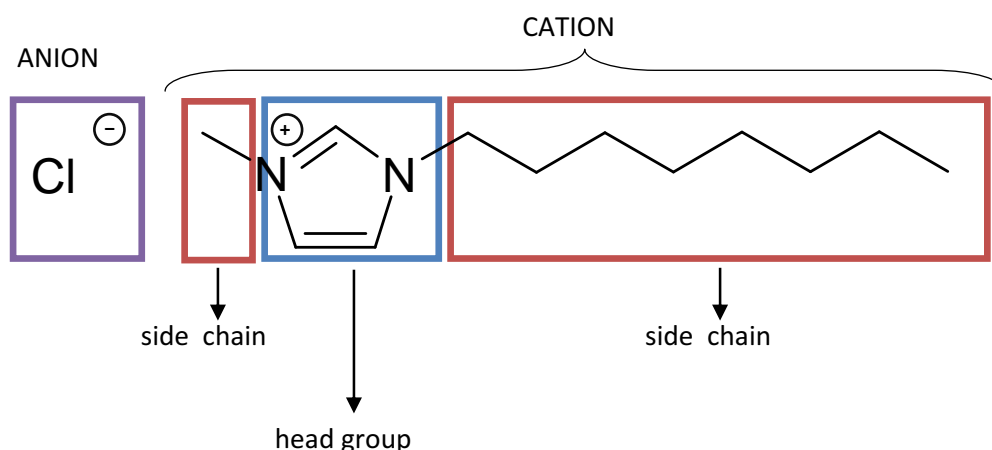
Figure 1. Chemical structure of 1-methyl-3-octylimidazolium chloride

The history of ILs dates back to 1914, when Walden described low-melting ethylammonium nitrite, and develops into so called first generation ILs - mixtures of aluminium (III) chloride and organic salt, usually pyridinium or imidazolium. A major disadvantage of all chloroaluminate (III) ionic liquids is their moisture sensitivity, meaning that an ILs itself needs to be prepared and handled under the inert gas atmosphere and presents a technological difficulty for an industrial application, as moisture must be excluded from all the reagents and equipment involved in all operations [4] [3]. This problem was partially solved at the beginning of 90's of XX century when first air and water stable ILs known as second generation ILs were obtained. These were not only far less moisture sensitive, even though some of them were shown to release hydrogen fluoride in the presence of water, but showed significantly higher electrochemical

stability. Since then, a variety of water stable ILs incorporating many different anions and cations were synthesized and studied. As in the early stages of IL's development the main goal was to obtain a low-melting electrolyte, water and electrochemical stability were main objectives in research conducted at that stage. As the result ILs with hydrophobic anions like: trifluoromethanesulfonate ($[CF_3SO_3]^-$), bis(trifluoromethylsulfonyl)imide ($[N(CF_3SO_2)_2]^-$) were developed providing not only hydrolytic stability but also a remarkable electrochemical window of up to 6 V, some examples of common ILs structures are given in Figure 2.
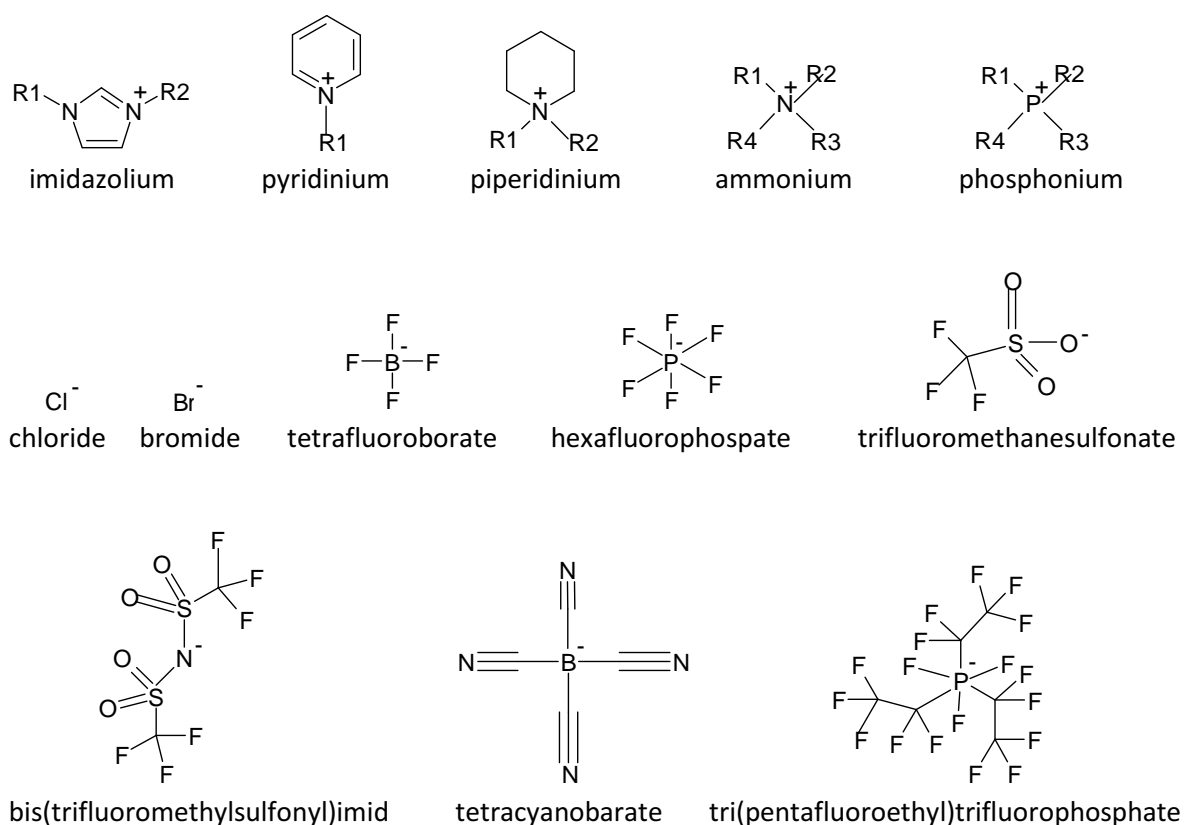
Figure 2. Commonly used ILs anions and cations

1.2. Applications and technological importance

ILs possess physicochemical properties which are perceived as beneficial for many technological processes and which can be additionally fine-tuned to match specific demands. The most significant ones are: good thermal stability, wide electrochemical potential window, extremely low vapour pressure, high electric conductivity and miscibility with water or organic solvents which make them interesting for application in different fields "limited simply by

imagination and the time required for investigation" [3, 5]. So far ionic liquids are used in metal plating [6-8], gas separation, compression and handling [9, 10], lithium-ion batteries [8], dye-sensitized solar cells [11] and as paint or polymer additives [12].

The unique feature of ILs is the tuneability of their physicochemical properties achievable by changing the structural elements forming the molecule. Properties such as: density, viscosity, hydrophobicity, melting point and solvating properties can be adjusted. As a simple example water solubility of imidazolium IL can be decreased by increasing the length of alkyl substituent of the cation or IL can be changed from completely water miscible to almost virtually insoluble by changing anion from simple halide to bulky bis(trifluoromehtylsulfonyl)imide. This offers the possibility of obtaining biphasic system with water as well as with organic solvents for liquid/liquid separation systems [13]. The range of possible applications of ionic liquids is constantly expanding currently covering fields of physicochemistry, electrochemistry, biochemistry, engineering, analytics, synthesis and catalysis (Figure 3).
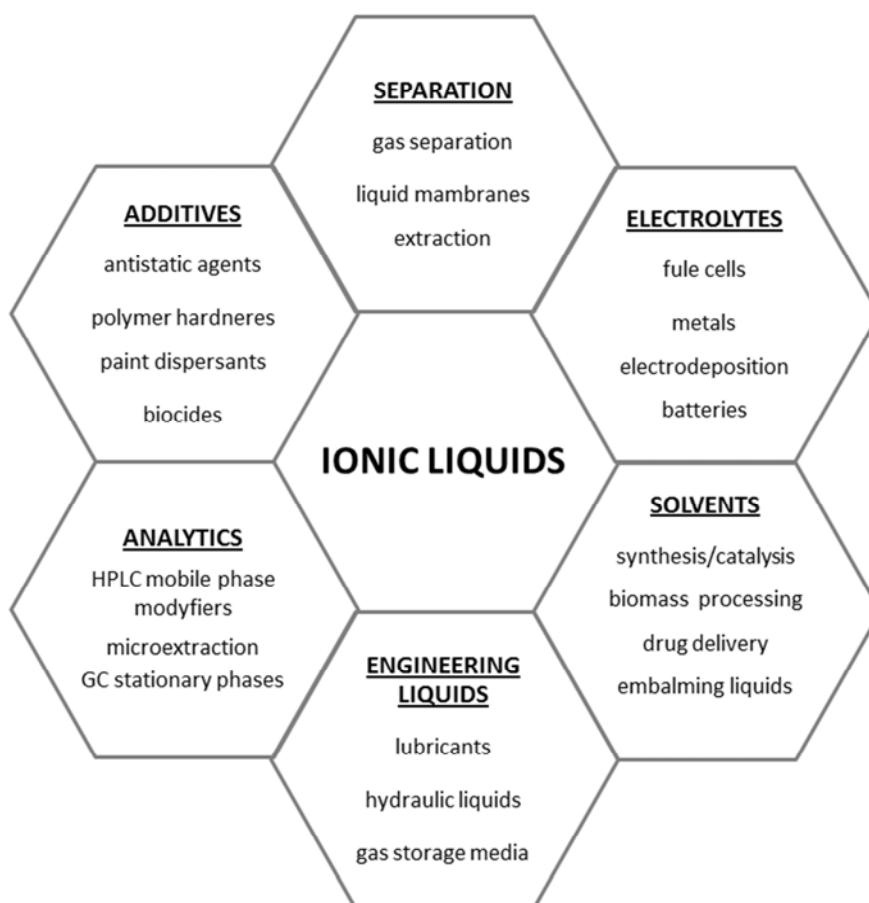
Figure 3. Potential applications of ionic liquids

One of the main forces driving research into ILs is the perceived benefit of substituting traditional industrial solvents with non-volatile ILs. Six hundred organic solvents are used industrially, most of which are volatile organic compounds (VOCs). It is estimated that 20 billion kg of VOCs are released to the atmosphere from industrial processes every year [14]. The most often cited attribute of ILs' greenness is their minimal vapour pressure even at elevated temperatures which offers advantages such as ease of containment, product recovery, recycling, and operational safety. Replacement of conventional solvents by ILs would prevent the emission of VOCs, a major source of environmental atmospheric pollution and is therefore economically and ecologically beneficial. Together with tuneable properties and stability, ILs' lack of volatility presents a unique opportunity for green chemical processes. For these reasons ILs were initially, commonly declared green solvents. It took some time before it was acknowledged that the property of low vapour pressure does not make ILs entirely green. Although they possess many undeniable advantages over conventional solvents (organic solvents or even water) they are not completely harmless. Some ILs have the potential to become persistent pollutants due to high stability towards abiotic and biotic degradation [15, 16]. Therefore it is important to determine the hazard associated with the presence of ILs in the environment.

1.3. Key compound

From among all ILs one exemplary compound, 1-methyl-3-octylimidazolium chloride ([OMIM][Cl]), was selected as key structure of current investigation. This selection was based on:

- technological relevance – up to date approximately 200 patents exists regarding application of [OMIM]$^+$ cation in technological processes and over 4 000 regarding other structures based on imidazolium core (according to GooglePatents as of 28.05.2012). Imidazolium based ILs can be applied as electrolytes in dye-sensitized solar cells [17], solvents in catalytic reactions [18], lubricant additives [19] etc. (Figure 3). [OMIM]$^+$ cation can be applied in preparing polymer/ionic liquids mixed membranes for gas separation [20], solid electrolytic capacitors [21], anti-electrostatic coatings [22], as a medium for catalytic reactions [23], heat transfer medium [24] or in separation of azeotropes [25] etc. Additionally a flagship of industrial success of ILs – BASF BASIL® process also employs an imidazolium IL [26].
- chemical structure offering a good representation of molecular interaction potentials (charge and medium hydrophobicity) which are important in interface interactions and self-assembling but also show average biodegradability and toxicity,

− coupling with simple anion resulting in complete miscibility of IL with water thus allowing for performing all planed test in wide concentration range. It is accepted that simple halide (Cl⁻, Br⁻, BF₄⁻, PF₆⁻) or cyano-based (N(CN)⁻, C(CN)₃⁻, B(CN)₄⁻) anions do not influence biodegradability of ILs' cation as they cannot serve as a carbon source, more complex anions like $[CF_3SO_3]^-$, $[N(CF_3SO_2)_2]^-$ or $[(C_2F_5)_3PF_3]^-$, though containing carbon, show negligible biodegradability. For these reasons the influence of anion on biodegradability was neglected here. Nevertheless the coupling with different anions which has a profound influence on hydrophobicity, water solubility and partition coefficients and might therefore influence sorption will be addressed later in Conclusions section.

1.4. Environmental release and distribution

In Europe the EU Water Framework Directive (WFD - 2000/60/EC) [27] and later Groundwater Directive (2006/118/EC) [28] established environmental objectives for protecting groundwater, the water bodies and ecosystems dependent on groundwater. ILs are considered xenobiotics and are therefore undesired in natural environment due to hazard they pose. The hazard arises mainly from ILs toxicity towards living organisms including humans, potential for bioaccumulation and mobility. So far imidazolium ILs are used in technological processes from small scale to pilot plant to large-scale industrial applications, what comes along with the increased probability that they will be eventually released into the biosphere, for example, as process effluents or consumer products, or in larger amounts as accidental spills [15].

Within this section a brief overview on release and environmental fate of chemicals is given. These concepts determine environmental concentration and thus are the basis for the understanding of hazards that [OMIM][Cl] may pose to biota when released into the environment. Generally the release of chemical can occur in several ways:

Spill - unintentional release into the environment as a result of accidents (e.g. fires, explosions, mechanical damage of industrial infrastructure) or malfunction of storage and transportation equipment (e.g. leakage from storage tanks, pipelines, transporting vehicles etc.). This form of release threats discharge of considerable amounts but is expected to be a rare, low risk event.

Leaching - unintended and unpreventable discharge into the environment due to normal usage of compound e.g. release from products containing IL (like paints, lubricants, polymers) during their usage. This type of release is expected to be small but might be difficult, if not impossible, to prevent.

Waste streams - release into wastewaters treatment facilities e.g. from plants producing ILs or manufacturing products containing ILs or utilizing technologies employing ILs as reaction media/solvents etc. or release from the landfills or waste collecting facilities storing spent products (such as batteries and solar panels). Various amounts of ILs can be released depending on waste management of companies.

Depending on the load/dose in particular place and potential for spatial spreading three major processes might be employed to remove ILs (or xenobiotic in general) from environmental compartments:

Degradation – can be a result of physical (like photo- or thermal degradation), chemical (e.g. hydrolysis, oxidation) or biological (biodegradation) process. All these processes result in transformation of xenobiotic to mostly simpler but not necessarily less harmful compounds. In environmental conditions biodegradation is the most important way of removing imidazolium ILs since they do not undergo hydrolysis easily and show stability towards photodegradation, thermal decomposition and oxidation due to inherent lack of susceptibility or absence of sufficiently harsh conditions in the environment. Biodegradation has undeniable advantage of transforming xenobiotics ultimately into simple, inorganic compounds provided it can proceed fully. Factors determining whether complete biodegradation can occur will be addressed later. Nevertheless biodegradation often proves to be ineffective and xenobiotic becomes recalcitrant or persistent.

Immobilization – containment in point of release might be a good option for compounds having chemical structure ensuring fast biodegradation, released in relatively small amounts. In such cases it might be sufficient to wait for natural attenuation assuring that contaminant will not spread. If relatively high amounts were discharged or the chemical is expected to persist or spread, due to its structure or environmental conditions immobilisation might be a temporary measure before treatment of contaminated site is employed. Nevertheless care should be taken since immobilisation lowers bioavailability of xenobiotics and might therefore hinder biodegradability.

Transfer to a different compartment/medium - moving xenobiotic from one environmental compartment to another e.g. evaporation from soil/water to atmosphere, soil washing or flushing and extraction techniques might be employed here. Those processes usually require cost intensive investments in infrastructure and cause dilution of xenobiotic, making treatment processes even more expensive.

2. Aims and scope

The scope of this work covers the assessment of processes involved in removal of [OMIM][Cl] from environmental matrices. Especially two distinct biotopes were taken into account as being of direct ecological and technological importance: activated sludge and natural soils.

To gain better understanding of processes affecting [OMIM][Cl] in the environment this dissertation follows the approach illustrated in Figure 4.
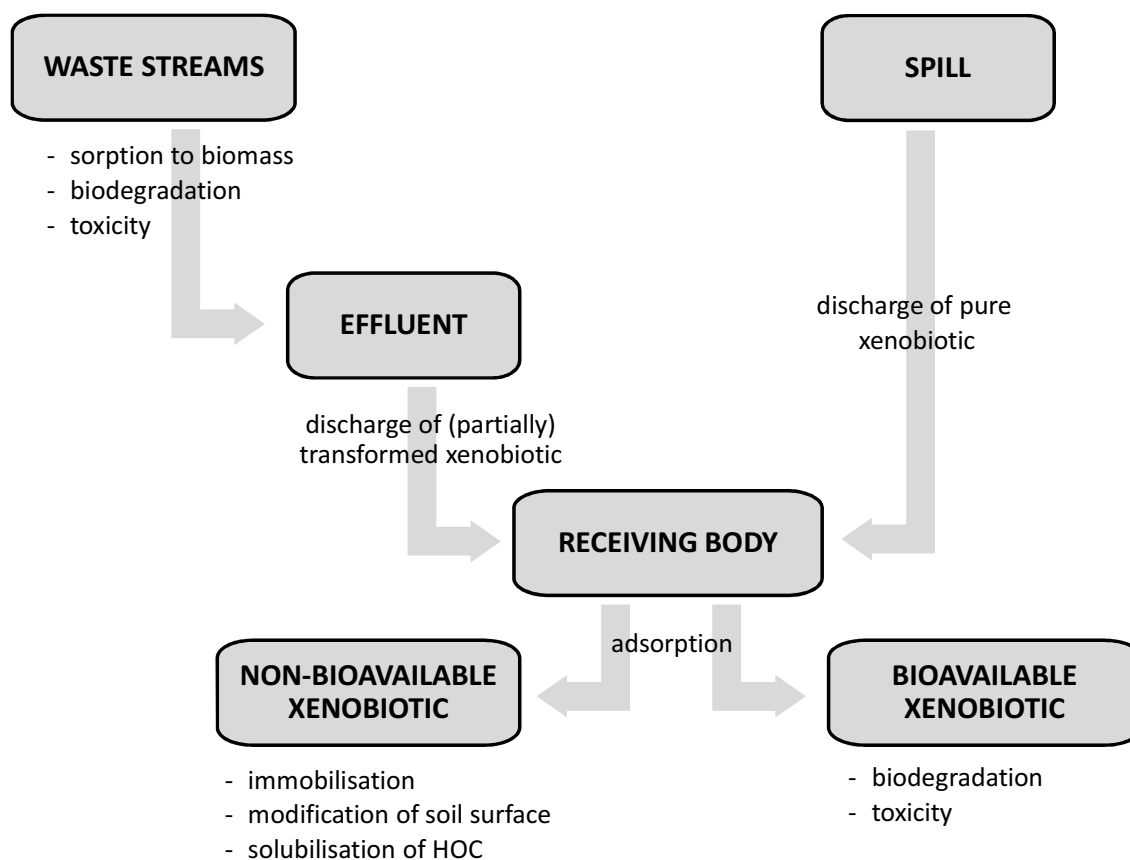
Figure 4. Diagram of possible release pathways of imidazolium ILs

When released into waste streams (e.g. wastewaters or landfills leachate collection systems) ILs will be directed into treatment facilities where they should be eliminated provided that efficient method is available. In WWTP xenobiotics will be (partially) removed due to biodegradation and adsorption on solids.

Biodegradation should ultimately result in complete breakdown of the xenobiotic to carbon dioxide, water and other simple, inorganic compounds and is a key element of xenobiotics removal in WWTP (Chapter 3.2). Nevertheless, it was shown previously that some xenobiotics are,

to large extent, removed by adsorption on solids – mostly flocs of activated sludge [29, 30]. This phenomenon will be addressed in Chapter 3.3. If, for some reason, treatment proves to be inefficient, effluent still containing ILs might be discharged from WWTP into receiving bodies.

Introducing high concentrations of chemicals showing significant toxicity can have a negative impact on activated sludge process, manifesting itself not merely by the lack of degradation of xenobiotic but also preventing removal of other, typically easy to degrade compounds. The issue of toxicity of imidazolium ILs towards activated sludge biocenosis will be discussed in Chapter 3.4.

Release as a consequence of spills or leaching from products will result in immediate appearance of ILs in the receiving body not allowing for any additional treatment. In the case of spill, high amounts of mostly unchanged ILs should be anticipated. Leaching from products (like paints or lubricants) is not expected to occur in high concentrations. ILs emitted in any of the above mentioned ways will eventually reach the soil or natural water bodies.

It was shown before that ILs sorb onto soils or bottom sediments. This results in partition of xenobiotic into bioavailable (freely dissolved) and non-bioavailable fraction (bound to solids). Bioavailable fraction can be uptaken by microorganism and degraded (Chapter 4.4) or distributed into deeper layers of soil (Chapter 4.3). Adsorption contributes to lowering of dissolved amount of xenobiotic and often mitigates the detrimental effect xenobiotic can have on microbial community [31]. Non-bioavailable fraction is immobilised by adsorption onto solid matter, the extent of which is defined by soil/water partition coefficient. Aggregation on soil mineral and organic fraction occurs due to different interactions and will be addressed in Chapter 4.2. Additionally this aggregation can change properties of soil e.g. affect charge of mineral fraction (Chapter 5.2) or amount of dissolved organic matter (Chapter 5.3). Lastly due to amphiphilic structure [OMIM][Cl] can cause enhanced pollution due to solubilisation of hydrophobic organic compound present in terrestrial environment (Chapter 5.4).

In order to obtain repeatable experimental data of scientific significance allowing comparison of results, suitable examination system had to be designed to fill the gap between knowledge build on the basis of standard laboratory tests and a need to evaluate fate of xenobiotics in realistic conditions.

3. Fate of IL in wastewater treatment

3.1. Background

Constant increase in the amounts of chemicals used by our civilisation in both qualitative and quantitative sense forced humanity to develop waste collection systems and processes treating these wastes in order to protect most basic natural resources like air, soil and water from pollution. What started as a way of removing mostly organic wastes from households in ancient Rome underwent great changes and improvements to be able to deal with tremendous amount of man-made chemicals discharged into wastewater systems nowadays. Yet the heart of wastewater treatment, the activated sewage sludge process, remains fairly conceptually unchanged for a long period of time.

Degradation of chemicals by microorganisms has a great potential due to broad, naturally occurring, microbial metabolic diversity allowing for the transformation, degradation or even accumulation of an enormous range of compounds [32]. There is however, a group of compounds, known as xenobiotics (from Greek: *xenos* = foreign, and *bios* = life), which resist biodegradation sometimes to significant extent. Compound that does not undergo biodegradation in specific test conditions is named *persistent*, compound that resists any degree of biodegradation in any test conditions is named *recalcitrant*. Although persistence is relatively straight forward to demonstrate, recalcitrance might not be as easy to prove [33].

Possible reasons for persistence of chemicals in the environment include [15, 33]:

− substrate incompatibility with a microbial metabolic capacities including: lack of uptake to the cell, lack of enzymes/inability of induction of enzymes showing activity towards substrate, break down to products which do not integrate into normal metabolic pathways or which are more toxic than the parent compound,

− lack of appropriate microflora capable of degrading chemical or insufficient number of microorganisms,

− lack or insufficient level of nutrients to support degrading community,

− physicochemical conditions (temperature, pH, redox potential) outside of microflora's optimum or range of tolerance,

− too high concentration of the chemical,

− unavailability of chemical due to adsorption on minerals/organic matter,

− inaccessibility of chemicals due to its size or insolubility,

− too short time period.

The first of reasons mentioned is perhaps the most important one as it is an inherent property of xenobiotic and cannot be influenced in any way.

The rate of xenobiotics microbial conversion is also governed by the rate of transfer to the cell (mass transfer) and the rate of metabolism. Escalating microorganisms' metabolic conversion capacities will not lead to higher level of transformation if mass transfer of xenobiotics to the cell is the limiting factor. This phenomenon is known as bioavailability and is controlled by a number of physicochemical processes such as sorption and desorption, diffusion, and dissolution. Usually water soluble compounds are biodegraded faster than insoluble ones, this is because the latter tend to adsorb on environmental matrices which limits their availability [31]. Nevertheless it does not necessarily mean that water insoluble compounds will be recalcitrant or totally unavailable. Examples of hydrophobic substances which are relatively easy to biodegrade are plentiful (crude oil, vegetable oil, fats etc.), additionally many microorganisms secrete biosurfactants to their surrounding enhancing bioavailability of these compounds [34].

Sorption to solids temporarily removes xenobiotics from aqueous solutions thereby preventing their spreading and limiting toxic effect. It can be perceived as a negative process as substances bound to solid cannot be uptaken by microorganisms and degraded [33]. On the other hand sorption can be beneficial, especially in case of presence of high concentration of toxic xenobiotics. In the latter case adsorption on soil matrix can lower the amount of xenobiotic that is bioavailable to concentrations that are not harmful to microflora so that the biodegradation may proceed.

Chemical structure of a compound

Certain chemical structures were proven to enhance/reduce overall molecule's biodegradability. Summary of desirable/undesirable structures which became a part of 'benign by design' concept of ILs synthesis are presented in the Table 1 [35]. Structural variations mentioned should be, however, treated with caution and more as indicators than actual rules as multiple deviations from them exist. Introduction of oxygen atom into a structure of xenobiotic usually initiates the aerobic degradative pathway. The initial oxidation is usually the limiting step and already partially oxidised substrates are degraded more rapidly. If no oxygen is present in a molecule it is inserted enzymatically by mono- or dioxyganases and usually drawn from molecular oxygen. Linear, over four carbon atoms long alky chains or phenyl rings are especially susceptible for oxygenases attack [33, 34].

Table 1. Structural features promoting and reducing biodegradability

Reduced biodegradability	Enhanced biodegradability
Halogens – Cl and F in particular, especially if more than three atoms are present in small molecule	Oxygen present as hydroxyl, aldehyde, ketone and carboxylic groups but not ether (except ethoxylates)
Chain branching – especially presence of quaternary carbon	Unbranched hydrocarbon chains (over four carbon atoms)
Polycyclic congeries – especially with more than three fused rings	Ester groups
Tertiary amine, nitro, nitroso, azo and arylamino groups	Amide groups – though examples to prove otherwise exist
Heterocyclic residues (imidazoles)	Phenyl rings
Aliphatic ether bonds – except ethoxylates	
Any other strongly electron withdrawing groups	

Microbial community and metabolisation

Microbial community colonizing given biotope is a group of taxonomically different microorganisms which are mutually interconnected, usually on a food web basis, forming a dynamically changing biocenosis. The more diverse the biocenosis, in terms of number of microbial species and their taxonomic variety, the more stable it is. Such biocenosis reacts to changes in environmental conditions in qualitative and quantitative way. Qualitative reaction encompasses: genetic mutations leading to formation of more fit individuals among one species, horizontal gene transfer between representatives of one species or between species allowing them to acquire features giving advantage over other organisms etc. Quantitative changes are based on the fittest organisms multiplying the fastest and outnumbering other members of the community [36].

Microorganisms can vary from metabolizing whole groups of compounds showing moderate structural similarities to organisms which are able to metabolise only one compound [37]. In the case of soils and activated sludge microbial community is diverse and shows remarkable metabolic flexibility especially the latter one. Nevertheless microorganisms can only perform the reactions for which they possess the genetic capability, meaning they can only

degrade xenobiotics when enzymes able to convert them evolved. Enzymes possess certain degree of specificity, yet this specificity is exhibited mainly with respect to the type of catalytic reactions they perform and specificity towards substrate is less stringent. Though many enzymes were not deliberately 'designed' to degrade xenobiotics they can target certain range of structurally similar compounds. The more similar is the structure of xenobiotic to naturally occurring substrates the grater is the possibility that already existing enzymes can degrade it. Such activity of an existing enzyme towards foreign substrate is known as *fortuitous* or *gratuitous metabolism* and is believed to be the major mechanism of xenobiotics degradation [33]. If only xenobiotic resembles any of enzyme's natural targets to such an extent that it can be accommodated into an active site there is a great possibility that it may undergo degradation. Certain compounds, containing structural elements, described in Table 1 as reducing biodegradability, but still recognisable by enzymes can be metabolised. Cometabolism is defined as a transformation of a substrate that is unable to support growth of microorganism in an obligatory presence of another growth-supporting substrate. It is possible that a substrate that was partially cometabolitically transformed by one microbial species can be further transformed by another until the degradation is completed [38].

In sites contaminated with recalcitrant pollutants indigenous bacteria colonizing the site may not possess the capacity to degrade the compound. One of existing solutions in such case is bioaugmentation – addition of microorganisms or mixtures of microorganisms which are known to be able to degrade the xenobiotic [39]. However, added bacteria may not be optimally fit to environmental conditions. If genes coding enzymes responsible for degradation of xenobiotic are present in a plasmid and the plasmid is conjugative (has the gene sequence which provides the ability to transfer this plasmid to another cell), has a sufficiently broad host range, and if it provides a selective advantage, it will be disseminated into suitable recipients. The resulting bacterial plasmid combination which in each micro-environment has the highest local fitness will prevail [32].

Nutrients

In order to sustain growth and assure continuity of a specie microorganisms need a supply of energy and building blocks, mainly carbon, oxygen, hydrogen, nitrogen, sulphur and phosphorus. Microorganisms are remarkably flexible with respect of carbon sources and there is virtually no naturally occurring molecule that cannot be used by at least some of them [37]. It was proven that addition of easily available sources of carbon and other nutrients might improve degradation rates of xenobiotics most probably by reducing the toxic impact, helping to overcome

cell inhibiting effect as well as providing reducing power or inducing degradative enzymes [40]. Therefore augmentation might be a way of enhancing degradation of xenobiotics. Nevertheless presence of easily available nutrients in sufficient amounts might as well redirect microbial metabolisms to those compounds excluding xenobiotics completely. This is especially important, because in wastewater treatment plants or natural environments, other organic substrates are present, which might be preferentially degraded or cometabolised with the primary contaminant resulting in increased/decreased biodegradation rates [41]. Standard biodegradation tests are usually conducted in a mineral medium which does not provide additional organic nutrients.

Examples of both positive and negative impact of supplementation of additional nutrients on degradation of xenobiotics can be found in literature. Lewis *et al.* [42] found that the addition of organic carbon significantly decreased the degradation of p-cresol. Similar results were obtained by Swindoll *et al.* for p-nitrophenol [43].

On the other hand Loh and Wang [40] found improved rates of 4-chlorphenol degradation when glucose and sodium glutamate were supplied and Piekarska *et al.* [44] showed that the addition of other sources of organic carbon and nitrogen increased the efficiency of degradation of diesel oil.

Environmental conditions

The growth of microorganisms is greatly affected by the nature of surrounding environment [33]. Certain physical and chemical parameters e.g. pH, salinity, temperature, pressure, water activity and amount of oxygen have to be maintained within range of tolerance for microorganisms to survive. Most microorganisms present in subsurface soils, surface waters and wastewater treatment plants share similar environmental requirements regarding those parameters. As most of them are fairly constant in mentioned biotopes they are rarely examined with respect of their influence on biodegradation [45].

Concentration threshold

As once said by Paracelsus, every substance can become toxic when it is applied in sufficiently high dose. Therefore, it is easy to imagine that when xenobiotic shows toxicity towards microbial community there will be a concentration above which this xenobiotic will not be degraded due to its harmless influence.

The interdependence of biodegradability and toxicity of chemicals can be clearly observed. Any adverse effects of chemicals on the biological activity of a WWTP will generally reduce the

quality of the wastewater discharged to the receiving water bodies or applied to agricultural soil as fertilizer [46]. Additionally if a chemical shows toxic effects towards the microbial sludge community the biodegradation experiments may deliver false negative result, with lack of biodegradation being the effect of chemical's toxicity towards inoculum and implying that lower concentration of tested compounds should be used for biodegradation testing.

Timeframe

Four phases of bacterial growth can be distinguished when microbial community is placed in a new environment [47]:

- lag phase – when cells are adapting to new conditions and not dividing,
- log phase – phase of logarithmic growth when nutrients are abundant and conditions favourable, this phase lasts until one or more of necessary growth substrates is exhausted or there is a build-up of inhibitory substances,
- stationary phase – cell division subsides,
- death phase – due to lack of nutrients the lysis of cells begins and population dwindles.

Since biodegradation depends on the presence of sufficient number of organisms it often mirrors the growth curve and two main time periods can be distinguished: lag phase and the actual degradation phase.

Adaptation is defined as a change in the microbial community that leads to decrease in biodegradation timeframe or an increase in the biodegradation rate or maximal biodegradable concentration of a given xenobiotic as a result of previous exposure. The general representation of degradation by adapted and non-adapted community is shown in Figure 5. Lag phase is significantly reduced or does not occur at all for adapted community allowing for elimination of delay in biodegradation.
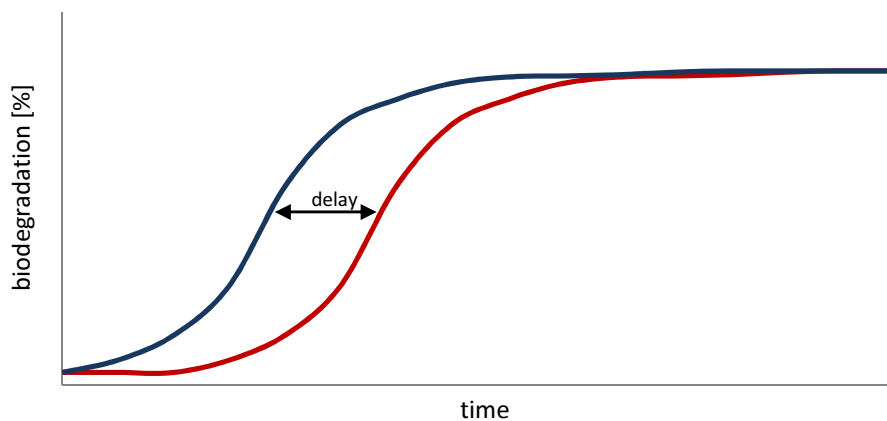

Figure 5. Biodegradation conducted by non-adapted (red line) and adapted microbial community (blue line)

Examples of such adaptation processes are: rapid degradation of p-nitrophenol by aquatic microorganisms [48] and enhanced degradation rates of m-cresol, m-aminophenol and aniline by subsurface soil communities [49] and improved degradation of nonylphenol ethoxylate by sludge community [50]. Mechanisms of adaptation usually involve processes such as genetic mutation or horizontal gene transfer, induction of specific enzymes which enhance the degradative capacity of the entire community, and population change such as selective growth of certain strains. Therefore use of mixed microbial communities that were pre-adapted to certain chemicals for treatment of wastewaters containing this chemical can be beneficial [51].

3.2. Biodegradability of ionic liquids in wastewater treatment

The studies on biodegradation and potential accumulation of ILs in the environment have recently been started. Based on current knowledge it is possible to predict which structures of ILs are benign and which have a potential to persist in the environment based on the SAR (Structure – Activity Relationship) concept [52, 53]. Nevertheless technological requirements not always allow for application of the most biodegradable ILs and a compromise between applicability and environmental hazards must be made.

Over the past years many papers [15, 54-60] and a several reviews [15, 61-63] have reported on the biodegradability of different ionic liquid structures, mainly according to "ready biodegradability" test procedures under aerobic conditions. A broad analysis of the influence of the structure on biodegradability is presented in recent review of Stolte *et al.* [15]. As this work is focused on imidazolium ILs in particular, the biodegradability of only those compounds will be discussed below.

Imidazolium ring was previously proven to be degradable through so called "histidine pathway". Similarly imidazolium ring substituted on any of the three carbon atoms is biodegradable. The situation is very different for N-substituted compounds and except for current work no imidazolium ring cleavage in N-substituted ILs was proved beyond doubt. Rorije *et al.* suggest that the imidazole ring itself is not inherently resistant to biodegradation it is rather the nitrogen substitution which could be a problem [64].

Short chained imidazolium derivatives show negligible levels of degradation. Elongation of the substituent improves biodegradability: hexyl- or octyl- substituted compounds reach fair to significant levels of biodegradation, nevertheless none of them could be classified as readily

biodegradable [54, 55, 57]. Further increase in side chain length (12 to 18 carbon atoms) causes serious inoculum inhibition [55].

Stolte *et al.* performed a comprehensive study of ionic liquids biodegradation utilizing two types of microorganisms. As the test substances imidazolium substituted with alkyl chain or short functional group (ether, hydroxyl, carboxyl, nitril or amide), with simple counter ions (Cl, Br, I) were used. Presence of terminal carboxyl or hydroxyl group enhanced biodegradability to small extent. For imidazolium compounds containing short ($\leq C_4$), functionalized or not, side chain no biodegradation was observed. However imidazolium ionic liquids substituted with octyl side chain showed complete primary degradation [54].

Gathergood *et al.* tested the influence of incorporation of an ester or an amide functional group to the said chain of imidazolium ionic liquids on biodegradability. According to their research, amide functionalised ionic liquids showed poor to negligible degradation, though it was previously shown that introduction of this functional group into alkyl chain improves biodegradability of surfactants. Compounds with ester group were significantly more degradable, particularly those with alkyl chain containing 4 and more carbon atoms [58, 59]. Introduction of the methyl group in position 2 of imidazolium ring does not significantly influence degradation [58]. Presence of phenyl ring, known to enhance degradation, did not exhibit any positive influence [54, 65]. Similarly zwitterionic IL, imidazolium-butyl-sulfonate did not show any appreciable levels of biodegradation [65]. It has been shown that most ILs investigated so far cannot be classified as "readily biodegradable" [54], however, the structural design of ILs with substantially improved biodegradability has been found possible using IL cations (such as N-alkyl-3-methyl-pyridinium and N-(ethoxycarbonyl)-pyridinium compounds) and anions (e.g. octylsulfate or acetate, propionate or benzoate) [58, 66].

Garcia *et al.* tested also the influence of the anion on the biodegradation and toxicity of imidazolium based ionic liquids of two series 1-butyl-3-methyl-imidazolium and 3-methyl-1-(propoxycarboyl)methylimidazole. Anions subjected to the test were: Br^-, BF_4^-, PF_6^-, NTf_2^-, $N(CN)_2^-$, $C_8H_{17}OSO_3^-$. None of tested compounds were described as readily biodegradable, nevertheless those containing octylsulfate anions were significantly more susceptible to biodegradation than others. In addition compounds of the second series showed higher level of biodegradation. Regardless of the type of counter ion, with octylsulfate being the only exception, all of the compounds tested showed similar and very poor levels of degradation. The high level of biodegradation of ionic liquid containing octylsulfate anion was mainly due to this anion degradation and was not surprising as linear alkyl sulfates were proven to be readily

biodegradable. Due to that fact the statement has been made that anion does not have considerable influence on biodegradation of ionic liquids [60].

Standard biodegradation testing procedures generally permit pre-conditioning of inoculum but do not allow for pre-exposition to the test substance [67]. Though very valuable, as a point of reference, the standard biodegradation tests do not fully represent real conditions. In *in situ* conditions, for example in wastewater treatment plants or soils and natural water bodies, the presence of readily available sources of energy and nutrients as well as the process of adaptation may often alter the fate and metabolic pathways of xenobiotics.

Microbial community and metabolisation

As mentioned before Stolte *et al.* performed a biodegradation test of (among others) [OMIM][Cl] utilizing two types of microorganisms: commercially available freeze-dried mixture of bacteria and activated sludge bacteria from WWTP. The comparison of both inocula showed that the freeze-dried bacteria were not able to degraded any of the tested ionic liquid cations, whereas taxonomically more heterogeneous WWTP activated sludge inoculum was able to degrade some of the investigated structures including [OMIM][Cl] [54]. Docherty *et al.* [57], who analysed the structure of the microbial community using molecular biology tools (DNA polymerase chain reaction followed by denaturing gradient gel electrophoresis) found that microbial communities exposed to [OMIM][Cl] were richer in certain bacteria species. Additionally Docherty *et al*. in previously mentioned work [57] found that N-butyl-3-methyl-pyridinium did not undergo biodegradation within 43 days, whereas in later study the same compound, in the same test system underwent 88% mineralization within 41 days [68]. The last result was also confirmed by Pham *et al.* in primary biodegradation test [69]. The encountered differences were attributed to the nature of the inoculum and its biodiversity (subject to spatial and temporal variations). It was suggested that inocula from different origin should be incorporated into the standard biodegradability test procedure in order to obtain a wider range of microbial composition. Also, the identification of microorganisms capable of degrading compounds would be desirable for improving understanding and perhaps facilitating modelling of biodegradation.

Abrusci *et al.* [70] reported that *Sphingomonas paucimobilis* bacteria were especially predisposed to biodegrade imidazolium ILs e.g. 75% of mineralisation was observed for [BMIM][Br] whereas only up to 5% of mineralisation was reported before in tests using wastewater microorganisms [58-60]. The same specie was able to mineralise [OMIM][Cl] in 37% within 28 days (initial concentration 6.5 mmol L^{-1}). This suggests that *Sphingomonas paucimobilis*

is an excellent candidate for bioaugmentation tests. On the other hand several conclusions that are inconsistent with previous research were drawn in that work, like: very fast mineralisation for short chained ILs, much higher than primary biodegradation ever reported, very slow mineralisation of long chained ILs normally degraded relatively fast, clear dependence of biodegradation on type of simple inorganic anion. For these reasons more research is needed to confirm findings of Abrusci *et al.*

Once uptaken into a living cell xenobiotics can undergo countless transformations via multiple enzymatic pathways. Although literature knows innumerable examples of such reactions they can, in general, be divided into four groups: oxidative, reductive, hydrolytic and conjugative [35]. Biodegradation of [OMIM][Cl] and other imidazolium ILs most probably starts with the transformation of the alkyl chains via oxidation of the terminal methyl group (ω - oxidation) conducted probably by monooxygenases e.g. the cytochrome P_{450} system. The formed alcohol is further oxidised via aldehydes to carboxylic acids by dehydrogenases. The resulting carboxylic acids then can undergo β-oxidation and the two released carbon fragments can enter the tricarboxylic acid cycle as acetylCo-A. Pathways of imidazolium ring biodegradation are known mostly from the histidine metabolism. Histidine degradation begins with nonoxidiative deamination of side chain performed by histidine ammonia lyase (histidase) to urocanate. Urocanate containing double bond created by histidase after ammonia removal is subsequently hydrated to 4-imidazolone-5-propionate by urocanase. These steps are followed by hydrolytic breakdown of imidazolium ring to form N-formiminoglutamate that is further metabolised to glutamate and formiminotetrahydrofoalte [71, 72].

Nutrients

A number of research groups have performed biodegradation tests with alkyl substituted imidazolium cations using activated sludge as inoculum. In most cases ILs were used as a sole source of organic carbon and organic nitrogen [54, 58-60].

Romero *et al.* [73] discussed the biodegradability of imidazolium ILs in the presence of additional carbon source. It was found that the ILs tested were not biodegraded when D-glucose was available. However, ILs with no additional carbon were also not degraded (2–10%), which is in contrast to other research where, e.g. complete primary biodegradation of 1-methyl-3-octylimidazolium chloride [OMIM][Cl] was shown [54, 56]. Results of Romero *et al.,* though very interesting, should be treated with caution due to the very short duration of the test (five days).

Environmental conditions

Most of WWTP employ open top tanks in which temperature is kept constant owning to heat evolved by microorganisms and pH is maintained in optimal range. Out of all environmental parameters the influence of availability of oxygen is the most widely studied. The nitrogen removal during wastewater treatment is a very important example of biological process that takes place in a sequence of aerobic/anaerobic reactions. Initially organic nitrogen present in peptides, amino acids, nucleic acids *etc.* is transformed to ammonia or ammonium (ammonification) and then to nitrites and nitrates (nitrification) in aerobic conditions. Subsequently nitrates are reduced to molecular nitrogen in anaerobic conditions (denitrification). For this reason examining degradation of nitrogen containing compounds in anoxic conditions is very important [37, 74].

The primary biodegradability of a set of different ionic liquid cations was investigated under anaerobic conditions [75]. No biodegradation was observed for imidazolium ILs including [OMIM][Cl] over a period of 11 months except for 1-(8-hydroxyoctyl)-3-methylimidazolium. Neumann *et al.* [64] reached the conclusion that in aerobic biodegradation processes, the initial oxidation of the octyl side chain of the [OMIM] cation probably involves molecular oxygen as a reactant. The oxygen is inserted by monooxygenase and the alkyl side chain can be further degraded via ß-oxidation. Under anaerobic conditions, no molecular oxygen can be inserted by monooxygenase and biodegradation is not initiated. In contrast, the pre-oxygenated compound with a terminal hydroxyl group can be biodegraded under anaerobic conditions.

Concentration threshold

So far no data are available on the concentration threshold that limits biodegradation of ILs. Researches into ILs degradability were so far focused on qualitative analysis (e.g. identifying structures prone to degradation, metabolic routes or metabolites). Most experiments were performed at relatively low concentrations (around 0.2 mmol L^{-1}) allowing for observation of those qualitative features without introducing more parameters [15].

Timeframe

In most cases biodegradation tests for ILs last 28 days as specified in OECD guidelines [55, 58-60, 65]. There are, however, cases when extending this time resulted in reaching higher levels of degradation [54, 57, 76]. It was additionally showed that six-fold increase in rate of degradation was achieved for [OMIM][Cl] after repeated exposure [54].

3.3. Sorption and bioavailability

Activated sludge is mostly composed of organic fraction which can be further divided into living cells and so called extracellular polymeric substance. This substance is a mixture of compounds excreted by microorganisms, lysed cells and organic matter obtained from solution and is believed to act like a protective barrier; it is also responsible for cohesion of sewage flocs. Both living cells and extracellular polymeric substance have the ability to sorb chemicals through van der Waals or charge - charge interactions [77]. Kördel *et al.* found significant sorption of e.g. aniline, benzamide, phenathrene, phenylacetamide on activated sludge [30]. Kümmerer *et al.* [29] identified adsorption on biomass as a main mechanism of elimination of quaternary ammonium compounds (QACs) during biodegradation testing. Approximately 50-65% QACs was removed in this way. Therefore it is interesting to investigate the possibility of removing ILs from wastewaters in the same way.

Up to date no adsorption of ILs on activated sludge was observed in direct adsorption test [78] nevertheless, data available from abiotic controls of some biodegradation tests can also be used for this purpose. Within OECD 301 'Ready biodegradability' test panel 301 A (DOC Die-Away) and 301 E (Modified OECD Screening) allow for observing adsorption as either depletion of dissolved organic carbon or tested compound (direct measurement of concentration in aqueous phase) is measured. All remaining tests are based on measurements of parameters that are direct function of biodegradation (CO_2 evolution, O_2 consumption etc.) and do not take adsorption into account [67]. In OECD 301 A and E test no adsorption on inoculum was observed, probably due to very low cell density [54]. Additionally no adsorption of 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]) was observed on pure culture of *Bacillus subtilis* [14] or composted organic matter [78].

3.4. Toxicity towards activated sewage sludge biocenosis

Toxicity of imidazolium ILs was extensively analysed up to date utilising many tests subjects including cell lines [79], bacteria [73, 80, 81], fungi [82, 83], algae [84, 85], crustaceans [86], plants [87] invertebrates [88] and fish [89, 90]. So far little is known about the toxic effects of ILs towards activated sludge biocenosis. Marine luminescent bacteria – *Vibrio fischeri* is often used as representative microorganism to describe inhibitory effects to wastewater microorganism communities and a multitude of ionic liquids have been tested in this system.

Wells and Coombe measured inhibitory effects of a series of N-methyl-imidazolium compounds with C12, C16 and C18 alkyl side chains based on inhibition of biodegradation of glucose. Increasing inhibitory effect on the microorganisms was found with increased number of carbon atoms in the chain [55]. This phenomenon, known as cut-off effect, can be observed for *Vibrio fischeri* (Figure 6) and was also observed for other organisms [82, 91, 92].

The toxicity span of butyl- to octadecyl- homologues covers five orders of magnitude, from over 20 mmol L^{-1} for 1-methyl-3-octylimidazolium chloride to approximately 0.6 µmol L^{-1} for 1-methyl-3-tetradecylimidazolium chloride. The mode of toxic action is believed to be mainly based on permeabilisation of cell membrane causing leakage of macromolecules, osmotic pressure unbalance and inability to maintain cell integrity [93].
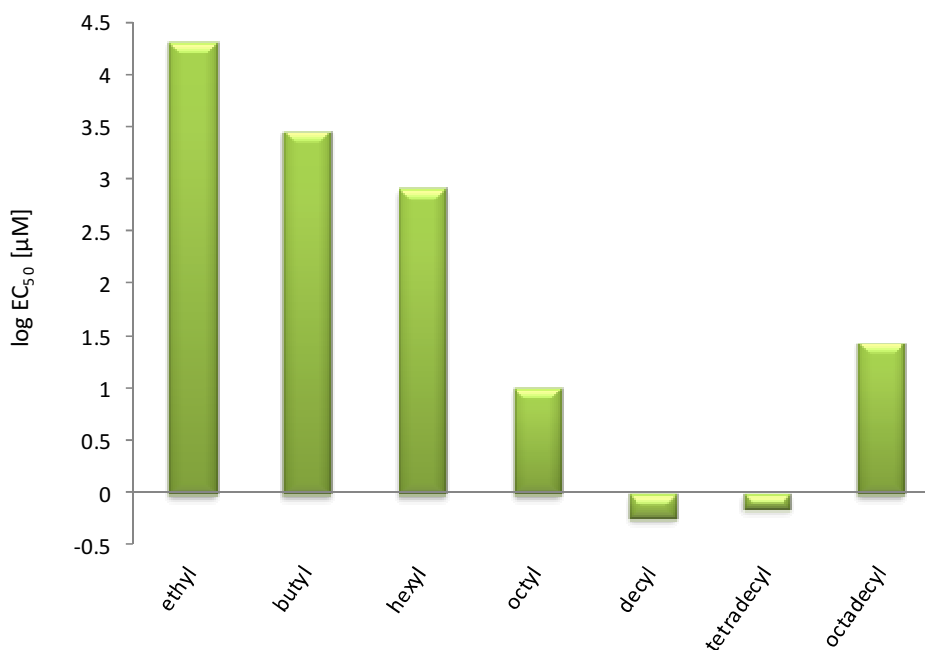
Figure 6. Ecotoxicity of 1-methyl-3-alkylimidazolium chloride homologues towards *Vibrio fischeri* [93]

Most of available toxicological data are reported as EC_{50} values and concern single species of organisms. This way of measuring toxicity allows for standardisation of the test conditions and comparison between tested substances.

The bioluminescence inhibition is a highly standardised and widely used bioassay [46, 94]. It is not recommended to base assumption of ionic liquids toxicity towards activated sewage sludge on results obtained for single organisms, however [95]. A direct conclusion about the influence of ILs on the sludge performance on the basis of available toxicity data is difficult due to

variability of microorganisms present. It is so mainly because sludge is a taxonomically complex consortium forming interconnected biocenosis which is usually more metabolically flexible [96]. Additionally the existence of the multitude of single species toxicity data so far failed to deliver the answer to the very difficult but in the same time extremely basic and important technological question: *what concentration of a xenobiotic is safe for activated sewage sludge process and what already causes malfunction?* Any adverse effects of ILs on the biological activity of a WWTP will generally reduce the quality of the wastewater discharged to the receiving water bodies or applied to agricultural soil as fertilizer [46]. Additionally if an IL shows toxic effects towards the inoculum the data of biodegradation tests may deliver false negative result implying that lower concentration of IL should be used for biodegradation testing [15].

Gutiérrez *et al.* [96] compared standard bioluminescence test targeting *Vibrio fischeri* with oxygen uptake inhibition test towards activated sludge. Single species test yielded lower EC_{50} values than community tests proving that the same xenobiotic has smaller toxic effect on the latter. Gray *et al.* [97], searching for a method suitable for measurement of toxicity of acid mine drainage, concluded that respiration inhibition test is less sensitive than single organism Microtox test. Authors established that even though single species tests are far more sensitive it is problematic to apply obtained results to real environment being a complex ecosystem [46, 95].

3.5. Experimental approach

As discussed in sections 3.1-3.4 the knowledge concerning the fate and biodegradability of ILs in wastewater treatment plants is based on simplified standard biodegradation tests using conditions that do not reflect realistic processes. To fill this data gap and to derive a more realistic picture on how ILs behave in wastewater treatments plants detailed experimental program has been executed for [OMIM][Cl] considering parameters such as toxicity towards sludge biocenosis, adsorption to biomass, microbial adaption and supplementation of nutrients.

3.5.1.   Biodegradation by direct concentration measurement

The biodegradation experimental conditions were based on OECD 301 procedure 'Ready Biodegradability' [24]. Half a litre of activated sewage sludge was suspended in approximately the same volume of demineralised water and mineral medium was added. Mineral medium was composed of 8.5 mg L^{-1} KH_2PO_4, 21.75 mg L^{-1} K_2HPO_4, 22.13 mg L^{-1} $Na_2HPO_4 \cdot 2H_2O$, 1.7 mg L^{-1} NH_4Cl, 27.5 mg L^{-1} $CaCl_2$, 22.5 mg L^{-1}, $MgSO_4 \cdot 7H_2O$ and 0.25 mg L^{-1} $FeCl_3$ dissolved in water (all chemicals provided by POCH Gliwice, Poland). Appropriate amounts of ionic liquid stock solution were

added to yield the desired concentration (0.02 mM, 0.2 mM, 1 mM, 2 mM, 3 mM, 30 mM, 75 mM, 150 mM). Flasks with test substances were accompanied by blank samples (inoculum and mineral medium without test substance) and negative controls (inoculum, mineral medium and given amount of IL poisoned with $HgCl_2$ in concentration 0.4 g L^{-1}) to exclude biodegradation and to consider sorption processes onto sewage sludge. A positive control using sodium benzoate (POCH Gliwice, Poland) was measured. Glucose and artificial sewage composed of synthetic sewage feed: 16 g L^{-1} peptone, 11 g L^{-1} meat extract, 3 g L^{-1} urea, 0.7 g L^{-1} NaCl, 0.4 g L^{-1} $CaCl_2 \cdot 2H_2O$, 0.2g L^{-1} $MgSO_4 \cdot 7H_2O$ and 2.8 g L^{-1} K_2HPO_4 were added to test samples in supplementation tests. All samples were continuously aerated, losses due to evaporation were adjusted with water on the mass basis and analytical samples were withdrawn at specific time intervals. All experiments were conducted at least in duplicate.

Analytical samples were centrifuged and a supernatant was collected and frozen for the further analysis. The method of degradation detection was by direct measurement of the substrate concentration by HPLC/UV. A Perkin Elmer Series 200 HPLC consisting of a chromatographic interface (Link 600) binary pump, UV/VIS detector, vacuum degasser and Rheodyne injection valve was used. For ILs cation separation C_6-Phenol (Phenomenex) 150 × 4.6 mm column was used in conjunction with detection by UV adsorption at a wavelength of 218 nm. Sample injection volume was 10 μl. As a mobile phase 27% acetonitrile/water +0.1% (v/v) trifluoroacetic acid at the flow rate of 0.8 mL min^{-1} was applied. For preparation of HPLC mobile phase HPLC – grade acetonitrile form Lab-Scan (Dublin, Ireland) and spectrophotometric-grade trifluoroacetic acid (Sigma-Aldrich, Germany) were used. Sodium benzoate was analyzed by HPLC/UV using a C_8 Waters (XTerra) 250 × 4.6 mm column with gradient elution (acetonitrile and water/acetic acid) and UV detection at 254 nm.

3.5.2. Analysis of metabolites

Additional analytical samples were taken from vessels containing live and chemically sterilized inocula at the end of adaptation tests. Samples were centrifuged to remove solids and the supernatant was diluted hundredfold (biotic sample) or thousandfold (abiotic sample) with a 9:1 methanol–water mixture resulting in approximately 5 μM concentration of parent compound in all samples. Subsequently samples were analyzed for the parent compound and metabolites by electrospray ionization mass spectrometry equipped with ion trap detector (Brucker-Daltonic GmbH, Germany). Mass spectra for cations were acquired in the positive ion mode in the scan range of $m/z+$ 50–300. The ESI source conditions were set to a capillary voltage of 3500 V, drying gas flow-rate of 5 L min^{-1}, drying gas temperature at 300 °C and nebulizer at 70 psi [98].

3.5.3. Toxicity by respiration inhibition

OECD 209 Activated Sludge - Respiration Inhibition Test [99] was performed as a toxicity test towards activated sewage sludge biocenosis. The method is based on the lowering of respiration rate caused by toxic action of the tested substance. Synthetic feed composed of easily available nutrients (16 g L^{-1} peptone, 11 g L^{-1} meat extract, 3 g L^{-1} urea, 0.7 g L^{-1} NaCl, 0.4 g L^{-1} $CaCl_2 \cdot 2H_2O$, 0.2g L^{-1} $MgSO_4 \cdot 7H_2O$ and 2.8 g L^{-1} K_2HPO_4) was fed to activated sewage sludge together with test substance or reference substance (3,5-dichlorophenol) and the mixture was aerated for 30 minutes. Simultaneously test mixture was transferred to Karlsruhe bottles and oxygen probe was inserted in such a way that no headspace was created over the liquid. The oxygen content was recorded immediately and after 10 minutes. The inhibition rate was calculated in accordance with formula 1.

$$\% \ inhibition = 1 - \left[\left(\frac{2R_s}{R_{c1} + R_{c2}} \right) \cdot 100\% \right]$$

(1)

where: R_s – respiration rate in sample with test substance, $R_{c1(c2)}$ – respiration rate in first (and second) reference sample.

Several concentrations of each IL were tested. Obtained % inhibition was then plotted against concentration on log-normal scale and the value of EC_{50} was derived as concentration causing 50% inhibition of oxygen uptake.

3.5.4. Adsorption on biomass

Adsorption on biomass can be observed in negative controls (samples poisoned with sterilising agent) of biodegradation experiments. From those samples small aliquots were taken during the whole duration of the tests like it was done with non-sterilised samples. The solids were centrifuged and the supernatant was recovered for HPLC/UV analysis. The dry mass of sludge was measured by gravimetric method. Ten mL^{-1} of sewage was dried at 100°C and weighed, resulting in 5 g L^{-1}. All experiments were performed in duplicate. The amount of test substance adsorbed was calculated as a difference between initially added amount and amount remaining in solution at equilibrium. Samples taken 24 hours after the start of the experiment were used to create sorption isotherm of [OMIM][Cl] on activated sludge.

3.6. Results

3.6.1. Concentration threshold

Biodegradation tests were performed using [OMIM][Cl] in different concentrations (from 0.02 mM up to 150 mM). The biodegradation measured here is a primary biodegradation, which is understood as defined by OECD in standard number 301 [67]. The results, as shown in Figure 7 A and Figure 7 B indicate that concentrations higher than 1 mM did not undergo biodegradation.

1 mM [OMIM][Cl] was biodegraded after approximately 20 days as displayed in Figure 7 B. In all cases a decrease in the [OMIM][Cl] concentration was observed. This concentration decrease was attributed to the sorption of the ionic liquid onto the sewage flocs. The negative control experiments were conducted for all concentrations, and the decrease of the concentrations was found to be similar to corresponding test samples for high concentrations of IL (>1 mM). This would indicate the decrease in the concentration of the IL is solely due to adsorption. Adsorption of ILs onto biomass was not observed before when it was tested directly [78, 100] or indirectly as most of biodegradability tests conducted up to day used inocula of very low biomass content [54, 57]. The sorption of ILs onto the sewage sludge flocs can be beneficial as it lowers the bioavailable concentration of [OMIM][Cl] below the toxic levels. Huang *et al.* [101] stated that the partitioning of organic compounds is to a great extend governed by the similarity (or the lack of similarity) between this organic compound and the medium into which the partitioning takes place. This suggests that compounds containing aliphatic entities (like side chain substituents of IL) will more favourably partition into aliphatic-rich matrices (like biological membranes).
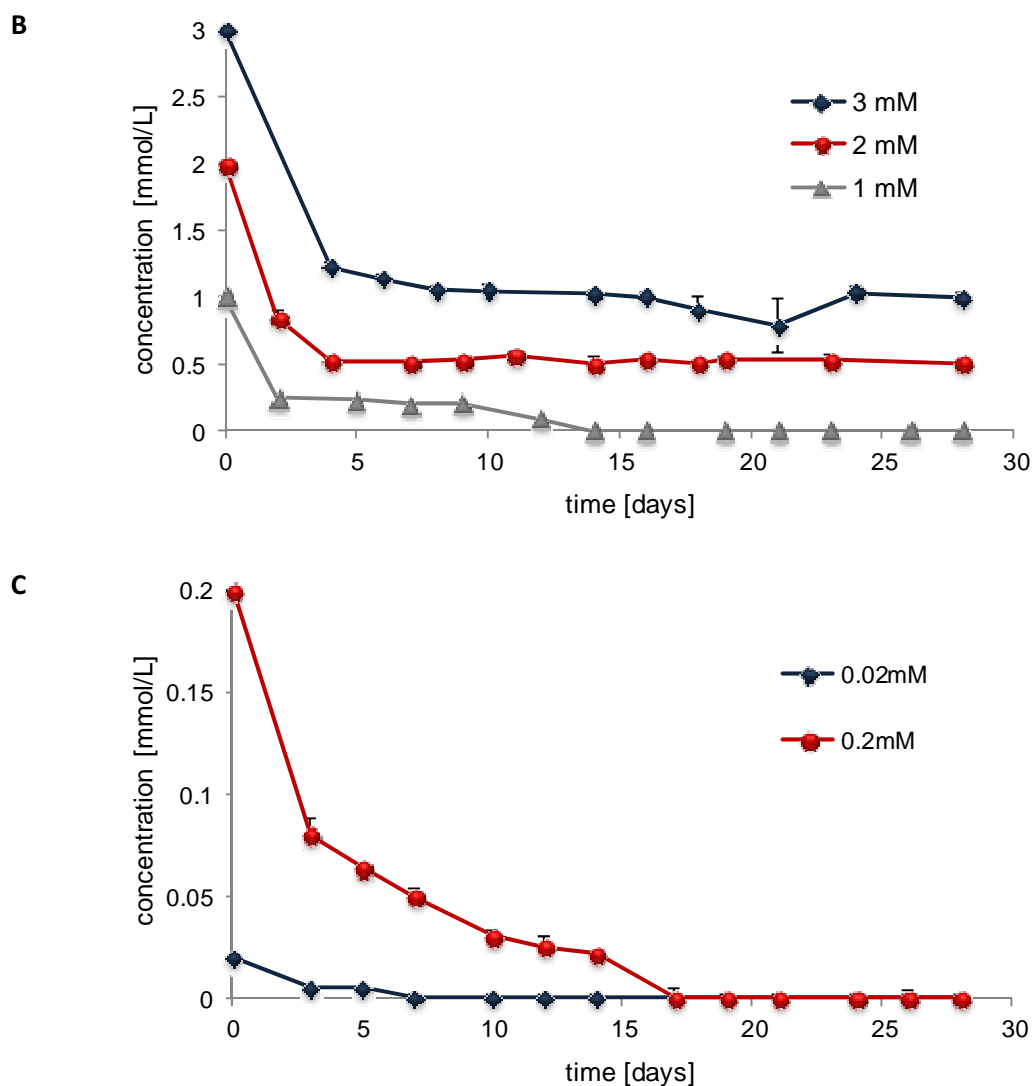
A

B

C
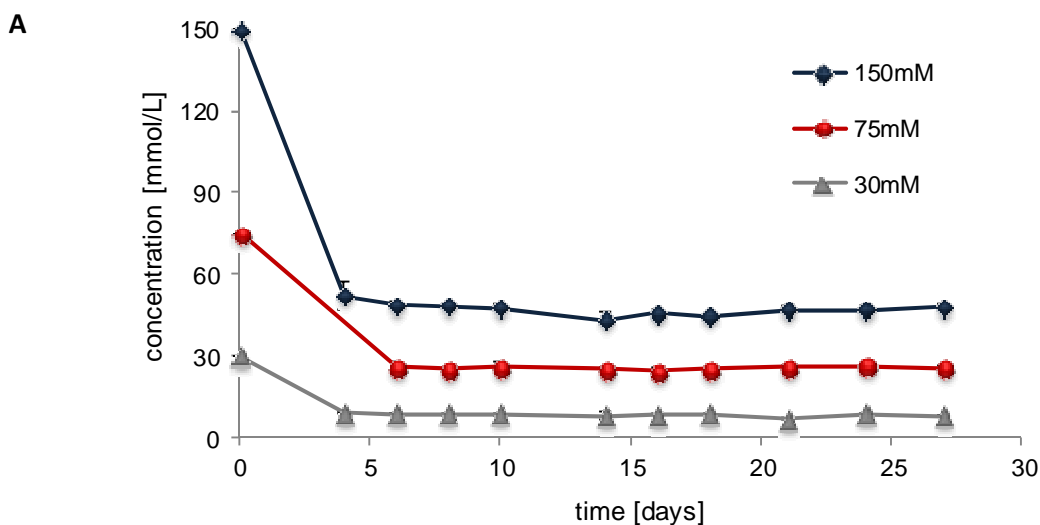
Figure 7. Examination of biodegradation threshold of [OMIM][Cl] in activated sewage sludge

Depletion from test medium is faster than reported before for [OMIM][Cl] in concentration of 0.2 mmol L^{-1} – 100% primary biodegradation within 17 days as compared to 100% primary biodegradation after 24 days and 81% after 17 days reported by Stolte *et al.* [54]. Unexpectedly, [OMIM][Cl] at 1 mM concentration was removed in approximately the same time. This is most probably due to the higher cell density used in current tests as well as additional removal by adsorption on sewage sludge flocs. At the concentration of 0.02 mmol L^{-1} [OMIM][Cl] was degraded within 5-7 days (primary degradation).

3.6.2. Adaptation

The test vessels were prepared as previously described in section 3.5.1. Sewage sludge from aeration tank of municipal WWTP Gdańsk 'Wschód' was used (dry mass 5.5 g/L). Solutions with increasing concentrations of [OMIM][Cl] (1 mM, 1.5 mM, 2 mM, 2.5 mM) were added every fortnight. The total time for the adaptation test was two months. Progressive accumulation of the xenobiotic can be observed in abiotic control (Figure 8). Theoretical total concentration anticipated in all samples at the end of the test, excluding biodegradation and sorption processes, is 7 mM. Concentrations measured in the aqueous phase of test media in biotic and abiotic control are presented in Figure 8.

In non-sterilized sample the sludge was able to adapt to the IL. An initial lag phase of around two weeks was observed. The biodegradation rate for each addition increased from 0.01 mmol/day to 0.15 mmol/day, to 0.26 mmol/day after the final addition. The adaptation allowed the sewage sludge to degrade concentrations of [OMIM][Cl] previously reported to be too high [56]. A small decrease in [OMIM][Cl] concentration in sorption control was observed possibly indicating that sterilizing agent did not inhibit biodegradation completely, however, sufficiently to allow for distinction from sorption.
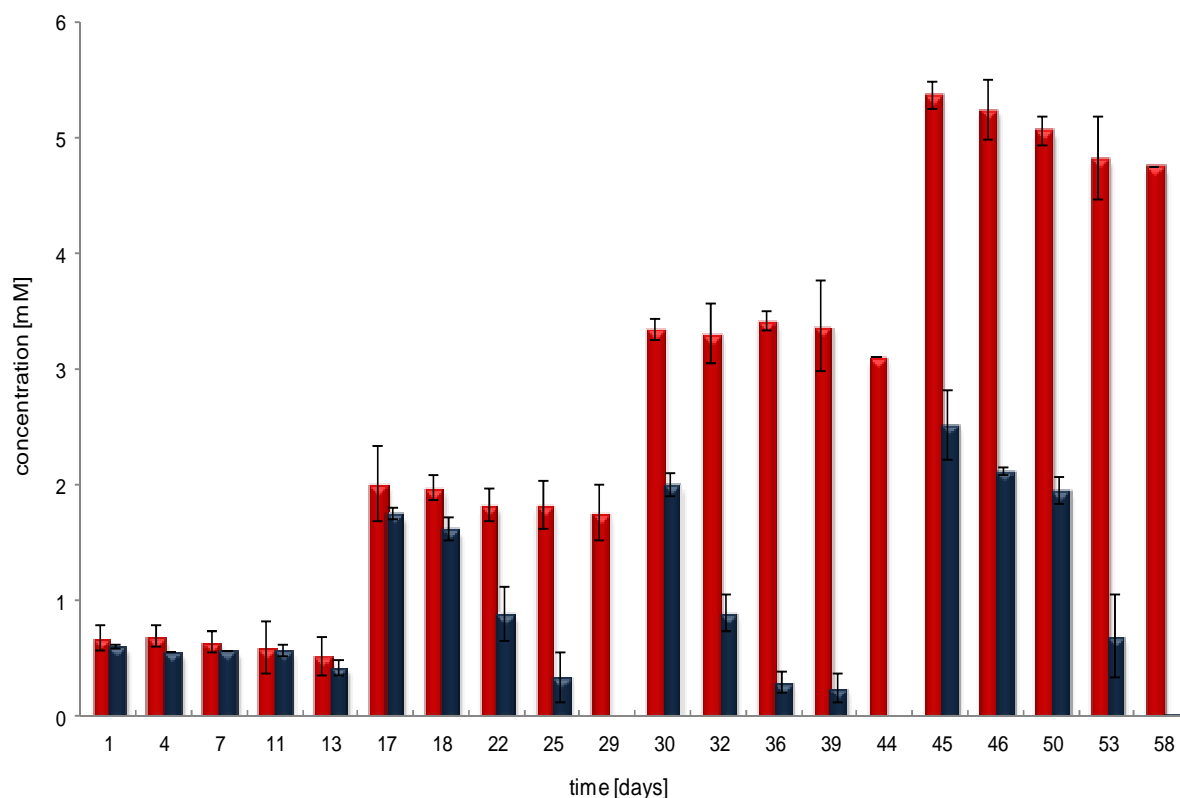

Figure 8. Biodegradation of [OMIM][Cl] conducted by adapted sewage community (blue bars) and abiotic control (red bars)

Figure 9 shows biodegradation curves for adapted and non-adapted communities. No biodegradation of [OMIM][Cl] in concentration of 2 mM was observed for the non-adapted community. The initial decrease in concentration is due to sorption on sewage sludge flocs, as previously shown. The adapted community, however, was able to utilize the IL. Primary biodegradation was completed within 15 days.
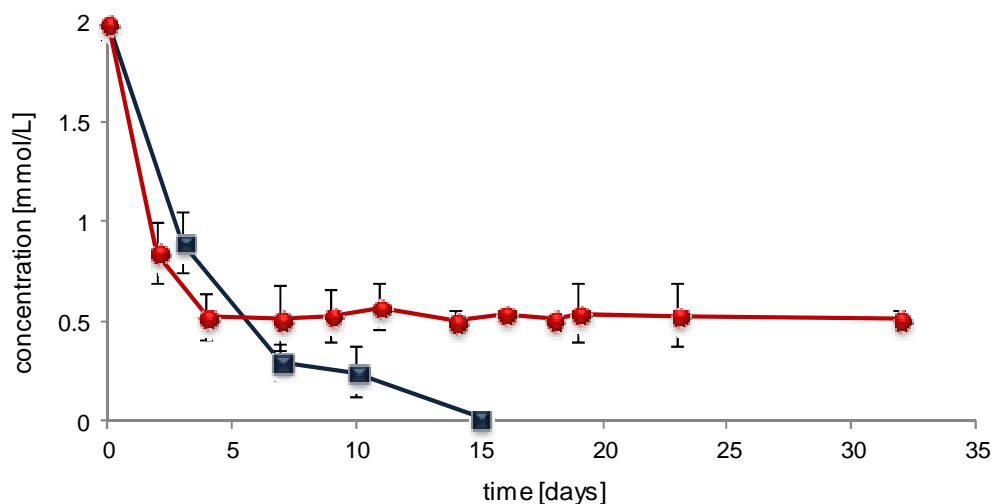

Figure 9. Comparison of biodegradation of 2 mM [OMIM][Cl] conducted by adapted (blue line) and non-adapted (red line) activated sewage sludge community

Results of the analysis of biodegradation products by ESI-MS (electrospray ionisation mass spectrometry) confirmed that on the 53rd day of the test the concentration of the parent compound ([OMIM][Cl]) in the abiotic sample was approximately ten times greater than in the biotic sample (Figure 10). Small amounts of degradation products were detected in the abiotic controls confirming that inhibition of inoculum was not complete. In the biotic sample [OMIM][Cl] was detected as the main compound, however relatively high signals corresponding to ketone, hydroxyl and carboxyl functionalized methyl-octylimidazolium chloride and a carboxyl-derivative of methyl-butylimidazolium chloride were observed. Degradation products were not quantified, however, based on the signal intensities it is possible to state that metabolites of the parent compound were present at a much lower concentrations than the theoretical concentration of the parent compound in the biotic sample. Therefore, it is reasonable to believe that the whole structure, including the imidazolium core, was biodegraded to some extent. Otherwise much higher amounts of intermediates should be present in the sample.
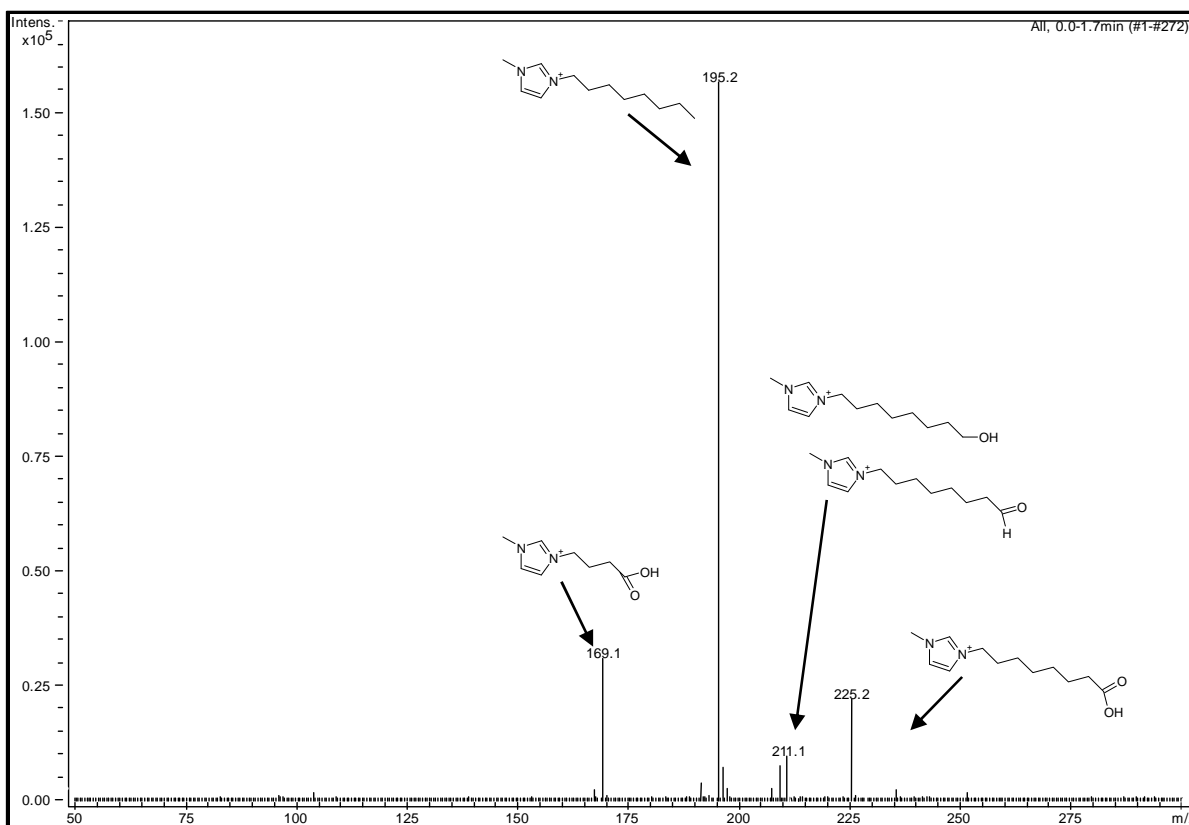
Figure 10. Mass spectra of a biotic sample at 53rd day of biodegradation

The adaptation is an important process during which a biocenosis adjusted to certain conditions and nutrient sources is formed. This biocenosis is able to degrade xenobiotics, to which it is adapted much faster and without noticeable lag phase. Adaptation is expected to occur in any environment where repeated exposure of microbial community to xenobiotic takes place e.g. WWTP treating ILs wastes.

3.6.3. Supplementation

The supplementation with glucose and synthetic feed increased the time of [OMIM][Cl] primary biodegradation. It can be observed that after approximately 14 days the biodegradation of the remaining (after sorption) 0.2 mmol L^{-1} [OMIM][Cl] was completed only when no supplements were added. After more than 20 days biodegradation was accomplished in the sample where synthetic feed was added. In the vessel with glucose supplementation complete biodegradation was not observed within the test timeframe.

The initial, fast decrease of [OMIM][Cl] concentration in Figure 11 was due to sorption of [OMIM][Cl] onto flocs of activated sewage sludge. The sewage sludge organic matter (especially

extracellular polymeric substances) can act as a 'buffer' for the IL, initially decreasing the bioavailable concentration and thereby mitigating its toxicity.
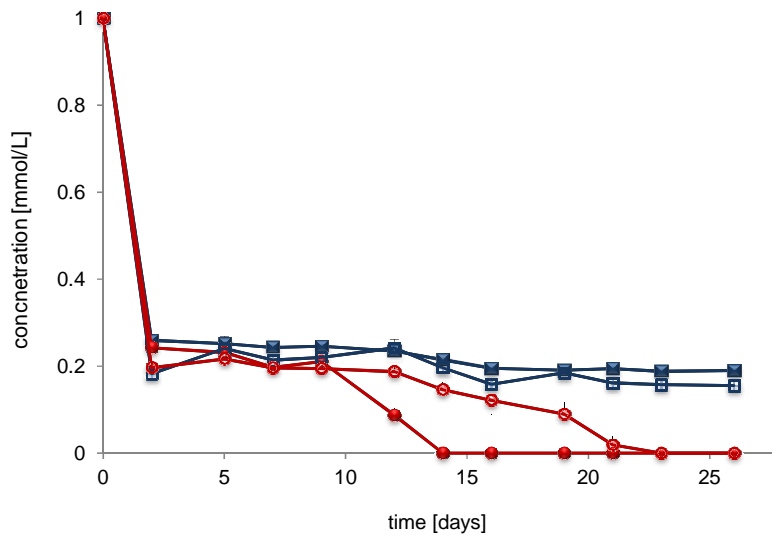

Figure 11. Biodegradation curves of [OMIM][Cl] as a sole source of carbon (closed circles), with supplementation of glucose (open squares), with supplementation of synthetic feed (open circles), sorption control (closed squares)
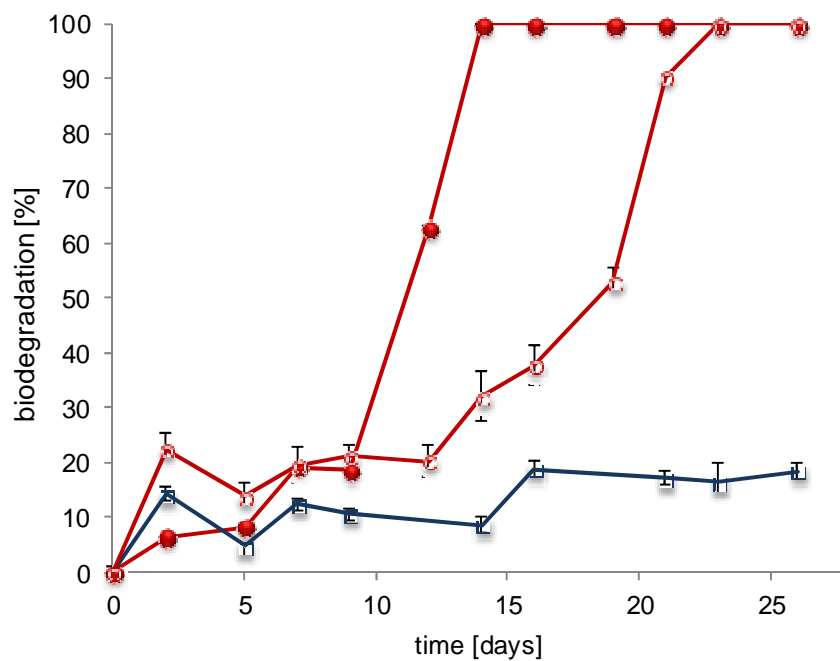

Figure 12. Biodegradation % of [OMIM][Cl] normalised for sorption on sewage sludge floc as a sole source of carbon (closed circles), with supplementation of glucose (open squares), with supplementation of synthetic feed (open circles)

The phenomenon of diauxie is known to occur in microbial communities fed with two growth substrates from which one is preferred over the other. In such cases organisms utilize this preferred substrate and an increased growth is observed. After the first nutrient is depleted the metabolism of second one starts together with the second growth phase [102]. The diauxie effect could not have occurred here however, as the feed was added continuously for the duration of the test so there was no possibility of nutrients to be depleted.

For [OMIM][Cl] as a only source of organic carbon and organic nitrogen complete primary biodegradation is achieved within 14 days. When synthetic feed is present the biodegradation rate is clearly reduced, even though complete primary degradation is achieved within 23 days. When supplemented with glucose, providing easily available source of organic carbon, [OMIM][Cl] is utilized in less than 20%. Therefore, the presence of other nutrients in the sewage or within the environmental media in general can have a strong influence on the biodegradability of ionic liquids. Also compounds which have been classified as "readily biodegradable" might be poorly degraded under real environmental conditions what has to be taken into account when evaluating their fate.

3.6.4.   Sorption on activated sludge

The removal of ILs from the solution due to sorption onto activated sludge is a very important mechanism of their removal from wastewaters [103]. In batch sorption experiments the equilibrium concentrations in the solution were used to determine the amount adsorbed on the sewage flocs. From these data an adsorption isotherm was created. Then the sorption coefficient was determined as a ratio of the amount adsorbed to the amount in solution, assuming that sorption is linear, as shown in Figure 13. The sorption coefficient for [OMIM][Cl] on sewage sludge flocs was determined to be 98.2 L kg^{-1}. The high sorption coefficient can be attributed to the strong interaction between the sewage floc rich in organic matter, and the alkyl chain of the imidazolium IL. This is in agreement with previous research which states that the dominant mechanism of interaction of ILs with organic matter rich solid phase is dependent on the lipophilicity of the IL and the amount of organic content of the solid phase.

Adsorption on activated sewage sludge is of a profound technological importance for wastewater treatment process. It was shown in the literature that hydrophobic organic compounds are adsorbed on sewage sludge to a significant extent [29].
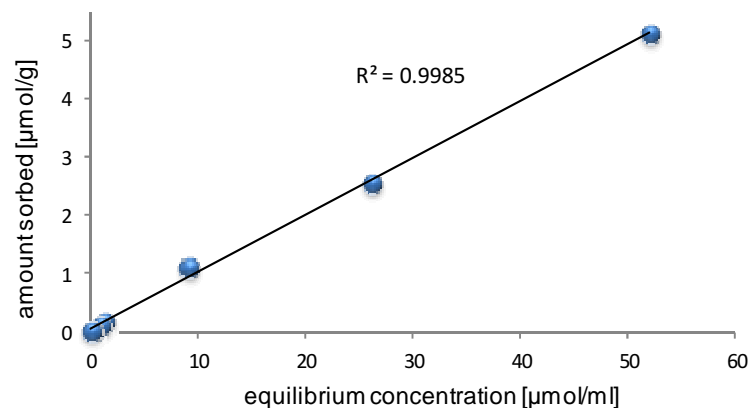
Figure 13. Sorption isotherm of [OMIM][Cl] on activated sewage sludge

It was also shown that the extent of adsorption depends mainly on lipophilicity of a compound that is being removed and lipophilicity of an activated sewage sludge (which is mainly defined by the amount of organic matter). Influence of place of origin and pre-treatment on sorptive capacity of sewage was examined in the literature and no significant differences were observed for e.g. fresh and dried sludge or sludge originating from different WWTP [30]. Within this work a concept and installation for utilising excess activated sludge as sorbent for reducing concentration of ILs and other xenobiotics of similar structure was proposed [104]. This concept provides fast, concentration independent way for reducing the amount of xenobiotics before they enter the activated sewage sludge bioreactor. In this way the more efficient treatment of wastewaters is assured and activated sewage sludge community is protected from instantaneous concentration surge. This result additionally points out that care should be taken when interpreting biodegradability results of this kind of system as depletion of xenobiotic from solution can be attributed to adsorption as well as biodegradation.

3.6.5.   Toxicity with respect to activated sludge

Toxicity of a series of imidazolium ILs towards activated sludge as biocenosis was determined based on inhibition of oxygen uptake rate of sewage community exposed to ILs relative to the control sample containing 3,5-dichlorophenol. Dose response curves were plotted and EC_{50} values were calculated as concentrations causing 50% inhibition in respiration rate. The results are summarised and compared with literature results for marine bacteria *Vibrio fischeri* in Table 2.

Table 2 Comparison of toxicity of ILs towards *Vibrio fischeri* and activated sludge

Compound	Log_{10} EC$_{50}$ [μM]		
	Vibrio fischeri	Municipal sludge	Industrial sludge
[EMIM][Cl]	4.33 [93]	> 5.00	> 4.70
[HMIM][Cl]	2.91 [93]	4.04 ± 0.06	N.A
[OMIM][Cl]	1.01[93]	2.32 ± 0.07	2.19 ± 0.05
[DMIM][Cl]	-0.23 [93]	1.71 ± 0.14	1.43 ± 0.07
[EMIM][B(CN)$_4$]	N.A	4.25 ±0.09	3.90± 0.03
[EMIM][N(CN)$_2$]	4.0 [81]	5.24	4.39 ± 0.08
[EMIM][(CF$_3$SO$_2$)$_2$N]	3.2 [81]	4.45± 0.09	3.56 ± 0.03
[EMIM][(C$_2$F$_5$)$_3$ PF$_3$]	N.A.	3.24 ± 0.08	3.26 ± 0.12

*N.A. – data not available

A clear relationship between the length of IL cation substituent and toxicity can be observed. 1-ethyl-3-methylimidazolium chloride ([EMIM][Cl]) did not show 50% inhibition within the concentrations measured, therefore EC$_{50}$ value was reported as higher than 100 mM. Elongation of alkyl chain results in increased toxicity towards activated sludge biocenosis yielding log EC$_{50}$ values of 4.04 for 1-hexyl-3-methylimidazolium chloride ([HMIM][Cl]) (municipal sewage), 2.32 (municipal sewage) or 2.19 (industrial sewage) for 1-methyl-3-octylimidazolium chloride ([OMIM][Cl]) and 1.71 (municipal sewage) or 1.43 (industrial sewage) for 1-decyl-3-methylimidazolium chloride ([DMIM][Cl]).

The influence of anion on toxicity towards activated sewage sludge was also examined for [EMIM][Cl] cation coupled with five different anions. For *Vibrio fischeri* toxicity increased with hydrophobicity of an anion, being the lowest for simple chloride and over three orders of magnitude higher for tri(pentafluoroethyl)trifluorophosphate.

For all ILs tested activated sewage sludge showed to be less susceptible than marine bacteria. This result was anticipated as activated sewage sludge is a complex mixture of organisms exhibiting different vulnerability but generally believed to show more flexibility in response to toxins.

Additionally the change in dehydrogenase activity of microorganisms was investigated as a way of evaluating IL's influence on microbial community. Two concentrations of [OMIM][Cl] were used in comparison with $HgCl_2$ as a well-known sterilizing agent.
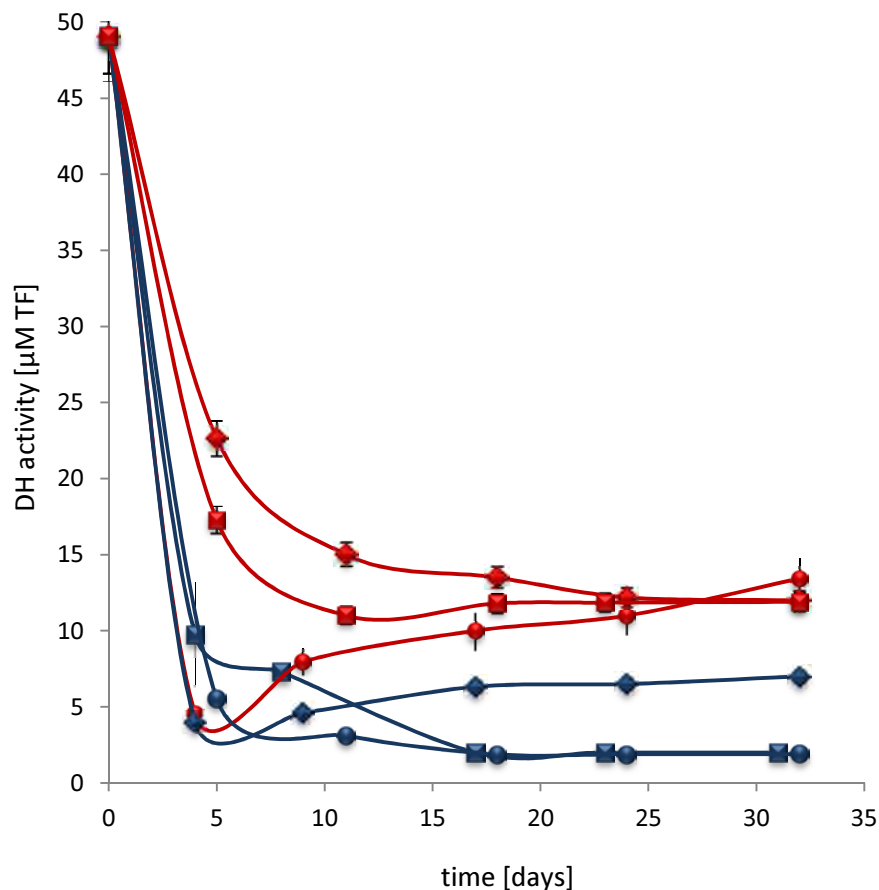

Figure 14. Dehydrogenase activity: 2 mM [OMIM][C] (blue squares), 0.2 mM [OMIM][Cl] (red circles), 2 mM [OMIM][Cl] abiotic control (blue circles), 0.2 mM [OMIM][Cl] abiotic control (blue diamonds), positive control (red diamonds), blank (red squares)

The dehydrogenase activity of 0.2 mM of [OMIM][Cl] after 25 days was comparable to that of glucose, indicating that the metabolism of the cell adapted to allow for the degradation of the IL. When investigating the dehydrogenase activity, it was found that [OMIM][Cl] in concentrations of 2 mM and higher inhibited the enzyme activity to comparable levels as $HgCl_2$, as shown in Figure 14. This would lead us to assume that high concentrations of [OMIM][Cl] can inhibit cell activity. Due to the structural similarity between surfactants and [OMIM][Cl] it can be assumed that the mode of action is similar to cationic surfactants such as quaternary ammonium compounds (QACs). According to the literature QACs are membrane-active agents. A target site is mainly cytoplasmic membrane of bacteria and yeast [10]. The mode of action of surfactants

includes their interaction with phospholipids components in the cytoplasmic membrane followed by loss of its selective permeability [23].

3.7. Summary – scientific novelty

Sorption of [OMIM][Cl] to activated sewage sludge flocs was noticed for the first time. It was shown to be an efficient mechanism of decreasing IL' concentration. The sorption of ILs onto the sewage sludge flocs can be beneficial in the biological removal of ILs from wastewater streams (e.g. in activated sewage sludge process) as it lowers the bioavailable concentration mitigating the toxicity. Unlike biodegradation, sorption is not limited by concentration of treated xenobiotic. IL, in concentrations two orders of magnitude higher than biodegradable concentration reported so far, can be to significant extent removed, from waste streams depending on IL's sewage organic matter/water partition coefficient. Sorption can be applied as a separate process of physicochemical removal of IL showing fast kinetics (few hours in comparison to days necessary for biodegradation to occur). During the course of research a patent application was developed describing a new way of purification of wastewaters containing ILs.

The proposed technology has introduced additional step of adsorption of ILs onto excess sewage sludge prior to biodegradation as shown in Figure 15. Dewatered excess sewage should be applied as its organic matter content is significantly higher and positively correlates with amount of adsorbed IL. Excess sewage which is treated as a waste and sequentially removed from the bioreactor is reused as sorbent for ILs and then disposed of. This modification allows for faster purification of waste containing even high concentrations of ILs. Moreover, it can act as a buffer preventing biological step of sewage treatment from unexpected, undesirable fluctuations in concentration of IL entering the bioreactor.
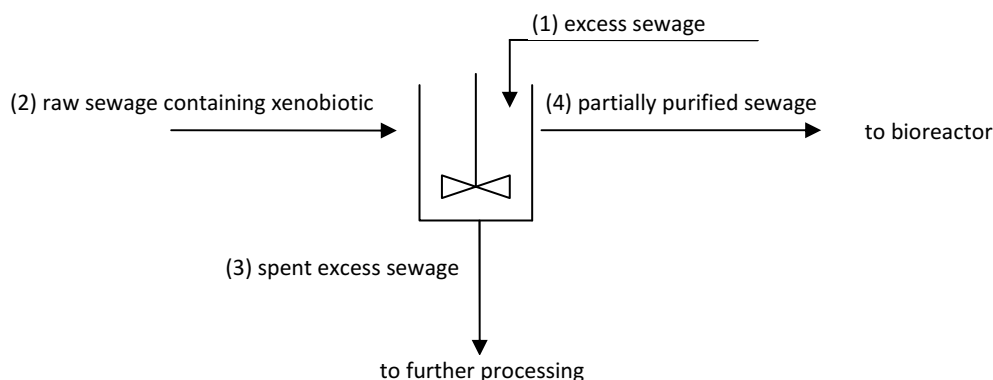

Figure 15. Schematic of modified sewage sludge treatment installation containing ILs adsorption reactor

It was discovered that biodegradability of [OMIM][Cl] can be enhanced by allowing activated sewage sludge community to adapt to it. Over 25 times higher rates of biodegradation were observed after two months of adaptation. Additionally concentration threshold was increased from 1 mM for not adapted sewage to 2.5 mM for adapted sewage.

This suggests that in industrial activated sludge process, where part of biomass in a bioreactor is retained, microorganisms might adapt to xenobiotic in a similar way. It is therefore expected that biodegradation of ILs, as well as xenobiotics in general, will be faster than biodegradation tests without pre-exposition would indicate.   .

On the other hand, presence of additional, easily available sources of carbon and/or nitrogen might impair degradation of ILs as these compounds can be utilised preferentially. Since presence of additional nutrients is obvious during wastewater treatment slowed down biodegradation can be expected.

Results presented in this chapter were published as:

Markiewicz M., Z. Lustig, S. Stolte, J. Łuczak, M. Skup, J. Hupka, C. Jungnickel, *Influence of microbial adaption and supplementation of nutrients on the biodegradation of ionic liquids in sewage sludge treatment processes,* Journal of Hazardous Materials, 2011, 195, p: 378-382

Markiewicz M., Jungnickel C., Markowska A., Szczepaniak U., Hupka J., *1-methyl-3-octylimidazolium chloride – sorption and primary biodegradation analysis in activated sewage sludge.* Molecules, 2009, 14:p. 4396-4405

Markiewicz, M., J. Hupka, and C. Jungnickel, *Sposób oczyszczania ścieków zawierających ksenobiotyki*. Zgłoszenie patentowe, nr wewnętrzny PG 53/2009.

4. Adsorption and biodegradation in soils

4.1. Background

Mobility is one of the most crucial issues in ecological risk assessment of ILs as it governs their bioavailability, toxicity and distribution among all environmental compartments. Mobility is influenced by water solubility and sorption to environmental matrix. The solubility of the IL will influences its ability to be transported in the soil pore fluid, as well as its ability to interact with the soil particles. The solubility of ILs depends largely on structural aspects – such as: the type of anion and chain length of the substituents. Many ILs have excellent water solubility, what makes them likely to enter the environment with industrial water/wastewater streams [105].

Sorption onto solid particles of minerals or/and organic matter in terrestrial or aquatic environments has a marked influence on the mobility of chemical substances. Investigation on the extent, strength and the mechanism of adsorption will allow prediction or modelling of the distribution of chemicals. Strong binding to soil particles can prevent leaching to groundwater which could further cause drinking water contamination. It can also decrease toxicity for flora and fauna since bound chemicals are not available for uptake [31, 106]. Similarly, partitioning between sediments and water bodies will decrease toxicity towards aquatic organisms [107].

Sorption of ILs may be reversible or irreversible and is rather rapid [105, 108, 109]. The extent of adsorption depends on the properties of soil (organic matter content, clay fraction content, cation exchange capacity, pH etc.) and the compound (e.g. size, shape, molecular structure, solubility, polarity, polarizability, charge distribution of interacting species, and the acid - base nature of the molecule) [110-112].

The bonding forces and the types of mechanisms involved in adsorption of xenobiotics onto the soil/sediment matrices may include [113-115]:

- *ion exchange* – adsorption by ion bonding between ion in the solution and ionised group on the surface of solid, due to prevalence of negatively charged sites soils show particularly high affinity toward cations;

- *hydrogen bonding* – formation of hydrogen bonds between functional groups containing highly electronegative atoms e.g. oxygen or nitrogen like hydroxyl or amino groups of organic matter and complementary groups in xenobiotics; in case of ILs a hydrogen present in C2 position can act as a donor of hydrogen bond;

- *adsorption by polarization of π electrons* – attraction between electron rich aromatic nuclei of the adsorbate and positive sites on adsorbent (i.e. previously sorbed molecules);

- *adsorption by van der Waals forces especially London dispersion forces* –resulting from instantaneous imbalance of a distribution of electrons in a molecule resulting in formation of momentary dipole interaction between electrons of one molecule and nucleus of the other; this force is proportional to molecular volume and number of polarizable electrons thus is more pronounced for compounds of high molecular weight ; London dispersion forces are weak, short-range interactions,

- *ligand exchange* – based on replacing of hydration water molecules or other ligand by xenobiotic,

- *charge transfer* – interactions between electron-rich (e.g. quinone-like structures in organic matter) and electron-deficient groups (e.g. amines, nitrogen or oxygen heterocycles),

- *covalent bond formation* - incorporation of xenobiotic into the structure of sorbent by formation of chemical bond; this type of interaction is often irreversible and occurs due to partial degradation of xenobiotic.

Not all mechanisms occur simultaneously, however, two or more may coincide depending on the nature of the functional groups of the molecules, the type of clay mineral, and the acidity of the system [111].

Sorption of ILs on soils is believed to depend mostly on the organic matter content and charge of organic matter and mineral fraction [107]. Charge developing on organic matter and mineral fraction is responsible for soil cation exchange capacity (CEC) which is defined as the amount of cations that can be exchanged between soil particles and soil solution. CEC of organic matter was reported to be approximately 200 meq/100g whereas CEC of minerals ranged from 100-150 meq/100g for vermiculite to 3-15 meq/100g for kaolinite [116]. Additionally sorption is influenced by the presence of other electrolytes, pH of soil solution, temperature and soil saturation with the liquid phase [117, 118].

Interaction with organic matter

The amount of organic matter has a strong influence on the properties of soil and on the behaviour of xenobiotics. The soil organic matter (SOM), which originates primarily from biologically degraded plant tissue, consists of a heterogeneous organic constituents, such as lignins, carbohydrates, protein, fats, and waxes which are collectively named humic substances [119]. Although molecular structures of humic substances have not been well characterized, they are known to be high-molecular-weight amorphous materials with significant polar-group contents. Humic substances consist of soluble fractions (humic and fulvic acids) and an insoluble

fraction (humin). Humic and fulvic acids are soluble organic acid macromolecules containing –COOH and –OH functional groups that, at pH $\geq$ 7, ionize in water releasing H$^+$ ions and providing negative charge centres on the macromolecule to which cations are attracted.

SOM presents the possibility of interactions through London dispersion forces, ion exchange as well as π-π interactions with [OMIM][Cl]. In this sense it is often seen as a separate, organic phase into which ILs can partition depending on their hydrophobicity. The partial dissociation of SOM carboxylic, hydroxyl and phenolic functional groups, gives the humic molecules a negative charge, the magnitude of which is pH-dependent, and which present sites for electrostatic interaction with positively charged ILs' core.

Interaction with mineral fraction

For soils, which have low organic matter content, the mobility of the contaminants is often related to the inorganic fraction, which is predominantly the clay-sized fraction [105]. The surface of most minerals is negatively charged in the soil environment allowing them to interact with ILs cations. The charge of minerals originates from:

– isomorphic substitution of original lattice elements with elements of usually lower valence e.g. substitution of Al^{3+} with Mg^{2+} or Si^{4+} with Al^{3+} will produce a negative charge known as pH independent charge. This mechanism has the most significant influence on the overall mineral charge,

– lattice defects – the empty spaces within the mineral lattice where an ion was removed leaving an unbalanced charge,

– formation of pH dependent charge on mineral oxides by adsorption of protons or hydroxyl groups to form positive or negative sites respectively. The pH dependent charge of mineral oxides depends on which of the two species dominates. Therefore H$^+$ and OH$^-$ ions are known as potential determining ions (PDI),
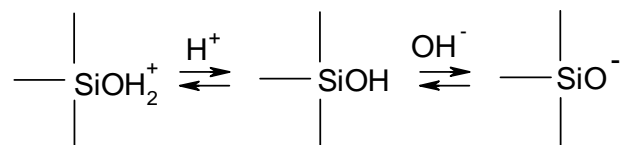

Figure 16. The mechanism of pH-dependent charge formation

– broken bonds - when the surface of minerals is fractured the bonds connecting elements can be broken leaving atoms with unsatisfied valence. The resulting charge can be positive or negative depending on the type of the bond that was broken [116, 120].

Diminishing negative electrical charge of minerals can cause: a destabilisation of colloidal systems, a decrease in the affinity for nutrients and metals etc. influencing agricultural utility of soil as well as performance of many technological processes employing suspensions of minerals. Electrostatic interactions must be considered for all charged organic compounds and are especially important for cationic xenobiotics due to the presence of negative charge on the surface of natural inorganic solids. Additionally, ILs can be forced to stay on solid surface by water molecules forming hydrogen bonds with each other from which ILs are largely excluded. Once in the proximity of surface weak and short ranged London dispersion forces may promote adsorption.

As mentioned previously, sorption of ILs to solids can limit their bioavailability and therefore the rate of their biological degradation. In order to establish the possible level of ILs removal from contaminated sites one needs to distinguish between removal due to sorption and actual biodegradation.

Importance of surface activity

Many ILs exhibit surface activity due to their amphiphilic structure. After exceeding certain concentration threshold – known as critical micelle concentration (CMC) – ILs spontaneously form aggregates in aqueous solution. When placed in suspension of solids analogous phenomenon takes place, ILs aggregate at the surface of solid after exceeding another threshold value – CAC (Critical Aggregate Concentration). CAC is usually lower than CMC since there are more interactions favouring formation of aggregates on a surface than in solution. Formation of surface aggregates is the result of two processes: minimization of contact area between surfactants molecules and water mentioned before and maximization of contact area between surfactant head-groups and negatively charged solid surface [107].

Aggregates in solution are mostly spherical since this shape exhibits a minimum contact area between aqueous bulk phase and the hydrophobic part of the surfactant. Hydrophilic head-groups screen hydrophobic core of the aggregate and are thought to be uniformly distributed with the maximum possible distance from each other corresponding to minimum electrostatic repulsive forces. The counter ions are arranged close to or between surfactants head-groups reducing the mentioned electrostatic repulsion and also screening the hydrophobic core. Their effectiveness is influenced by the counter ion size including a hydration shell. Weakly hydrated ions can approach the head-group closely and are more effective in charge compensation therefore promoting the growth of the aggregates stronger than more hydrated counter ions having larger hydrodynamic radius. In general counter ions obey the lyotropic series in promoting

adsorption of cationic surfactants as follows: $Cl^-<Br^-<NO_3^-<ClO_4^-$ [121]. The valence of a counter ion is of a great importance since two head-groups' charge compensation by one divalent ion favours the aggregation stronger than by two monovalent ions as the Schulze-Hardy rule states [122].

From an environmental point of view, the surface activity indicates that ILs may create foams, stabilize emulsions of hydrophobic phases and particle dispersions in surface or ground water. These functions are related to the surfactant adsorbing at a gas, liquid or solid interfaces with the hydrophilic part of the molecules oriented towards the aqueous phase [28]. Micelle formation of ILs may influence the interactions of the compound with the surrounding environmental compartments, including changing the transport parameters of ILs in soils, and the possibility of colloid facilitated transport of other contaminants [29]. The ability of ionic liquids to decrease the surface tension and micellar aggregate formation also means that ILs may increase the solubility of hydrophobic compounds, e.g. anthropogenic hydrocarbons, pesticides in aqueous environments. As a consequence, these chemicals will be more accessible and can therefore show higher toxicity and tendency to accumulate in living organisms but in the same time exhibit higher biodegradation rate.

Initially size and shape of adsorbed aggregates were calculated from the two-plateau sorption isotherm since no direct visualization techniques were available. For cationic surfactants it varies from 3-20 molecules and is considerably smaller than the bulk solution micelles composed usually of 20-50 molecules. Rupprecht *et al.* on the basis of calculations of surface aggregation number assumed that small spherical aggregates of the diameter approximately equal to the length of a hydrocarbon chain and isolated from each other are formed at the second plateau region of the sorption isotherm [123, 124]. Also the pH value of the solution can influence the size of aggregates and aggregation number (number of molecules forming one aggregate) e.g. the aggregation number of tetradecylpiridinium chloride can decrease from 7.2 at pH 3.5 to 2.1 at pH 9.5 [123].

Johnson and Nagarajan observed that during adsorption of cationic surfactant (CTAB) on a hydrophilic surface (mica) the so called composite hemicylinders are formed at CAC, that undergo morphological changes with the increase of bulk surfactant concentration, as shown in Figure 17. At 3.7 × CAC the mentioned above aggregates transform into composite hemispheres, at 14 × CAC into lamellar bilayers and into composite finite discs at 18 × CAC. The significant increase in CAC is needed to transform previous structures to fully cylindrical (4900 × CAC) and further on fully spherical (6600 × CAC). Therefore, the existence of full three dimensional structures is unlikely and can occur at very high concentrations in the solution. Hemicylinders and hemispheres are

minimizing contact area of surfactants with water and maximizing contact area with negative mineral surface, which can explain the difference between atomic force microscopy imaging (AFM) of adsorbed surfactant and free-energy based prediction of the shape of surface aggregates [125].
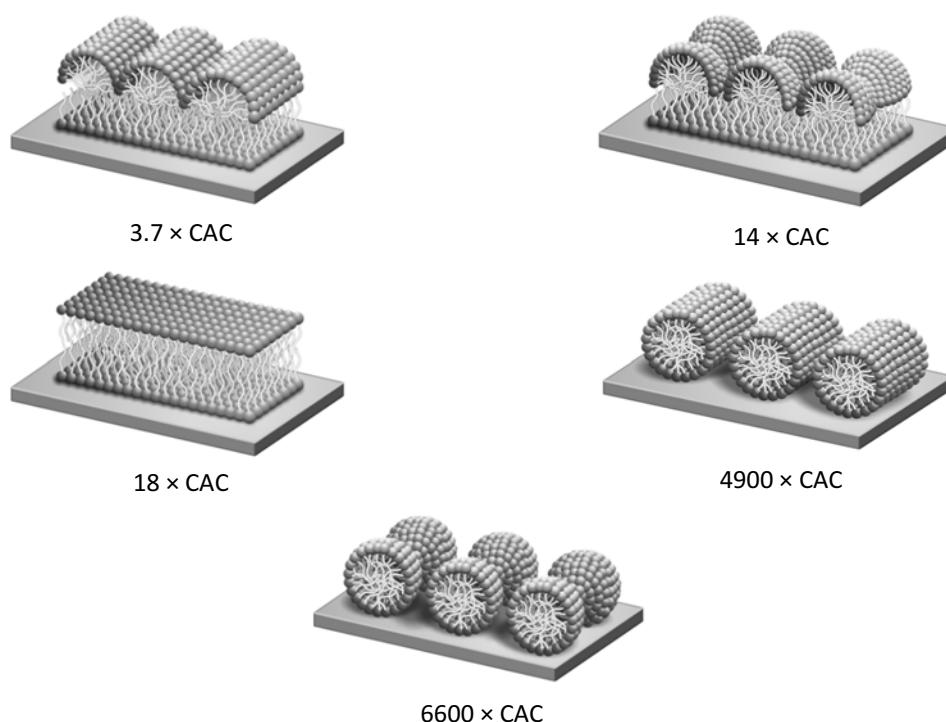

3.7 × CAC

14 × CAC

18 × CAC

4900 × CAC

6600 × CAC

Figure 17. Shape of cationic surfactant micelles on hydrophilic surfaces at different concentrations (expressed as magnitude of CAC) [107]

The cationic surfactant sorption on hydrophobic surfaces was mainly measured on graphite since it is easy to obtain, a molecularly smooth graphite surface [126]. Zettlemoyer postulated that at low bulk concentration (up to 10% CMC) surfactant molecules are adsorbed lying flat on the surface of adsorbent since in this way the minimal contact area with water molecules is obtained. As the bulk concentration increases the molecules start to attain more upright position to be oriented perpendicularly to the adsorbent surface at around bulk CMC value (Figure 18). However with a development of modern imaging techniques Zettlemoyer model was questioned. Using AFM measurements it was established that molecules arrange themselves along the graphite symmetry axis and parallel to each other on the graphite plane with periodicity of two surfactant molecules implying head-to-head tail-to-tail order. This arrangement serves as a template for full monolayer build-up with an increase of surfactant bulk concentration resulting in hemicylindrical monolayer formation. The periodicity of the template formation assumes tail-to-tail and head-to-head cross sectional arrangement. Whereas the first

one is to be expected due to existing London dispersive interactions, the latter seems less obvious since there is an electrostatic repulsion between head-groups that should prevent them from aggregating. The authors suggested that the head-group charge is screened by the counter ion.

This explanation certainly holds true for small counter ions having high charge density, the structure of cationic surfactants aggregates having more bulky counter ions and the efficiency of head-group charge screening of these have not been investigated so far [127]. Adsorption on soil organic matter is expected to proceed in similar, yet perhaps less orderly manner.
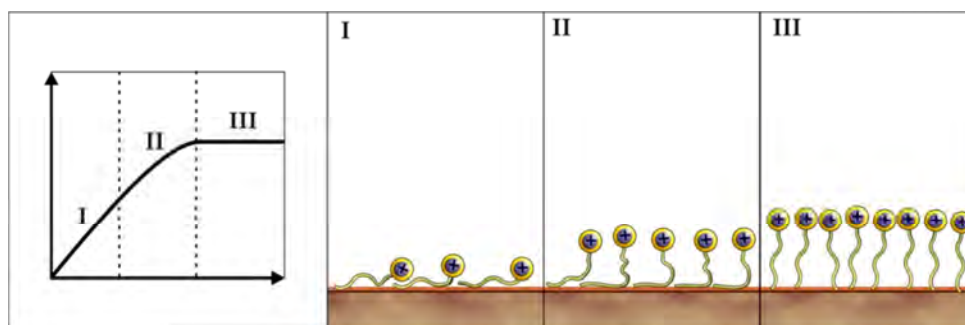
Figure 18. Sorption isotherm and surface aggregates arrangement of cationic surfactants on hydrophobic surfaces [107]

Fuerstenau *et al.* [128] investigated the surface interaction of the surfactants with a silica surface by contact angle measurements. They found that the sorption of dodecylpyridinium chloride (DPC) on silica takes place and significant changes of surface hydrophobicity/hydrophilicity are observed that remain in good agreement with sorption isotherm. At zero surfactant concentration silica is hydrophilic with contact angle equal to zero. However, with a small increase in DPC concentration (10^{-6}M) the contact angle is increasing to 24°, at around 4×10^4 M the hemimicelles are being formed and the sharp increase in contact angle is observed corresponding to sharp increase in both sorption isotherm and zeta potential curves – suggesting adsorption of surfactant molecules with the head-groups towards the silica surface. At DPC concentration of 2×10^{-2}M the contact angle begins to decrease with the increase of concentration attributed to the beginning of double layer formation that is additionally coinciding with continuous increase in zeta potential. Contact angle continues to decrease until it again reaches zero at the end of region III of sorption isotherm when the full bilayer is assumed to be formed.

Since molecular transfer is a prerequisite for the uptake of organic pollutants by organisms, the bioavailability of a given compound and thus its rate of biotransformation, is affected by sorption as well [129]. From a toxicological perspective, binding of xenobiotics to soil/sediment usually leads to reduction of the toxicity of the compound; a decrease of material

available to interact with biota; and reduction of its leaching and transport [130-133]. Therefore, to fully understand the behaviour of ionic liquids in the environment, it is of prime interest to analyse the possible interaction sites available as a result of the chemical structure of these entities. Different types of soils and sediments may vary strongly among each other in their physical and chemical properties and thus, in their sorption potential.

4.2. Adsorption

Gorman-Lewis and Fein [100] tested adsorption in simplified systems using 1-butyl-3-methyl-imidazolium chloride ([BMIM][Cl]) and minerals: quartz, gibbsite, montmorillonite – without organic matter and *Bacillus subtilis* cells. No sorption onto quartz, gibbsite and bacterial cells was observed. Since gibbsite's point of zero charge is pH=9.8, within the experimental pH range (6-10) it was mainly positively charged. Due to this fact electrostatic repulsion between gibbsite particles and IL cation, which limits the sorption by charge-charge interaction, should have been expected. Nevertheless quartz, having point of zero charge approximately 2-3, thus being negatively charged in the pH range of experiment, also did not showed any adsorption of [BMIM][Cl]. Authors claimed, on the basis of literature data, that the amount of binding sites was at least one order of magnitude higher than concentration of test substance and therefore attributed the lack of sorption to the chemical properties of IL. *Bacillus subtilis* is gram positive, common soil micro-organism, possessing carboxyl, phosphoryl and hydroxyl groups on the surface. Those functional groups deprotonate within pH range of the experiment, thereby creating a negatively charged surface capable of attracting IL molecules. Bacterial cell walls also present a possibility of hydrophobic interactions with organic compounds. Despite these factors no sorption of IL was detected. The lack of sorption onto bacterial cells was explained by rather low hydrophobicity of [BMIM][Cl]. On the contrary to the others, significant sorption was detected onto montmorillonite. It is due to the fact that montmorillonite differs in a composition from previous minerals by possessing a so-called 2:1 structure. Those minerals are well known to be by far more able to sorb positively charged chemicals by electrostatic attraction than 1:1 minerals of gibbsite and quartz type. Authors concluded that dialkylimidazolium ILs are unlikely to be retarded in most geological systems taking into account also bacterial community, unless significant amount of clay is present. Consequently they possess a potential to enter ground water systems and to act as contaminants.

Stepnowski [134] determined the behaviour of imidazolium based ionic liquids in soil and sediments in order to assess their potential to spread into the environment and predict possible

distribution compartments. Those ILs were strongly bound to the soil, with the sorption increasing with the lengths of alkyl side chain suggesting mostly hydrophobicity based retention and desorption decreasing in the same order. On the contrary to this bonding to the peaty soils (with the highest organic matter content) was relatively weak indicating that other mechanisms like electrostatic interactions might be involved. Even stronger affinity (with an order of magnitude higher partition coefficient) towards Baltic sediments was found. Authors speculated that this might be a result of strongly developed surface as well as presence of swelling clay (2:1 clays like montmorillonite) which were previously shown to sorb ILs to high extent [100]. In subsequent work of his group adsorption of two imidazolium type ILs on natural soils was examined. The highest partitioning of [BMIM][Cl] and 1-hexyl-3-methyl-imidazolium chloride ([HMIM][Cl]) in the soil matrix was found for fluvial and clayey agricultural soil which had high organic matter content and relatively high CEC (especially the first one). Strong correlation of sorption with fine clay particles content of a soil was found and authors attributed sorption to CEC of soil. Additionally the correlation with the alkyl side chain length of ILs was observed however only up to the concentration of 1 mM. Desorption experiments showed that [HMIM][Cl] was almost irreversibly bound to soils (only 2% desorption). Moreover adsorption of 1-ethyl-3-methyl-imidazolium chloride ([EMIM][Cl]) was increased in higher pH as a result of deprotonation of negatively charged sites of clayey fraction [135]. Within these two works multilayer sorption of ILs was suggested since the amount of IL sorbed onto the soil particles significantly excided CEC of this soil.

Adsorption of C2-C8 imidazolium homologue series was also tested on pure kaolinite (1:1 clay mineral). Double-plateau isotherms characteristic for surfactants were obtained and compounds containing longer alkyl substituents had also higher partition coefficients. Exothermic nature of adsorption characterised by second-order kinetics was demonstrated [136].

Bealieu *et al.* [14] conducted an experimental study aiming to describe dependence of the sorption strength on sediments/sand organic matter content and IL's side chain length. Four imidazolium ILs substituted with butyl, hexyl and octyl- alkyl chains were used, as they were expected to have different sorption behaviour due to an increasing hydrophobicity. No measurable sorption to sand was observed, whereas sorption to sediments was very strong and positively correlated with organic carbon content but not with alkyl chain length. This suggested that interaction with the organic matter is the primary sorption mechanism, although hydrophobic interaction cannot be the exclusive justification of this phenomenon. No correlation of partitioning with cation exchange capacity (CEC) of solids was proven, suggesting that electrostatic interaction is not a major sorption mechanism.

Matzke *et al.* examined the influence of enrichment of standard Lufa 2.2 soil in two types of clay minerals (2:1 and 1:1 type) and organic matter on adsorption of [OMIM][BF$_4$], [BMIM][BF$_4$] and [BMIM][CF$_3$SO$_3$]. Adsorption of all ILs on standard soil was between 30 and 50% and was higher for [OMIM] cation. Addition of 10-15% of smectite drastically increased retention of all ILs, in low concentrations it could hardly be detected in supernatant. The effect of kaolinite was ambiguous and did not show clear tendency. Sorption was decreased by approximately 20% for [OMMI] cation and increased by few per cent for [BMIM][BF$_4$]. Enrichment of Lufa 2.2 in organic matter was achieved by adding 5-10% flower soil (OM content 89%) and affected sorption more than kaolinite though less than smectite. Additionally less than 10% of [OMIM] was desorbed from all soils except those enriched with kaolinite. Remaining two ILs showed better recovery apart from samples where smecitite was present and desorption was negligible. Different influence of both minerals can be explained by differences in their structure as in the work by Bealieu *et al.*. Smectite, being swelling, 2:1 type clay has much higher specific surface and charge than 1:1 type, non-swelling kaolinite. Authors concluded that ionic interactions are more significant than dispersion interaction with organic matter [137].

Mrozik *et al.* tested adsorption of imidazolium and hydroxylated imidazolium ILs on costal soils covering vast OM and CEC spans. Well described in literature chain length dependence was observed here with [OMIM]$^+$ retained the most and [EMIM]$^+$ the least. Compounds containing terminal hydroxyl group in the side chain showed lower affinity to soils than those containing simple alkyl chain most probably due to increased polarity of the former allowing for more interaction with water [138]. In a later work, employing chemometric study Mrozik *et al.* concluded that CEC is the most important property of soils with respect to adsorption potential. Soils having high CEC, as well as relatively high OM content, were among the ones that retained ILs the most. Two of the soils used, particularly rich in organic matter, showed significant but not the highest K$_d$ [139].

Zhang *et al.* examined the adsorption of two imidazolium ILs onto model organic matter (Aldrich humic acid) in varying concentrations. Those ILs were shown to sorb strongly with partition coefficients between $10^{4.2}$ and $10^{4.6}$ for [OMIM][Cl] and $10^{4.3}$ and $10^{4.6}$ for [BMIM][Cl], so much higher than ever reported for natural soils. It was also stated that adsorption of [OMIM][Cl] correlated well with concentration of organic matter yet for [BMIM][Cl] relationship was less obvious and authors stated that at least in the case of latter other interactions (e.g. electrostatic) should be considered [140].

Paloamre examined adsorption of a series of imidazolium based ILs coupled with different anions onto activated carbon (AC). This sorbent showed higher affinity towards ILs than previously

reported for soils and sediments. Adsorption of [OMIM][PF$_6$] significantly decreased specific surface of AC and at 30% (w/w) load of IL the micropores were completely filled and the process continued by filling bigger pores (meso- and macropores). Regeneration of used AC with acetone proved to be efficient (90% of initial adsorption capacity and surface area was restored). Increasing affinity of ILs towards AC was observed with elongation of alkyl side chain. Similarly dependence of adsorption of anions on AC was based on increasing hydrophobicity [141].

The exact mechanism of ionic liquids sorption onto environmental surfaces has been the matter of assumptions since there is a lack of consistent data and many contradicting statements exist in literature. On the basis of cationic surfactants it was assumed that ILs are associating on solid/liquid interface mainly due to physisorption. Lungwitz and Spange claimed that 1-methylimidazolium chloride undergoes a physical sorption onto the surface of silica [142]. The existing sorption data for ILs have been focused on quantitative description of this phenomenon in order to establish, and later to predict, soil partition coefficients. Therefore, experimental data on the mechanism of ILs sorption available are scarce. Nevertheless an insight into this phenomenon can be obtained on the basis of cationic surfactants sorption data, due to structural similarities between ionic liquids and many cationic surfactants. Together with developing knowledge and availability of experimental techniques the adsorption models underwent a significant modification from a very simple one like one-step adsorption model to far more complicated like five-step adsorption model [107, 143]. Yet, no general model for ionic liquids or even surfactant sorption that would allow for the exact prediction of the structure and the mechanism of aggregates formation was proposed so far.

Sorption isotherm

The five-step adsorption model, as shown in Figure 19, was initially developed for cationic surfactants on charged cellulose fibres. In region I surfactant molecules are adsorbed due to electrostatic interactions, with hydrocarbon side chains lying flat on the surface to reduce the contact area with water molecules. At the end of this region the relatively high adsorption density prevents other molecules from adsorbing due to electrostatic repulsion forces between head-groups. The following rapid increase in the sorption isotherm is caused by conformational change from monomers lying flat on the surface to monomers oriented perpendicularly to the surface.
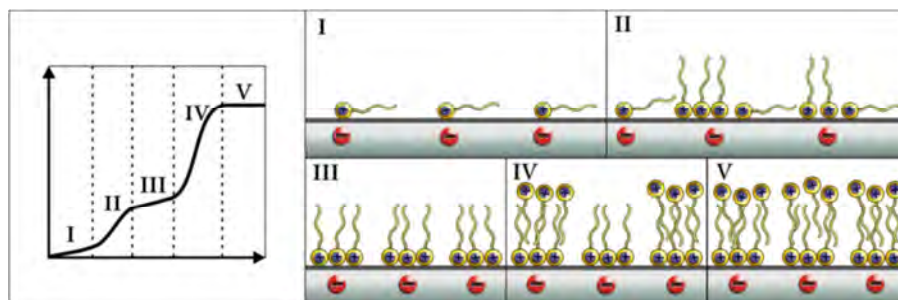
Figure 19. Five-step model of adsorption on negatively charged surfaces [107]

The contact area between water molecules and surfactants hydrocarbons is reduced by the aggregation of the latest. In region III slope of the isotherm decreases again due to high surface monolayer saturation and increasing electrostatic repulsion between surfactants head-group. The following region IV occurs when the bulk surfactant concentration is sufficient to overcome head-groups repulsive forces and allows for its association in bilayer structures. The last, region V is the plateau region where the full saturation of bilayer occurs. It is worth noticing, that with the increase of the sorbent surface charge density and/or hydrocarbon chain length the slopes in regions II and III become steeper. This can cause region II, III and IV of the isotherm to merge and become indistinguishable thus, at high charge density of the sorbent or for long chained surfactants only three-step isotherm can be observed (Figure 20).
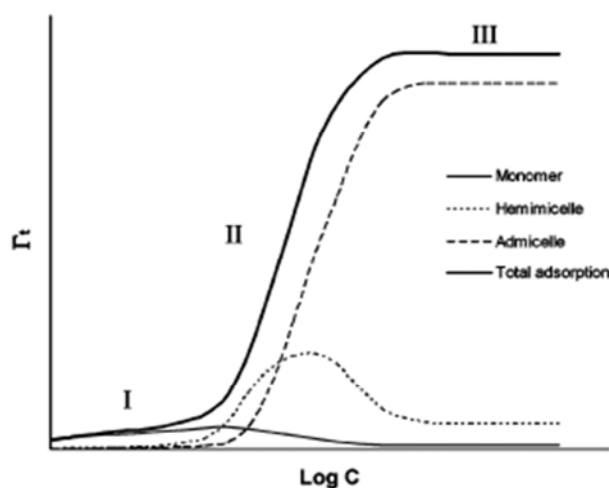
Figure 20. Three-step adsorption model with mono- and bilayer formation – hydrophobic interactions, high surface charge density or high ionic strength strongly promote aggregation, mono- and bilayers are formed simultaneously [107]

The addition of salt, so an increase of ionic strength of the solution, decreases the adsorption in the region I since both the surfactant head-group and the adsorption sites are screened by counter-ions, whereas in regions II, III and IV it has the aggregation-promoting effect

dues to reduction of electrostatic repulsion between surfactants head-groups. It is for the same reason why with the increase of ionic strength the transition from five- to three-region isotherm occurs [144]. When considering the side chain effect on the shape of the sorption isotherm the vertical part of the isotherm is shifted towards lower concentrations with the decrease of CMC values directly related to the length of the hydrocarbon chain. For short side chain surfactants there is a distinct separation between the first plateau of the isotherm attributed to electrostatic interactions and the second plateau believed to occur due to dispersive forces. For surfactants with longer hydrophobic chains first and second plateau merge because second layer formation starts before the first layer is completed.

4.3. Mobility

In natural conditions, water percolates through the porous structure of soil, bottom sediments or other solid media. As it moves the water will leach, transport and redistribute encountered natural or man-made species. The process is complex and involves sorption/desorption, advection, dispersion, (bio)degradation, precipitation, solubilisation and a multitude of other phenomena. As a result species (be they natural components of soils or xenobiotics) migrate and spread [145].

Phenomena that can mitigate the pollution of soil and water by ILs are sorption on solids and biodegradation. ILs sorbed on soil particles become immobilized (temporarily or permanently) and thereby less bioavailable. Sorption can prevent spreading into deeper layers of soil or groundwater.

Transport of ILs in soil columns was examined previously [138, 146, 147]. Mrozik *et al.* observed that short chained ILs were mobile in soils low in organic matter and the presence of terminal hydroxyl groups in side chain resulted in higher mobility. ILs containing longer alkyl substituents were retained strongly especially in soils rich in organic matter. It was concluded that upon entering terrestrial environments ILs will most probably be retained in the top soil and migration to deeper layers will be negligible [138]. Studzińska *et al.* came to a similar conclusions and additionally stated that leaching of ILs from soils inversely depends on the total organic carbon content of soil [146].

4.4. Biodegradation in soil

As discussed in chapter 3.2 there is a significant amount of data concerning biodegradation of imidazolium based ILs. In general, longer alkyl substituents (six and more carbon atoms) or side

chains with incorporated oxygen result in higher biodegradability [54-60, 65, 73]. In most biodegradation tests activated sewage sludge is used as an inoculum as it gives approximation of degradation during wastewater treatment. If ILs will emerge in soil environment their degradation might be different due to the differences in microbial composition as well as very nature of this biotope. Biodegradation of ILs by soil microbial communities was examined by Modelli *et al.* and degradation of 1-butyl-3-methylimidazolium cations ranging from 20 to 60% depending on anion was reported during a three month period [148].

4.5. Experimental approach

4.5.1. Sorption batch test

Sorption testing was performed according to OECD 106 'Adsorption-desorption using a batch equilibrium method' procedure [149]. Two grams of solid (soil/mineral/activated sewage sludge) was weigh into the test vessel and was equilibrated by shaking overnight with 0.01 M $CaCl_2$ solution. The IL stock solution was added to yield the final volume of 20 mL and the mixture was shaken for another 24 hours at room temperature, then centrifuged at 2000 g for 4 minutes to remove solids. The supernatant was recovered for HPLC/UV analysis. All experiments were performed in duplicate accompanied by blank and control samples to exclude sorption on the test vessel or degradation as potential factors influencing the final results. Amount of test substance adsorbed was calculated as a difference between initially added amount and amount remaining in solution at equilibrium. Sorption isotherms for each solid were plotted and solid/liquid partition coefficients were derived as the slope of the linear part of the isotherm.

4.5.2. Soil column test

Soil columns test was performed according to OCED 312 – Leaching in soil columns'. Cylindrical columns made of stainless steel 21 mm in diameter and 65 mm in length (internal volume 22.5 cm^3) were equipped with filters made of mineral wool to prevent flushing of solid. Columns were mounted vertically on stands and connected to multichannel peristaltic pump. Each column was initially equilibrated with 0.01 M $CaCl_2$ solution by flushing from top to bottom as long as conductivity of leachate measured using conductivity probe was equal to the conductivity of 0.01 M $CaCl_2$ solution.

Columns were filled with soil (35 g on dry mass basis) or soil/activated sewage sludge (35 g soil and 3.5 g of concentrated activated sewage sludge) or sand (35 g on dry mass basis) and

sand/activated (35 g sand and 3.5 g of concentrated activated sewage sludge). Activated sewage sludge was obtained from the aeration chamber of municipal wastewater treatment plant Delmenhorst, Germany. Sewage flocks were centrifuged at 2000g for five minutes and the supernatant was discarded. The remaining pellet (dry mass 42.7 g L^{-1}) was used for packing sewage amended columns. Columns were equilibrated with 0.01 M $CaCl_2$ solution.

Subsequently the feed was changed for IL solution and the direction of flushing was reversed and kept at the flow rate of 0.5 mL h^{-1} for 36 days. Samples of 15 mL volume were collected from each column and IL's concentration was analysed by HPLC/UV as described before. Additionally the composition of leachate was examined by LC/MS in order to check for possible metabolites. All columns were prepared in duplicate. After 36 days, IL solution was replaced with 0.01 M $CaCl_2$ solution and columns were flushed with a flow rate of approximately 10 mL h^{-1}, samples were collected from each column.

All collected samples were centrifuged at 2000g for 15 min in order to remove any remaining solids and transferred to 1 mL vials. Concentration of [OMIM][Cl] in samples was determined using the HPLC/UV VWR Hitachi system containing the L2130 pump, L2130 degasser, L2200 autosampler, L2300 column oven, L2450 diode array detector and the EZChrom Elite software. A cation exchange column (250/3 NUCLEOSIL 100-5 SA) purchased from Macherey-Nagel (Dürren, Germany) was used. The mobile phase consisted of 55% acetonitrile (HPLC grade) and 45% aqueous 20 mM KH_2PO_4 / 3.9 mM H_3PO_4 buffer. A flow rate of 0.7 mL min^{-1}, a temperature of 40°C and a detection wavelength of 212 nm were used.

4.5.3. Biodegradation by manometric respiromentry

The biodegradation experiment conditions were based on the OECD 301 F procedure 'Ready Biodegradability – Manometric Respiromentry' [24]. ILs stock solution was added to yield the concentration of 0.25 mM and 2.46 mL of raw municipal sewage was suspended in mineral medium composed of 8.5 mg L^{-1} KH_2PO_4, 21.75 mg L^{-1} K_2HPO_4, 22.13 mg L^{-1} $Na_2HPO_4 \cdot 2H_2O$, 1.7 mg L^{-1} NH_4Cl, 27.5 mg L^{-1} $CaCl_2$, 22.5 mg L^{-1}, $MgSO_4 \cdot 7H_2O$ and 0.25 mg L^{-1} $FeCl_3$ dissolved in water to give final volume of 246 mL. Test mixture was placed in a test bottle made of dark glass equipped with magnetic stirrer and gas-tight closed with manometric cap (Oxitop, WTW). Each test sample was run at least in triplicate and was accompanied by blank samples (to account for endogenous cellular breathing) and positive controls containing sodium glutamate in the same concentration as the test substance. Sodium hydroxide as a sorbent of carbon dioxide was used. Allylthiourea was added to each bottle as nitrification inhibitor. The temperature during the test was set at 20⁰C and controlled. Decrease in pressure inside the bottle caused by oxygen consumption was

measured, recalculated into biological oxygen demand (BOD) and recorded daily. BOD values from the bottles containing blank samples were deducted from BOD values from the bottles containing test sample and positive control according to the equation 2:

$$BOD = \frac{mg\ O_2\ uptake\ by\ the\ test\ compound - mg\ O_2\ uptake\ by\ the\ blank}{mg\ of\ test\ compound\ in\ the\ vessel}$$

(2)

Then % degradation was calculated by dividing BOD value obtained from equation (2) by theoretical oxygen demand (ThOD) of the test sample or positive control calculated according to equation 3.

$$\% \ degradation = \frac{BOD\ mg\ O_2/mg\ of\ test\ compound \cdot 100\%}{ThOD\ mg\ O_2/mg\ of\ test\ compound}$$

(3)

ThOD for each test sample was calculated as follows:

$$ThOD = \frac{\left[2C + \frac{1}{2}\left(H - Cl - 3N\right) + \frac{1}{2}Na - O\right]}{molecular\ mass} \left[\frac{mg\ O_2}{mg\ of\ test\ compound}\right]$$

(4)

4.5.4.   Biodegradation in soil columns – analysis of metabolites

Three samples from each column were selected and diluted 1:50 with methanol. Subsequently samples were analysed by electrospray ionization mass spectrometry equipped with an ion trap detector (Brucker-Daltonic GmbH, Germany). Mass spectra for cations were acquired in the positive ion mode in the scan range of $m/z+$ 50–400. The ESI source conditions were set according to [98] with a capillary voltage of 2000 V, drying gas flow-rate of 5 L min^{-1}, drying gas temperature at 300 °C and nebulizer at 50 psi.

4.6. Results

4.6.1.   Sorption isotherms

Sorption batch tests of [OMIM][Cl] were conducted according to OECD 106 procedure. Two soils: standard Lufa soil and Lindum soil, two minerals: quartz and kaolinite, and sand - which was treated as an intermediate between natural and pure system - were used. Adsorption was measured as a difference between initial concentration in the sample and aqueous concentration at equilibrium measured by HPLC/UV. Subsequently adsorption isotherms were plotted (Figure 21 to Figure 27). Two step sorption isotherm, characteristic for surface active agents, was observed.
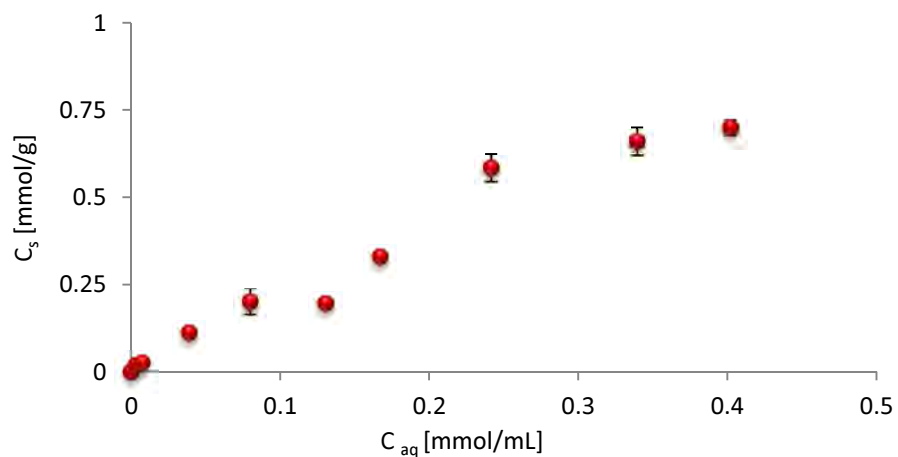
Figure 21. Sorption isotherms of [OMIM][Cl] on Lufa soil
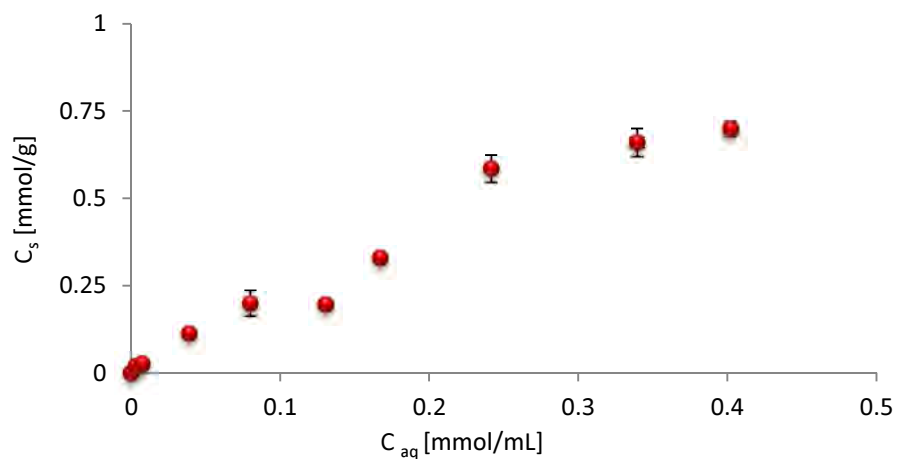
Figure 22. Sorption isotherms of [OMIM][Cl] on sand
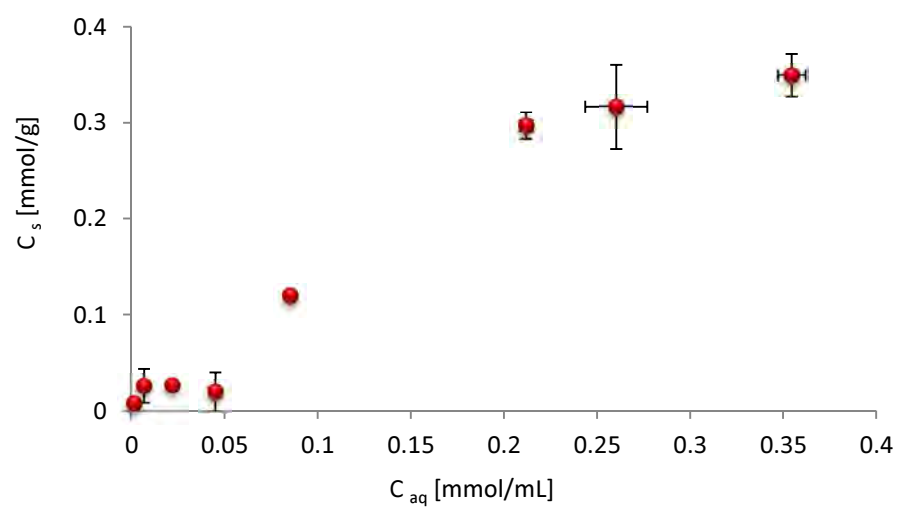
Figure 23. Sorption isotherm of [OMIM][Cl] on Lindum soil
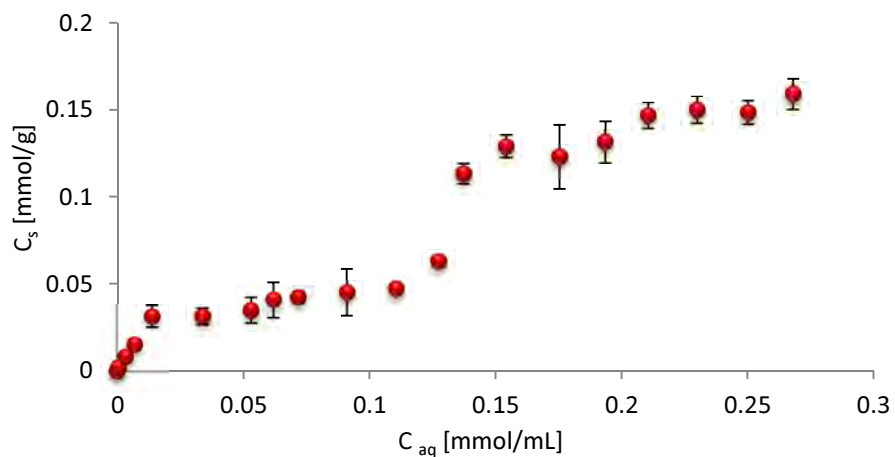
Figure 24. Sorption isotherm of [OMIM][Cl] on kaolinite
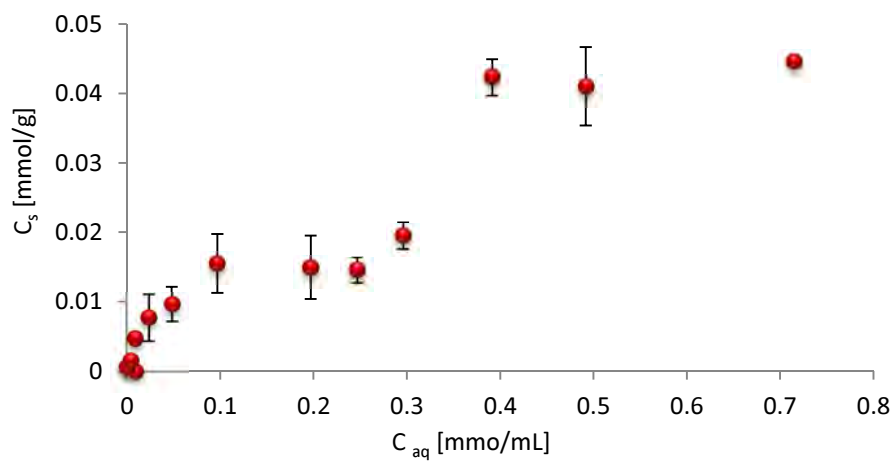
Figure 25. Sorption isotherm of [OMIM][Cl] on quartz
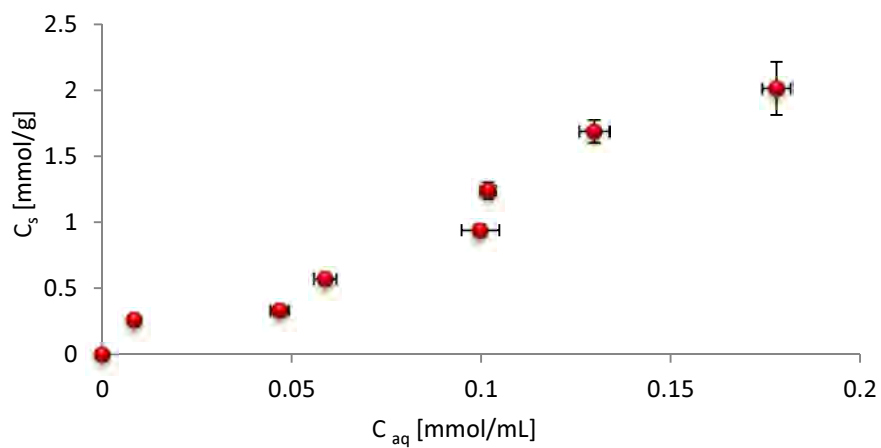
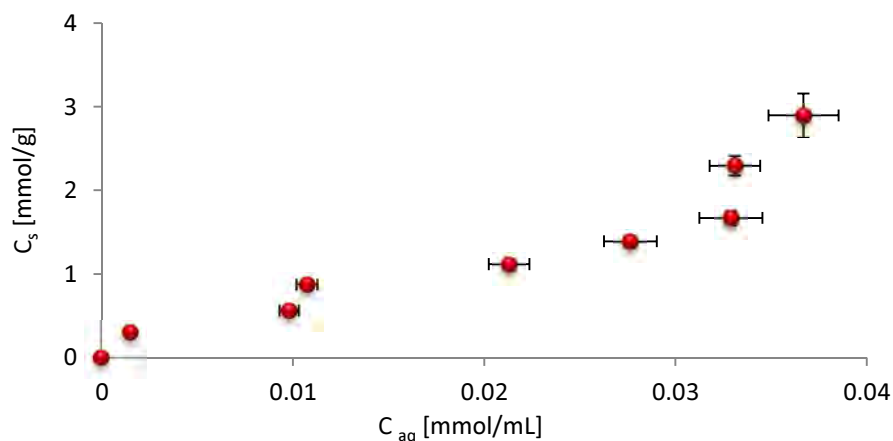Figure 26. Sorption isotherm of [BMIM][Br] on kaolinite

Figure 27. Sorption isotherm of [BMIM][Br] on quartz

Soils/solution partition coefficients were calculated from slopes of linear fragments of isotherms. Results are summarised in Table 3 together with basic parameters of soils influencing adsorption.

Table 3. Soil-water partition coefficients for 1-methyl-3-octylimidazolium chloride

K_{d1}/K_{d2} [mL/g]	OC [%]	CEC [meq/100g]
Lufa soil		
2.65	0.62±0.07	4.0±0.70
1.63		
Lindum soil		
3.46	2.5±0.04	3.24±0.36
1.51		
Sand		
2.47	0.12±0.05	2.78±0.37
1.81		
Kaolinite		
2.24	0	2.95±0.65
2.03		
Quartz		
0.195	0	1.74±0.45
0.199		

The K_{d1} corresponds to partitioning of IL between soil and solution, whereas the K_{d2} describes the sorption of the second layer of IL onto soil modified by the adsorption of the previous layer. The first K_d is noticeably larger than the second one, except for quartz. The

justification for this is that the first K_d accounts for both charge – charge and London dispersion interactions, and the second K_d only accounts for the London interaction between already adsorbed IL molecules and IL molecules forming second layer.Partition coefficients are the highest for soil and lower for minerals. The highest partition coefficient was observed for Lindum soil which has relatively high organic carbon (OC) content and CEC. Standard Lufa soil has higher CEC but much lower OC content and therefore lower K_{d1}. Relatively high K_d values for sand result from presence of trace amounts of organic matter as well as considerable CEC.
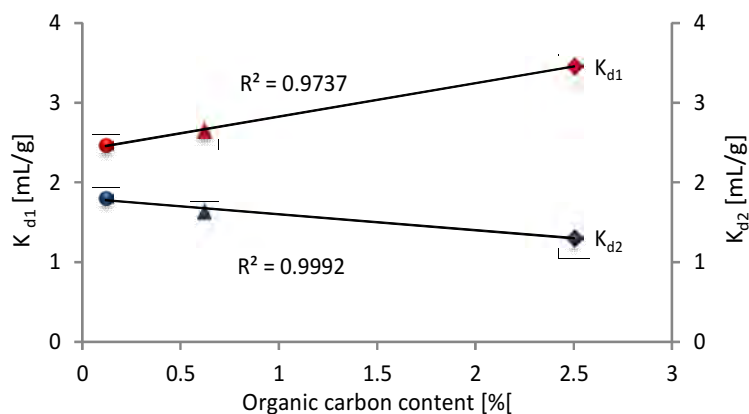
Figure 28. Correlation between soil/water partition coefficients and organic carbon content for [OMIM][Cl] and Lufa soil (triangles), Lindum soil (diamonds) and sand (circles); K_{d1} – red, K_{d2} - blue

Two natural soils (Lindum soil, Lufa soil) and sand showed positive correlation of K_{d1} with organic carbon content. Similarly organic carbon content correlated well but negatively with K_{d2} (Figure 28). During first layer formation on organic carbon rich soils ILs can adsorb both by charge-charge interactions and London dispersion forces as described in sections 4.1 and 4.2. Nevertheless it is believed that the more organic matter is present the more the mode of adsorption changes for the benefit of London forces, which means molecules adsorb tail-first as depicted on the right panel of Figure 29. The presence of head groups pointing towards solution renders soil positively charged and gives rise to repulsive forces between already adsorbed molecules and molecules freely dissolved in solution what may hinder further adsorption.
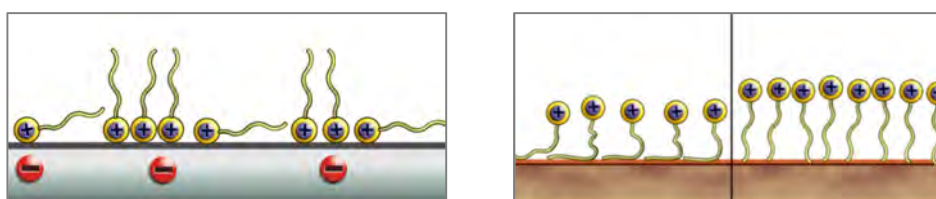
Figure 29. Model of ILs adsorption on OC-rich and OC-poor surface [107]

It was described earlier in sections 4.1 and 4.2 and will also be discussed later, that formation of the first layer on mineral surfaces in not solely electrostatically driven. ILs' molecules are forced to adsorb on solid surfaces in the same way they are forced to occupy e.g. water/air interface - by water molecules forming hydrogen bonds with each other. To gain the most energetically favourable state molecules will position themselves with side chains lying on the solid surface to minimise contact with water as it is shown on the left panel of Figure 29. This mechanism was perhaps underestimated before due to lack of experimental data, however it is theoretically viable and will be supported by examples.

For pure minerals (quartz and kolinite) and sand good, positive relationship bewteen K_{d1} and even better between K_{d2} and cation exchange capacity was found (Figure 30). ILs molecules forming first layer due to charge-charge interactions and London dispersion forces (2) render surface more hydrophobic. Clearly the more molecules are adsorbed in the first layer the more driving force for the formation of the second layer. There was no correlation of partition coefficients with CEC for natural soils and with OM for minerals and sand.
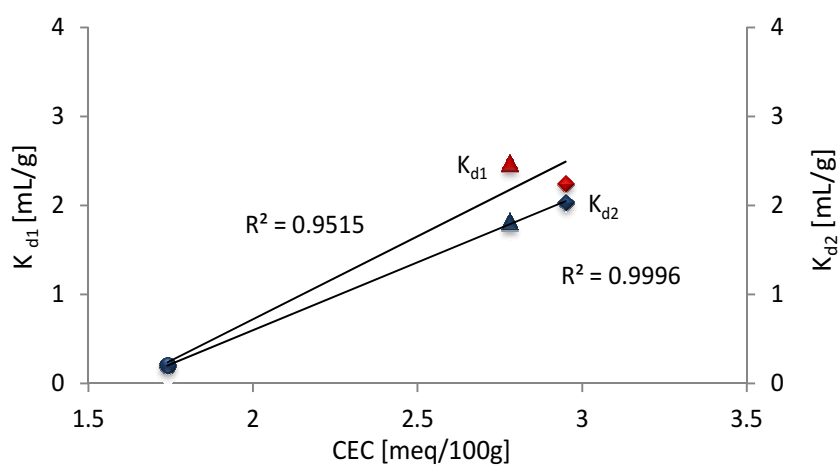
Figure 30. Correlation between mineral/water partition coefficients and cation exchange capacity for [OMIM][Cl] for quartz (circles), kaolinite (diamonds) and sand (triangles); K_{d1} – red, K_{d2} – blue

4.6.2.   Mobility and biodegradation in soil columns

A set of columns was packed with sand or Lufa soil to simulate natural, porous profile of soil. Another set of columns was packed with the same type of solid and additionally amended with dewatered activated sewage sludge (10% on the mass basis). The purpose of this is to

examine the usefulness of adding activated sewage sludge in order to: first enrich soil in organic matter and second diversify microbial community. Adding additional organic matter has a potential to increase sorptive capacity of soil, since it was shown previously that adsorption in soil correlates with amount of organic matter especially for more hydrophobic ILs [138]. It can therefore retard movement of IL into deeper layers. Diversifying composition of microbial community aims to provide organisms that might be able to degrade IL. A solution of [OMIM][Cl] was continuously pumped through all columns and leachate was collected and analysed for [OMIM][Cl]. In this way breakthrough curves (Figure 31 and Figure 32) were obtained showing how fast IL can move through porous bed of soil. The amount of flushing solution pumped through column was expressed as multiples of the pore volume (PV).
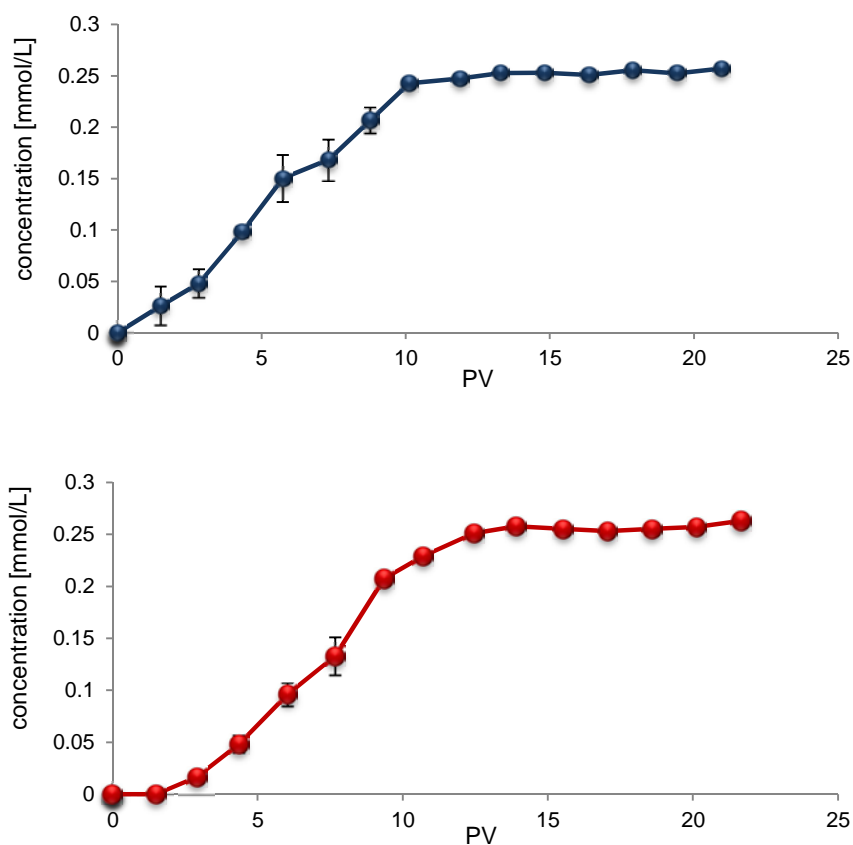
Figure 31. Breakthrough curves of [OMIM][Cl]: columns with Lufa soil (blue) and Lufa soil/sewage mixture (red)

The breakthrough occurs faster in columns filled with Lufa soil/sand only, as no lag is visible and concentration at the end of the column equals inlet concentration after 10.5 PV. For columns amended with sewage sludge lag of 2 PV occurs before ILs can be detected at the end of the column and maximum concentration is reached after 12.5 PV. A similar breakthrough pattern occurs in columns filled with sand or sand and sewage.
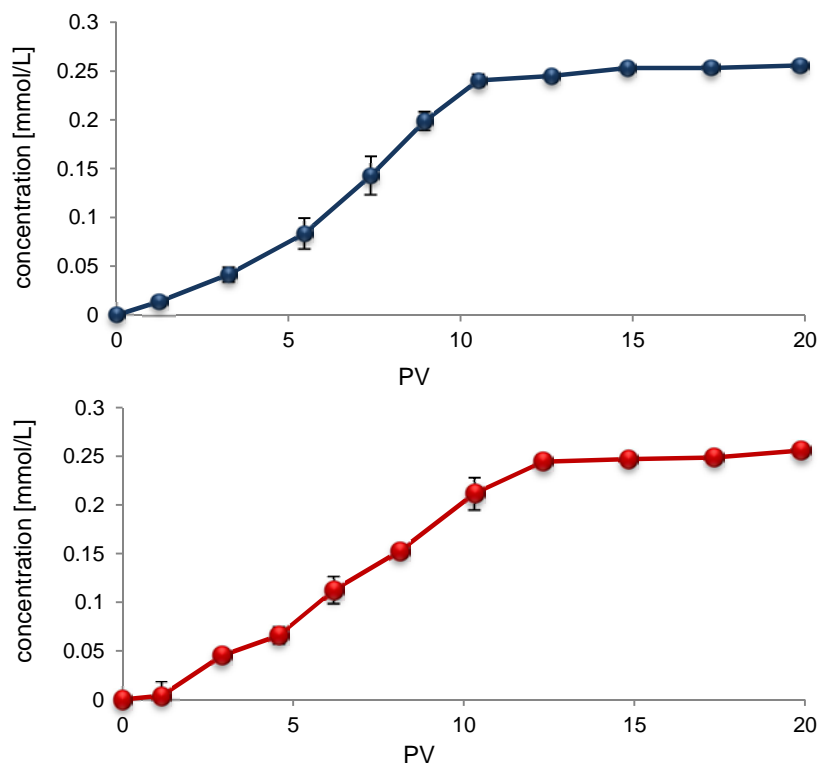
Figure 32. Breakthrough curves of [OMIM][Cl]: columns with sand (blue) and sand/sewage mixture (red)

After breakthrough of IL occurred the flushing solution was changed for 10 mM $CaCl_2$ to examine removal of IL from soil profile. Results are presented in Figure 33.
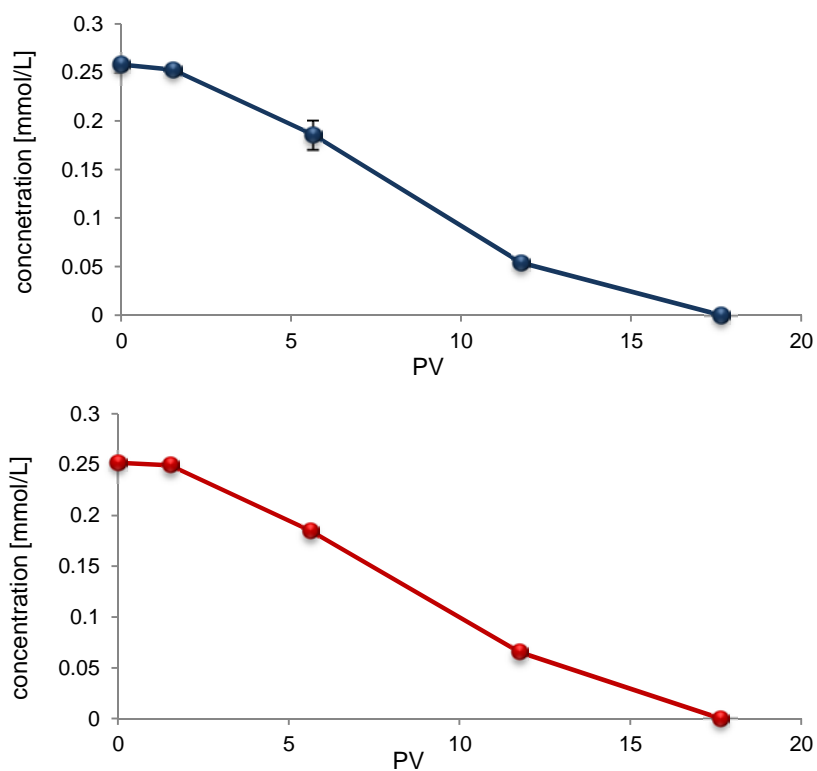
Figure 33. Desorption of [OMIM][Cl]: columns containing Lufa soil (blue) and Lufa soil/sewage mixture (red)

A slightly higher amount of artificial rainwater is required to flush IL out of soil pores in columns amended with sewage. [OMIM][Cl] is retained in all columns to a small extent. Based on mass balance about 97% (± 4%) and 80% (± 2%) of compound is removed from columns packed with Lufa soil and amended Lufa soil respectively.

4.6.3. Biodegradation by soil community

Biodegradation of [OMIM][Cl] and sodium glutamate by activated sludge and soil community is shown in Figure 34. Viability of both inocula was confirmed, as full biodegradation of sodium glutamate took place. Nevertheless, in samples containing activated sewage sludge sodium glutamate degradation was completed after three days and in soil derived community it lasted over twenty days. Degradation of IL proceeded much slower and was not completed within 60 days. The ILs degradation was lower for soil derived community and reached around 30% upon test termination. Biodegradation of [OMIM][Cl] exceeded 60% yet was still too low to classified as being readily biodegradable.

Based on the mass balance and considering recent investigations it is assumed that the biodegradation observed in the sewage microbial community was due to side chain degradation whereas the core structure remaining recalcitrant toward biodegradation [54, 56, 150].
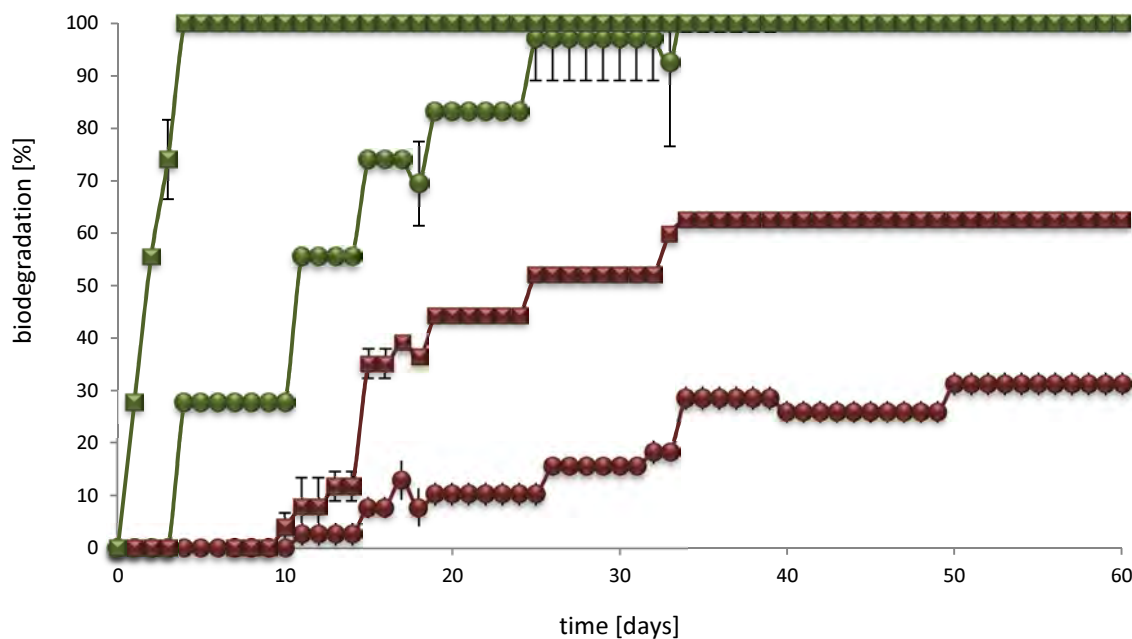
Figure 34. Ultimate biodegradation of [OMIM][Cl] conducted by soil (red circles) or activated sludge (red squares) and sodium glutamate conducted by soil (green circles) and activated sludge (green squares)

4.6.4. Biodegradation in soil columns – metabolites analysis

The leachate from the columns was in addition analysed by mass spectrometry, to detect possible metabolites. It was found that in all columns the level of the hydroxylated parent compound increased significantly (m/z^+ = 211). This indicates that both the indigenous bacteria and supplemented sewage bacteria are able to start to metabolize the [OMIM][Cl]. The degradation is of course not as efficient as that in the well mixed manometric respirometry biodegradation experiments, and therefore the same level of degradation cannot be expected. A number of other metabolites were detected (m/z^+ = 155, 169 and 185), which correspond to various hydroxylated and carboxylated imidazolium compounds, with shorter alkyl chains [54]. The intensities of these peaks however were small.

The difficulty to accurately determine and quantify (via HPLC) these metabolites lies in the fact that they, like the parent compound, continually sorb into the soil matrix. Therefore an accurate mass balance of the metabolites and parent compounds is not possible – unless the sorption coefficients of each metabolite are measured independently.

4.7. Summary – scientific novelty

In most natural soils organic matter content does not exceed 5% [119]. Nevertheless even such small amount considerably influences adsorption of [OMIM][Cl]. For soils poor in organic matter CEC proved to be a good indicator of soil/water partition coefficient. For soil rich in organic matter adsorption was driven by hydrophobicity. Only in systems lacking organic matter charge – charge interactions were playing significant role.

The transport of imidazolium type ILs coupled with simple anion in low organic matter soils will be relatively fast and only slightly hindered in soils amended with organic matter, unless it is present in relatively high amounts (higher than 10%). Those ILs will be retained on the surface of soil to small extend (5-20%) depending on OC content.

The biodegradation of [OMIM][Cl] in batch tests was shown to occur for both sewage and indigenous soil microbial communities. The soil bacterial communities, however, were less efficient – biodegradation was slower and reached lower percentage.

Metabolites were detected in columns containing both soil and sewage amended soil. Even though the contact time and oxygen availability were much more limited than in the case of batch test, biodegradation still occurred. Though in batch test IL was degraded by activated sewage sludge faster than by soil community the addition of sewage to the soil did not cause any observable difference (e.g. earlier appearance or higher amounts of metabolites). This might have

resulted from several limitations imposed by the soil column test. Limited mass transfer of IL to microbial cells as well as limited amounts of oxygen and lower cell density are expected to decrease the rate of degradation. Though metabolites of IL are generally less hydrophobic than the parent compound, and therefore are expected to have lower affinity for soil, they might have been sorbed preventing their detection.

One can speculate that in the case of release of [OMIM][Cl] to terrestrial environment due to insufficient removal during wastewater treatment or an accidental spill IL might be transported in soils to deeper layers or ground waters as it is fully soluble and was only slightly retarded by sorption on soil particles. Nevertheless the degradation in soils most probably will occur, although the rate will be lower that measured in batch biodegradation tests.

Results presented in this chapter were published as:

Jungnickel C., W. Mrozik, M. Markiewicz, J. Luczak, *Fate of ionic liquids in soils and sediments,* Current Organic Chemistry, 2011, 15(12), p: 1928-1945

5. Impact on surface chemistry of soil components

5.1. Background

Due to their toxicity, presence of ILs in soils is undesired and perceived as hazardous pollution. ILs possessing surface activity and positive charge in the core of cation exhibit behaviour similar to cationic surfactants. In aqueous environment surfactants exist as monomers only at low concentrations. Hydrophobic chain which cannot form hydrogen bonds breaks normal water structure in its surrounding increasing free energy of the system. Free energy is decreased again by minimizing hydrocarbon chain's contact with water. This is achieved by surfactants adsorption on solid surfaces (predominant at low concentrations) additionally aided by electrostatic interactions or by aggregation of surfactant's molecules into bulk or surface aggregates (predominant in higher concentrations). Aggregation occurs at specific threshold concentration known as critical micelle or critical aggregate concentration (CMC and CAC) [105, 143].

The core of surfactants micelle is lipophilic, therefore hydrophobic organic compounds (HOC) can partition into it and can be removed from soil matrix, as shown in Figure 35. Due to the ability to solubilize HOC surfactants are used in so called surfactant enhanced soil remediation. In this technology surfactant solution, above its CMC is pumped into soil, where it mobilises HOC, then recovered and treated. ILs as well as cationic surfactants are sorbed on soils to higher extent than ionic or non-ionic surfactants. Adsorption on soil results in significant losses and decreases the 'active' amount of surfactant.

In comparison to non-ionic surfactants cationic surfactants, also exhibit rather high CMC values resulting in high surfactant doses required to obtain effective solubilization. These phenomena not only decrease effectiveness of HOC solubilization shifting it towards higher surfactant concentration due to depletion of surfactant from soil solution but can also enhance HOC retention in soil by increasing its hydrophobicity. Therefore, cationic surfactants are not substances of first choice in soil remediation techniques such as soil washing or flushing [105]. However, the ability to retain and immobilize HOC in soil has become an alternative soil remediation strategy based on applying sorptive barriers [151, 152]. The surfactants used in soil remediation must be efficient, non-toxic and biodegradable. Imidazolium ILs cannot be classified as any of those which is another reason advocating against using them in soil remediation. Nevertheless, once present in soil ILs might be capable of solubilizing HOC and causing secondary pollution.
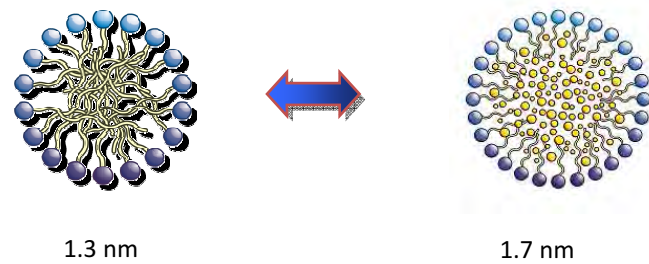
1.3 nm   1.7 nm

Figure 35. Schematic representation of micellar solubilisation of HOC inside IL micelles

Several processes are involved in HOC solubilization:

— surfactant sorption to soil and surface aggregate formation (hemimicelles)

— surfactant micellization in solution

— HOC sorption in soil

— HOC sorption on surfactant hemimicelles

— HOC solubilization inside of surfactant micelles [153]

Dissolved organic matter (DOM) present in soils can artificially increase apparent water solubility of HOC. Soil colloids can be perceived as hydrophobic carriers for HOC [152]. They are stabilized by their negative charge allowing them to stay suspended in solution. At low surfactant concentrations (well below CMC) adsorption of surfactants on DOM (having much higher surface area that bulk soil) is strongly increasing apparent aqueous solubility of HOC. However with increasing adsorption of cationic surfactants DOM losses its negative charge and dispersion stability and eventually precipitates resulting in no further increase in HOC aqueous solution concentration. At this stage solubilisation inside of micelles starts to play the main role in HOC release [154].

5.2. Impact on charge characteristics of mineral fraction

As previously described in section 4.1, most of natural minerals bear negative charge in neutral pH as a result of isomorphic substitution. Adsorption of ILs on the surface of minerals can change their properties dramatically.

When a solid is placed in water or in electrolyte solution the electrical double layer (EDL) is formed. The concept was introduced in XVIII century and underwent substantial conceptual changes since then evolving to what we now know as Gouy-Chapman-Stern model. This model

assumes that negatively charged particle is surrounded by positively charged ions compensating the charge. In the vicinity of the surface ions are held firmly forming so called Stern layer (further divided into the inner Helmholtz plane – IHP and the outer Helmholtz plane – OHP). Further toward solution the ions form a diffuse layer in which they are mobile. Both layers are divided by virtual boundary known as slipping plane or shear plane. The potential drops significantly within the Stern layer and more gradually within the diffuse layer. A potential measured at the slipping plane is known as zeta potential (ζ). The overall charge of double layer is equal to the charge of the particle and of opposite sign [155].
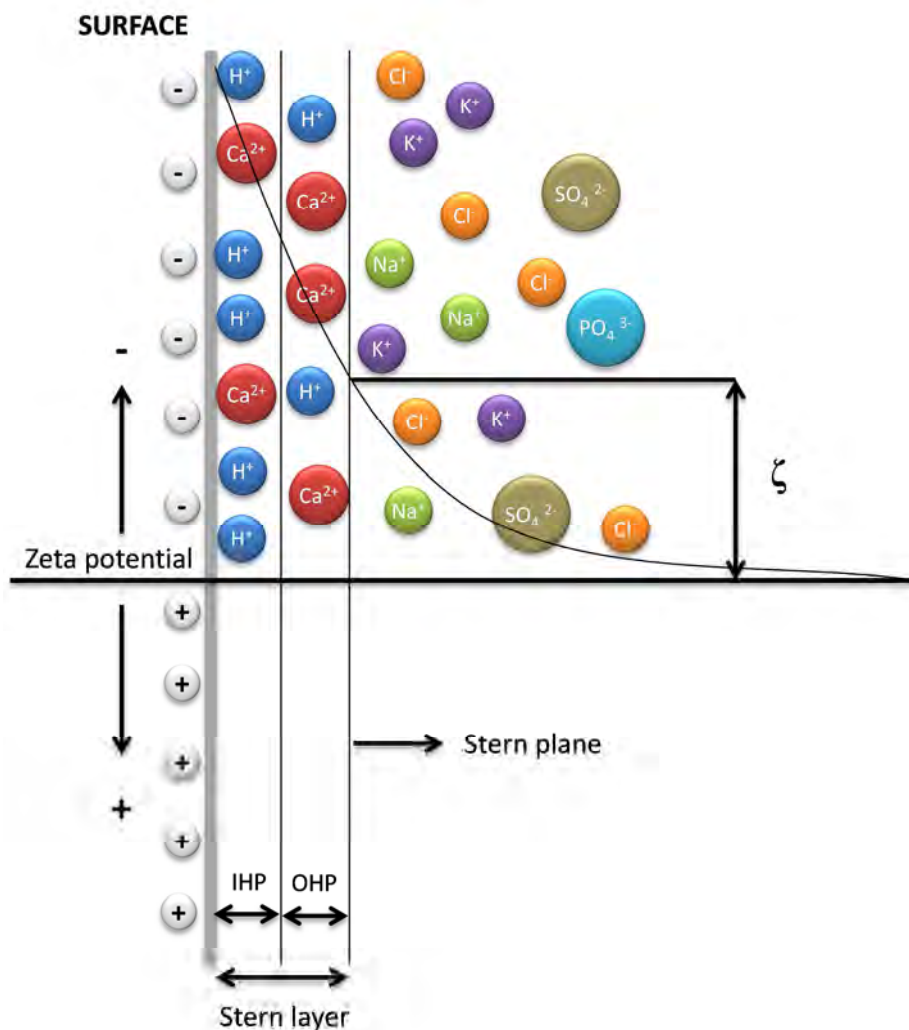
Figure 36. Schematic representation of electrical double layer - adapted from [156]

Colloidal systems are thought to be stable when the zeta potential of particles is smaller than -30 mV or greater than +30 mV [155]. A reduction of the negative charge of minerals can cause: destabilization of colloidal systems, decrease the affinity for nutrients and metals etc.

influencing agricultural utility of soil as well as performance of many technological processes employing suspensions of minerals. In order to understand the influence of ionic species on the ζ potential of solid surfaces it is important to distinguish between *indifferent ions*, *potential determining ions (PDI)* and *surface active electrolytes*. Indifferent ions are ionic species which can cause a change in the ζ potential bringing it close to zero. The only type of interaction between those ions and the surface is an electrostatic attraction which ceases to exist when the surface charge is compensated and thus does not allow for further adsorption of indifferent ions. On the contrary, potential determining ions are able to reverse the zeta potential of the electrical double layer (EDL) as they are interacting with the surface of the particle not only by electrostatic interaction [157]. Surface active electrolytes behave as indifferent ions, until a certain solution concentration is exceeded, and admicelles form on the surface, thereby altering the zeta potential.

5.3. Impact on organic matter in soils

Soil organic matter (SOM) is an organic fraction of soil comprised of (1) partially decomposed plant reside (containing carbohydrates, amino acids, fats, nucleic acids and lignins) and (2) humic matter or humus. The first fraction can be found in top horizons of soil and is transformed into humic matter by action of soil microorganisms and abiotic processes. Though very important for maintaining proper conditions in deeper horizons, dead residue is fairly chemically inert because of its low surface area. Humus is a heterogeneous, high molecular weight, acidic substance formed in the process of humification out of dead organic matter. Humic substances are traditionally divided into:

– humic acids (HA) – soluble in basic and insoluble in highly acidic solutions (pH 2-10)

– fulvic acids (FA) – soluble in both basic and acidic solutions

– humine – insoluble.

Humic matter is responsible for soil fertility and is an important part of soils sorption complex. Humic substances are organic macromolecules containing carboxyl, hydroxyl, phenolic functional groups that, at neutral or basic pH, ionize in water, releasing H^+ ions and providing negative charge centres to which cations are strongly attracted [119]. Humic and fulvic acids are of particular importance since they are very soluble in a broad pH range and can be therefore easily transported through soil profiles or in water as dissolved organic matter (DOM). Any chemical associated with DOM can be transported in soil while compounds associated with

particulate SOM are rather immobile. This may significantly impact environmental distribution parameters of xenobiotics such as: mobility, bioavailability and toxicity.

DOM interacts with SOM matter as the result of their hydrophobicity acting towards destabilization of the system; however, negative charge present in both DOM and particulate SOM causes mutual repulsion and acts towards stabilization of the system. Any disturbance of hydrophobicity or charge can shift this fine balance and cause precipitation or dissolution of DOM. Indeed, it was shown that adsorption of cationic surfactants can precipitate DOM [158]. If SOM is dissolved it is increasing apparent solubility of HOC in the aqueous phase. Yet precipitation of DOM causes simultaneous precipitation of HOC [152].

Imidazolium ILs were shown to sorb strongly onto model DOM with partition coefficients between $10^{4.2}$ and $10^{4.6}$ for 1-methyl-3-octylimidazolium chloride and $10^{4.3}$ and $10^{4.6}$ for 1-butyl-3-methylimidazolium chloride [140], which is orders of magnitude greater than partition coefficients normally found in natural soils or minerals. It can, therefore, be anticipated that ILs can influence the properties of soil organic matter.

5.4. Solubilisation of hydrophobic organic compounds

As mentioned previously, surfactants can cause solubilisation or precipitation of DOM and any other compounds associated with it [152, 159]. Evidence for both increased and decreased adsorption of HOC in soils treated with surfactants can be found in literature. It was suggested that whether one or the other occurs depends on structure and concentration of surfactant, properties of soil and HOC as well as on the order in which they contact soil [105]. It was reported that treating soil with low surfactant concentrations prior to addition of HOC decreases adsorption of the latter probably by reducing the number of adsorption sites. When the HOC is added to soil after surfactant the adsorption of HOC increased with surfactant load probably because adsorption sites were occupied by PAH and additional partitioning into surfactant hemimicelles occurred. At high surfactants concentrations adsorption of HOC decreased regardless of the order in which it was added due to solubilisation [160].

It was shown before that surfactants solubilise HOC mostly above CMC by incorporating them inside of micelles as described in Figure 35 [105, 160, 161]. The ability of surfactant to solubilise HOC is described by so called molar solubilisation ratio (MSR) defined as a ratio of the moles of HOC solubilised to the moles of surfactant present as micelles. It was shown that MSR decreased with increasing size of HOC and increase with increasing size of hydrophobic part of surfactant molecule [161]. Similar phenomenon was observed for ionic liquids. For small

molecular weight PAHs (naphthalene, acenaphthene and fluorene) micelle/water distribution ratio was increasing with elongation of alkyl substituent of imidazolium IL. For high molecular weight PAHs no such increase was observed and an growing discrepancy between those distribution ratios and octanol/water partition coefficient were observed [162]. Decyl- and tetradecyl substituted imidazolium ILs caused increased apparent water solubility of toluene, xylene, decane, dodacane and tetradecane above CMC (56.8 mM and 3.26 mM respectively, measured in pure water). Micellar solubilisation was confirmed by increased size of micelles in pure water and in water/HOC mixture [163]. Researches concerning solubilisation of HOC in ILs have been conducted in pure water up to date. No data pertaining to the release of HOC adsorbed in soils are available.

5.5. Experimental approach

5.5.1. Zeta potential determination

Zeta potential of mineral suspensions were obtained using Dispersion Technologies Acoustic and Electroacustic Spectrometer (DT1200, Bedford Hills, USA) equipped with titration unit, pH, conductivity and electroacustic probes (sound wave frequency 3MHz).

Samples for zeta potential determination were prepared by suspending 16 g of mineral (quartz or kaolinite) in 100 mL of solution containing IL in concentrations from 0-500 mM with a background electrolyte (KCl or $CaCl_2$) and equilibrating for 24 hours. The influence of background electrolyte on the zeta potential of mineral surfaces was examined. Two salts, bearing mono- and divalent cations in two concentrations were tested. Calcium chloride in concentration of 10 mM was chosen as a starting point as it is the recommended background electrolyte for the OECD 106 standard 'Adsorption-desorption using the batch equilibrium method'. Since, divalent ions are usually considered to have a greater influence on the zeta potential, we also used a monovalent salt (KCl) for comparison. The concentration used was double that of $CaCl_2$, thereby maintaining equal number of positive charges. Each sample was split into two subsamples one of which was titrated with acid (1M HCl) and the other with base (1M KOH). Curves of zeta potential versus pH were recorded.

When the sound wave travels through a dispersion of particles whose density differs from that of the surrounding medium, inertial forces induced by the vibration cause a motion of the charged particles relative to the liquid, causing an alternating electromotive force. This force can be measured as colloid vibration potential (CVP).

CVP was measured during each titration run and dynamic electrophoretic mobility was calculated according to equation 5 based on concepts developed by Dukhin, Goetz and O'Brien [164, 165].

$$\mu_e = \frac{\kappa^* \cdot \rho_m}{\rho_p - \rho_m} \cdot \frac{Z_g + Z_s}{Z_g \cdot Z_s} \cdot \frac{CVP}{C\varphi}$$

(5)

where:

μ_e - dynamic electrophoretic mobility [$m^2 s^{-1} V^{-1}$]

κ^* - complex conductivity of dispersed system [$S m^{-1}$]

φ – volume fraction

ρ_m – density of medium [$kg\, m^{-3}$]

ρ_p – density of suspended particles [$kg\, m^{-3}$]

Z_g – acoustic impedance of sound transducer [$N\, s\, m^{-3}$]

Z_s – acoustic impedance of dispersed system [$N\, s\, m^{-3}$]

CVP – colloid vibration potential [V]

C – calibration constant

Subsequently zeta potential was derived according to the equation 6

$$\zeta = \frac{3\eta\mu_e}{2\varepsilon\varepsilon_0 \cdot f(\kappa a)}$$

(6)

where:

ζ – zeta potential [V]

$f(\kappa a)$ – Henry's function (1 to 1.5)

η – viscosity of the medium [Pa s]

ε – electric permittivity of medium [$F\, m^{-1}$]

ε_0 – electric permittivity of vacuum [$F\, m^{-1}$]

μ_e – dynamic electrophoretic mobility [$m^2 s^{-1} V^{-1}$]

As the result series of pH versus zeta potential curves for each mineral and each IL were obtained. The average particle size of each mineral was measured using the same technique. Surface area of minerals was measured using nitrogen vapour adsorption BET method.

Crystalline quartz SIKRON SF 800 was obtained from Quarzwerke Gruppe (Frechen, Germany) and 'GHL BL' kaolinite from P.P.U. Standard (Lublin, Poland). The surface area of the

minerals was determined by BET nitrogen vapour adsorption method using a Gemini V Surface Analyzer (Micrometrics, Norcross, USA). Cation exchange capacity was determined by standard compulsive $BaCl_2$ exchange method according to Ross [166].

5.5.2. Organic matter solubilisation/precipitation

2 g of Lindum soil coming from Lindum waste storage facility (Oslo, Norway) and therefore named 'Lindum soil' were weighted into each test vial. Then 20 mL of [OMIM][Cl] solution in concentrations of 0 mM, 2.5 mM, 10 mM, 25 mM, 50 mM together with $CaCl_2$ to yield concentration of 10mM were added and shaken for 48 hours. Subsequently solids were centrifuged, samples of supernatant were diluted 1:100 with demineralised water and total organic carbon content was measured using organic carbon analyser LiquiTOC, Elementar with platinum catalyst.

5.5.3. PAH solubilisation experiments

2 g of Lindum soil, contaminated with PAH, coming from Lindum waste storage facility (Oslo, Norway) were weighted into test vials. Then 20 mL of [OMIM][Cl] solution in concentrations of 0 mM, 2.5 mM, 10 mM, 25mM, 50mM, 100mM, 250 mM, 300 mM or 400 mM was added. Additionally 0.1 g passive sampler made of polioxymethylene of 55 µm thickness was inserted into each test vial. Samples were closed and shaken for 30 days. After equilibration, solids were centrifuged and samples were taken from each test vial for HPLC/UV analysis of the concentration of IL. Remaining water phase was spiked with mixture of deuterated PAH – to serve as extraction efficiency standard – and extracted with 20 mL of hexane. Passive samplers were removed, placed in 20 mL of 80:20 hexane:acetone mixture spiked with mixture of deuterated PAH - as extraction efficiency standard and shaken for 30 days. Both extracts were vacuum centrifuged to reduce the volume to approximately 0.5 mL and concentration of PAH in extracts was measured using GC/MS.

Agilent 6850 Gas Chromatograph equipped with a Agilent DB-XLB Column (30 m $\times$ 0.25 mm and 0.1 µm film thickness, TeknoLab, Norway) was used. A carrier gas flow was set to 1 mL min^{-1} and the following temperature programme was used: 2 min at 50 °C, to 150 °C at 10°C min^{-1}, to 280 °C at 5 °C min^{-1}, 9 min at 280 °C, to 310 °C with 40 °C min^{-1}, at 310 °C for 8 min. Detection was performed with an Agilent 5973 mass spectrometer in the electron impact mode with a 70 eV ionisation energy and a dwelling time of 25 ms. Identification of the PAH was assured by using two compound-specific ions: a quantifier ion corresponding to the respective molecular weight (m/z=M+) and a qualifier ion ([M-2H]$^+$ for analytes and [M-2D]$^+$ for internal standards) with

a mass ratio similar to the one determined in the calibration. Four external calibration standards (1 ng mL^{-1} to 1 µg mL^{-1}) containing constant amounts of internal standards (20 ng mL^{-1}) were run before each series. Internal standards included: d8-Naphthalen, d10-Phenanthrene, d10-Pyrene, d12-Benz(a)anthracene, d12-Benzo(a)pyrene, d12-Benzo(ghi)perylene.

5.6. Results

5.6.1. Zeta potential

Quartz and kaolinite were chosen as sorbents since they are abundant in soils, well characterized and have many technological applications. Properties important for analysing zeta potential are listed in Table 4.

Table 4. Summary of basic properties of kaolinite and quartz (with standard errors)

Parameter	Quartz	Kaolinite
Mean size [nm]	450 ± 19	154 ± 8
Surface area [m^2/g]	4.03 ± 0.39	5.05 ± 0.88
Cation exchange capacity [meq/100g]	1.74 ± 0.45	2.95 ± 0.65

As described in subchapter 4.6.1 solid/liquid partition coefficients of [OMIM][Cl] were an order of magnitude higher for kaolinite than for quartz. Similarly, the first plateau region on quartz is at lower surface excess (Figure 24 and Figure 25). This indicates that there is a higher affinity of IL towards kaolinite which is consistent with higher CEC and surface area. Additionally, the first isotherm step corresponding to monolayer saturation of quartz is located below the mean CEC value, which is to be expected as some of negative sites will be occupied by other cations present in solution (background electrolyte cations). For kaolinite, however, the first plateau region exceeds the CEC suggesting that further aggregation proceeds but is not electrostatically driven.

A significant, gradual decrease in pH of kaolinite suspensions with increasing concentration of [OMIM][Cl] (from 0 to 320 mM) suggests that IL replaces protons at the surface of mineral, displacing them into solution.

Charge reversal of kaolinite due to adsorption of [OMIM][Cl] occurs for approximately 40 mM and 22 mM IL solution in KCl and CaCl$_2$, respectively. Reversal of the zeta potential of quartz takes place at slightly lower concentrations (approximately 23 mM of IL in KCl and 18 mM of IL for

CaCl$_2$).The charge reversal for both minerals almost ideally overlapped with CECs, nevertheless, the first layer was not saturated for kaolinite at this point.
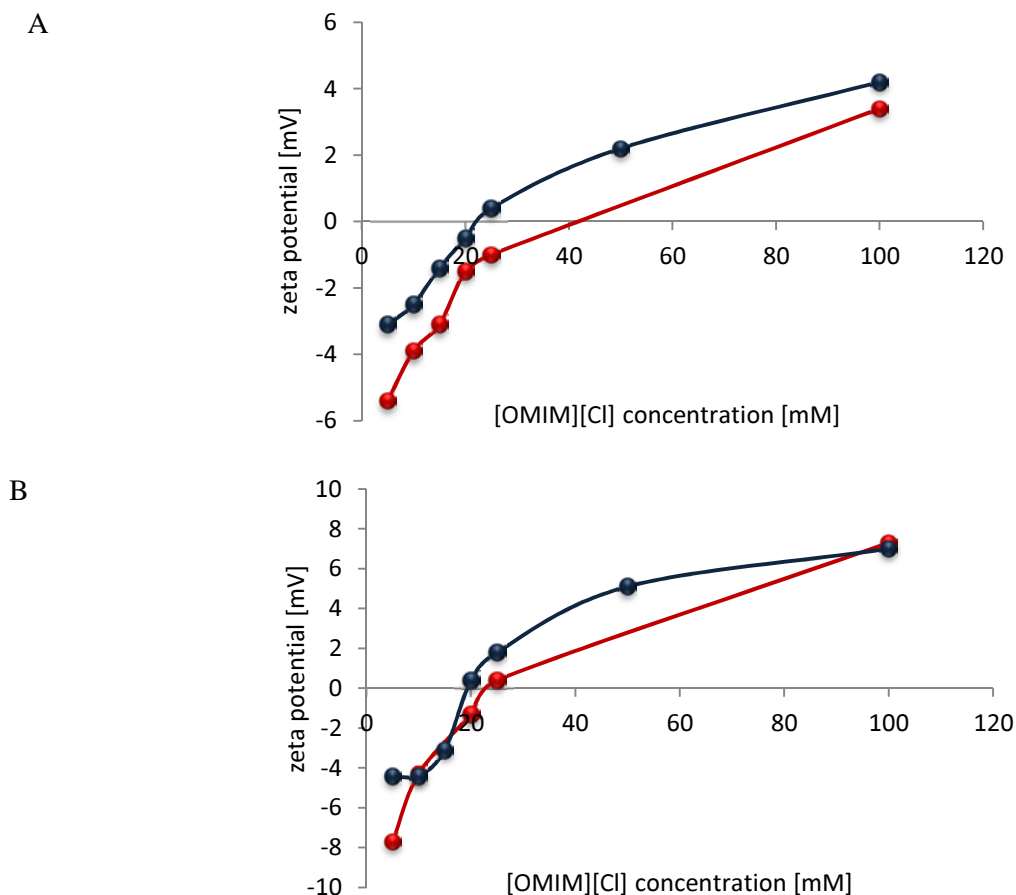
Figure 37. Changes of zeta potential of kaolinite (A) and quartz (B) due to adsorption of increasing concentrations of [OMIM][Cl] with 10 mM KCl (red) and 20 mM CaCl$_2$ (blue) as background electrolytes

The further aggregation, possibly due to interactions between alkyl chains of imidazolium substituents, continued before the second layer formation begun. Therefore, the adsorption of [OMIM][Cl] on mineral surfaces can be explained by the 'four-region model' describes in [107, 143]. The application of 'four-region model' in current work is presented in Figure 38. In the region I IL is electrostatically adsorbed with the imidazolium headgroup in contact with the mineral and the alkyl chain either pointed towards bulk solution or, more probably, lying flat on the surface. In region II, characterized by a smaller slope for kaolinite and almost horizontal line for quartz, lateral interactions between alkyl chains promote formation of hemimicelles. A difference in shape of both isotherms is caused by the fact that more molecules are adsorbed in the first layer on kaolinite due to higher CEC presenting more possibilities of lateral interactions, this is additionally supported by the fact that at this point zeta potential is already reversed so

interactions other than electrostatic must be involved. Further increase in ILs concentration, above CAC, causes molecules to interact tail-to-tail with head groups pointing towards solution.
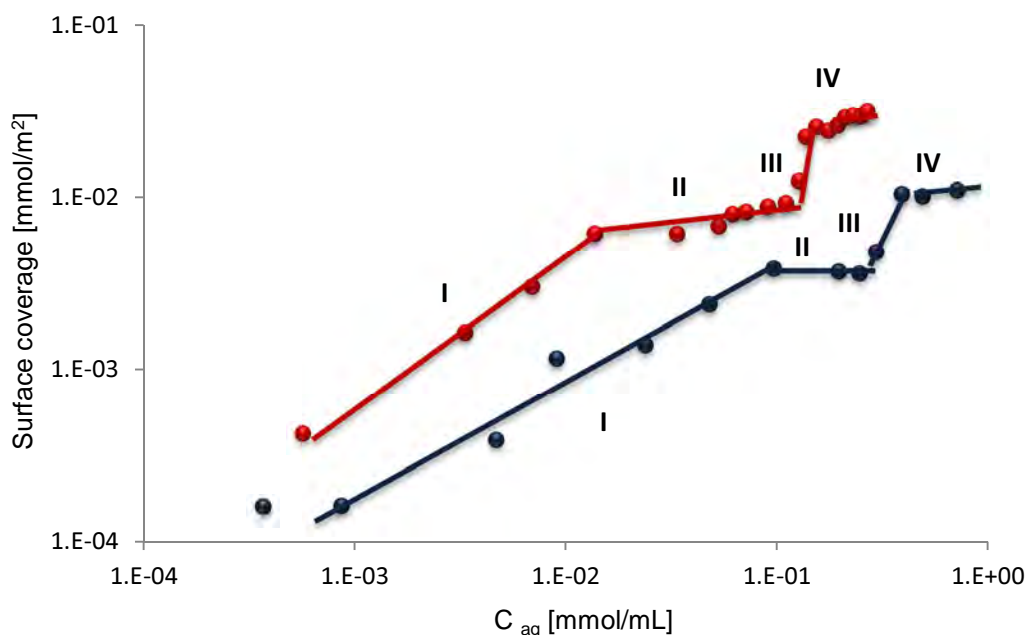
Figure 38. 'Four-region model' applied to [OMIM][Cl] on kaolinite (closed symbols) and quartz (open symbols) adsorption isotherms.

The original 'four-region model' assumed charge neutralization at the transition from second to third region, in our case this occurred earlier. In the last region no further increase in the amount adsorbed can be observed and the aggregates form a full bilayer. In our experiment, region II occurred at lower surface coverage for quartz, as the number of charge interaction sites is lower, which in turn caused region IV to appear earlier since the second layer is built on the first and therefore limited.

Though charge densities of calcium and potassium ions are much higher than of the imidazolium cation the influence of the latter on zeta potential of minerals is substantial. This is a result of the presence of hydrophobic substituents on the imidazolium ring which facilitate coverage of minerals' surface with IL molecules and cause considerable changes in zeta potential. It is important to notice that charge reversal in $CaCl_2$ environment occurs at somewhat lower IL concentration than the first plateau region on the adsorption isotherm, as Ca^{2+} cations present in solution are also able to cause charge reversal. The fact that adsorption of the first layer proceeds beyond this point suggests that interactions other than electrostatic must, therefore, be involved.

Zeta potential of kaolinite with a background electrolyte

The zeta potential of kaolinite in demineralised water was measured to be -78mV at pH 6.9, and decreased as pH increased (Figure 39). This phenomenon was observed before and is attributed to deprotonation of surface silanol groups or adsorption of OH- [167]. As expected, the electric double layer was more suppressed in calcium chloride solution than in potassium chloride solution having the same ionic strength, yet none of them reached isoelectric point. In order to examine if any of these salts behave like potential determining ions (PDI) concentrations of both salts were increased by the same factor, to 50mM (for $CaCl_2$) and 100 mM (for KCl). The zeta potential was reversed for calcium but not for potassium chloride, thereby confirming that divalent Ca^{2+} ions are potential determining ions and monovalent K^+ ions can be treated as indifferent ions [167-169]. No isoelectric point (IEP) was observed for kaolinite in water, as well as all KCl and 10 mM $CaCl_2$ solutions. However, IEP at approximately pH 10.5 was observed for 50 mM $CaCl_2$.
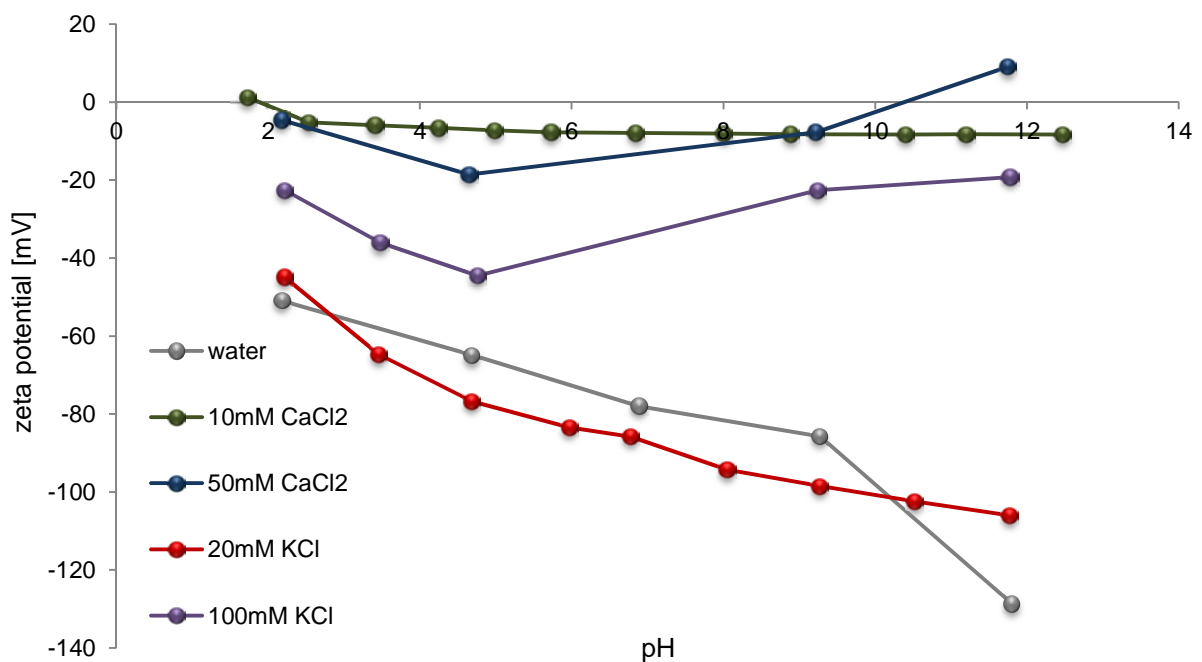
Figure 39. Zeta potential of kaolinite vs. pH for in $CaCl_2$ and KCl

Changes of the ζ potential of minerals with type of background electrolyte having different valences follow the Hardy-Schultze rule which originates from colloidal science and states that the ability of the electrolyte to coagulate colloidal system is proportional to the

valence of the electrolyte. It is believed that di- and trivalent cations can be specifically adsorbed on the surface of minerals (in the inner Helmholtz plane) causing charge reversal. Additionally, hydrated ions present in the outer Helmholtz plane can also increase zeta potential by compressing the EDL [156, 170]. One can deduce that divalent ions will reduce the double layer thickness more than monovalent ions, and trivalent ions will have an even more significant effect (given that they are all present in the same concentrations) causing a sharper potential drop across Stern layer [171, 172].

Zeta potential of kaolinite in [OMIM][Cl]/CaCl$_2$

Addition of [OMIM][Cl] caused a gradual change in zeta potential of kaolinite, until it became positive within the whole pH range of measurement in 25 mM [OMIM][Cl] solution (Figure 40). A maximum value of around 4 mV was recorded in 100-150 mM [OMIM][Cl] solution whereupon it started to decrease again when concentration of IL increases. This is the effect of EDL compression resulting from a very high ionic strength of the solution.
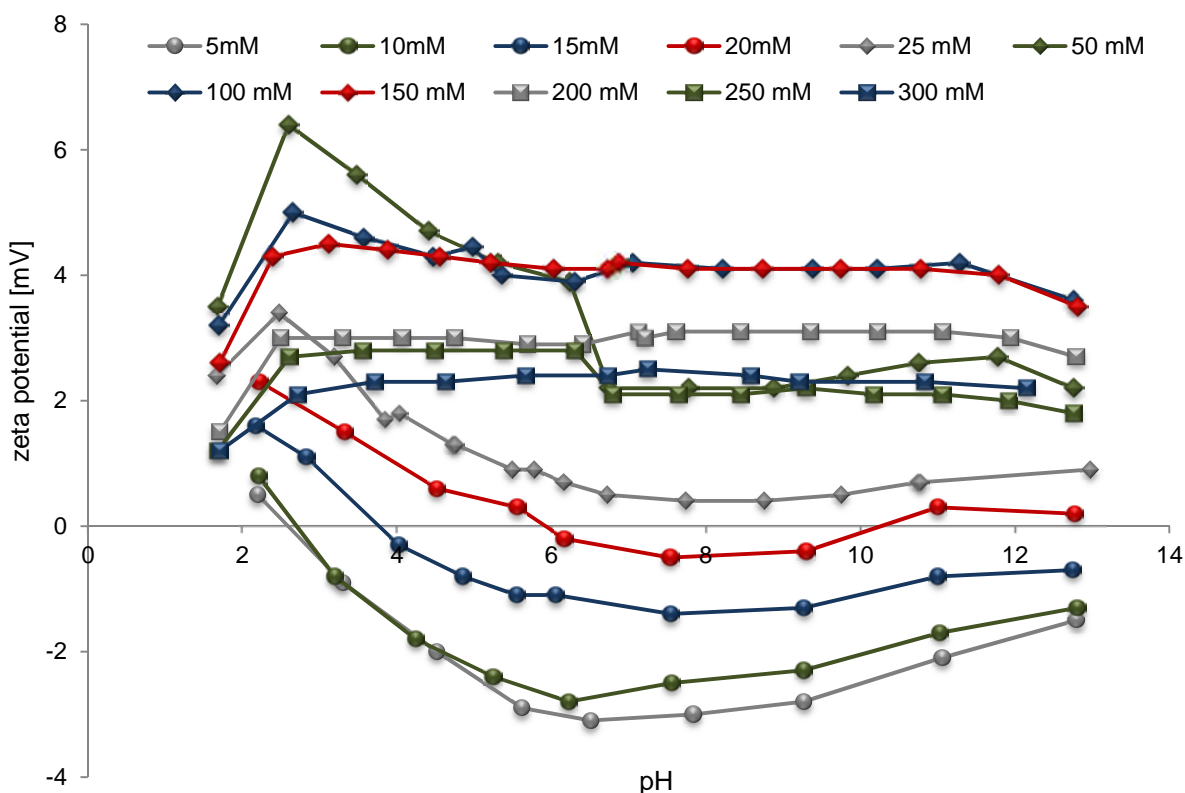
Figure 40. Zeta potential of kaolinite in 10 mM CaCl$_2$ at different concentrations of [OMIM][Cl]

Zeta potential of quartz in [OMIM][Cl]/CaCl$_2$

Quartz in solutions of [OMIM][Cl] of the same concentration has comparable zeta potential values as kaolinite (Figure 41). The ζ potential is reversed for 25 mM [OMIM][Cl] solution, reaches maximum value of approximately 8 mV in 150-200 mM [OMIM][Cl] solution and additional amounts of IL cause it to decrease again. Reversal of ζ at slightly lower IL concentrations for quartz is to be expected as it has lower surface area and lower CEC.
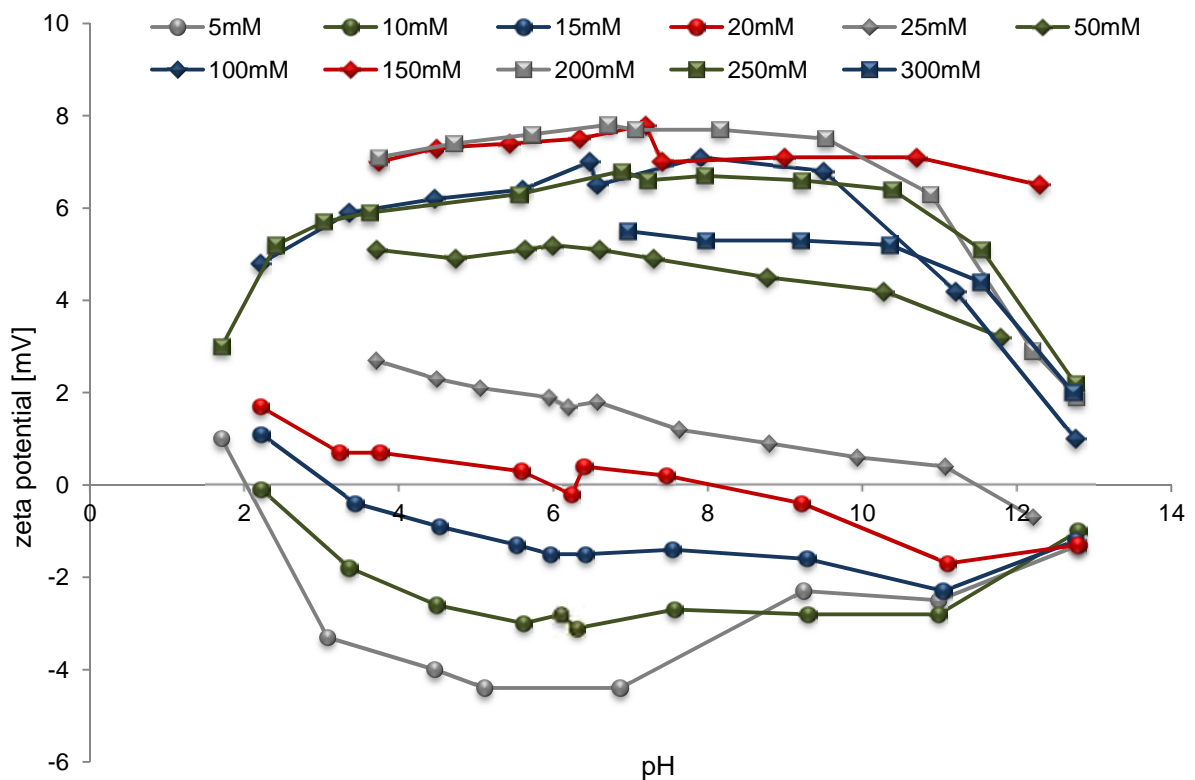
Figure 41. Zeta potential of quartz in 10 mM CaCl$_2$ at different concentrations of [OMIM][Cl]

The decrease of zeta potential at elevated pH values might be caused by the reaction of dissolved calcium cations with OH$^-$ groups to form calcium hydroxide which in turn removes some Ca^{2+} ions from solution.

Zeta potential of kaolinite in [OMIM][Cl]/KCl

With potassium chloride as a background electrolyte zeta potential reversal occurs later than with calcium chloride, and ζ values are lower than for corresponding samples in CaCl$_2$ (Figure 42). In 25 mM IL solution ζ potential of kaolinite becomes positive at pH ≤5 and stays positive in

the whole range in 100mM solution. This is consistent with results obtained for both minerals and $CaCl_2$ and results from higher CEC of kaolinite.
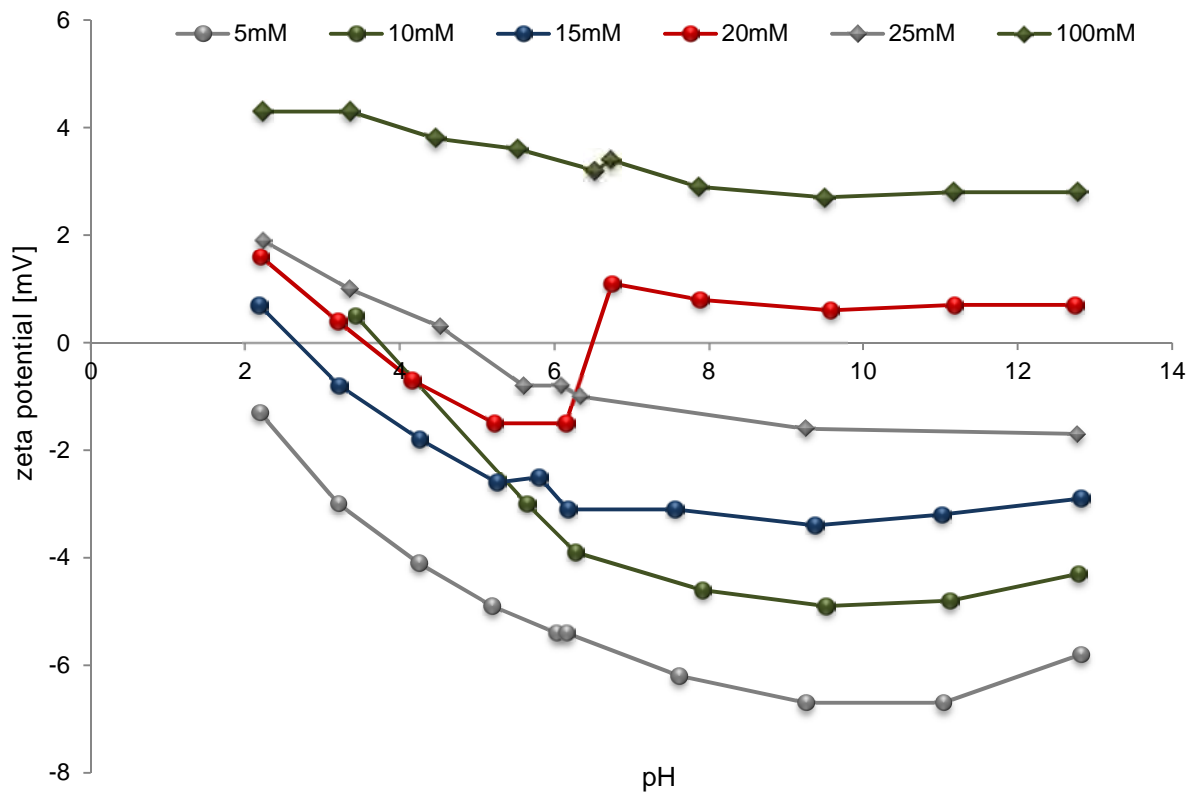
Figure 42. Zeta potential of kaolinite in 20 mM KCl at different concentrations of [OMIM][Cl]

Zeta potential of quartz in [OMIM][Cl]/KCl

As in the case of kaolinite, change in ζ potential of quartz is smaller in KCl. In 25mM [OMIM][Cl] solution ζ potential becomes positive in acidic and neutral solution and eventually reverses in 100 mM IL solution (Figure 43). The ζ potential titration curves have different shape in KCl solution and a continuous increase of the ζ potential is observed as pH changes from very basic to very acidic. A less prominent influence of pH was observed for $CaCl_2$.

The adsorption of protons on mineral surface is influencing ζ potentials in all cases as protons were previously shown to be potential determining ions. Nevertheless, this influence is 'screened' in $CaCl_2$ solution as calcium ions are also potential determining ions which are additionally divalent, therefore, more effective in charge neutralization.
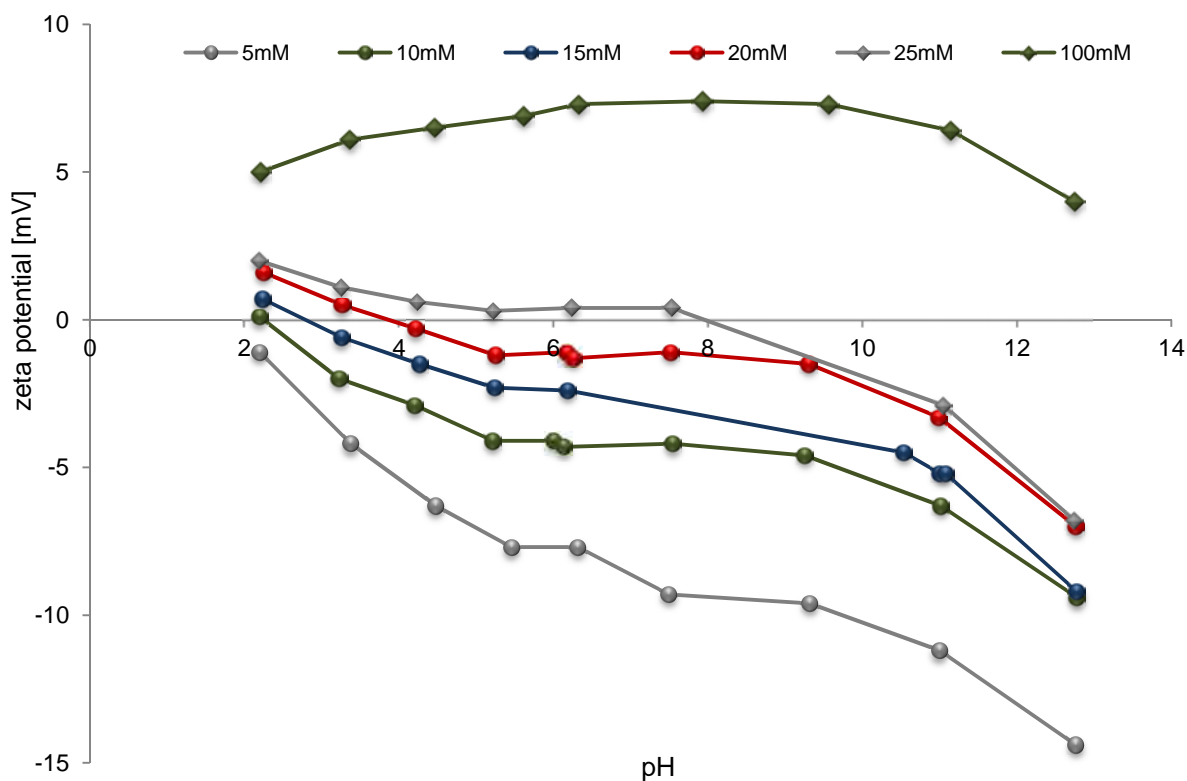
Figure 43. Zeta potential of quartz in 20 mM KCl at different concentrations of [OMIM][Cl]

Zeta potential of [BMIM][Br] on kaolinite in CaCl$_2$

Even though the concentration of background electrolyte was kept constant the zeta potential was increasing with higher concentrations of [OMIM][Cl], the [OMIM]$^+$ cation is therefore a PDI. If coulombic interaction is assumed to be the main driving force for adsorption of the first layer then the imidazolium ring should play an integral part in the process. *Ergo* ζ potential reversal should be observed for all ILs containing the imidazolium ring (but not limited to these). To verify this hypothesis [BMIM][Br] – another imidazolium IL substituted with a shorter chain was examined.

Small shifts in zeta potential with increasing [BMIM]$^+$ concentration were observed (Figure 44). Nevertheless ζ potential was not reversed until pH ≤3. The reversal in this case is also caused by adsorption of protons on the surface of kaolinite. Since 25 mM [OMIM][Cl] in the identical experimental setup (kaolinite/CaCl$_2$) was rendering the mineral surface positive the same would be expected for [BMIM][Br]. However, this is not the case (Figure 43). In addition, even concentrations as high as 500 mM [BMIM][Br] did not reverse zeta potential of kaolinite. This suggests the adsorption of imidazolium homologues must be influenced by interactions other than coulombic.
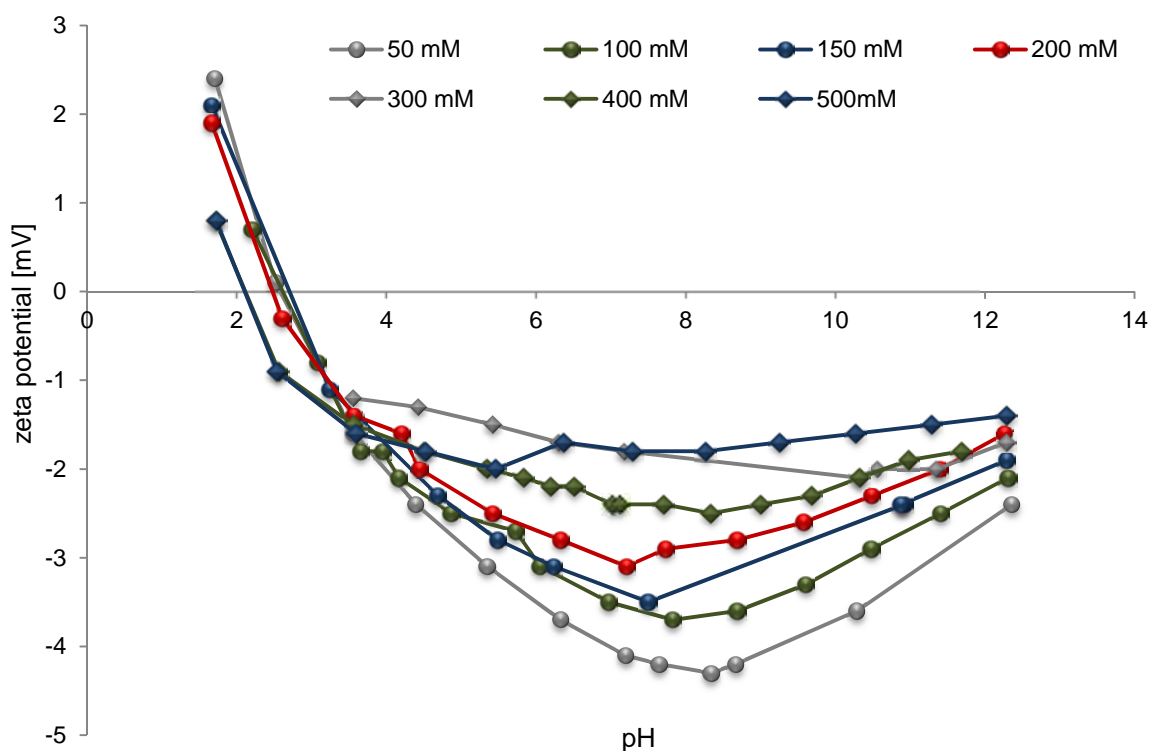
Figure 44. Zeta potential of kaolinite in 10 mM CaCl$_2$ at different concentrations of [BMIM][Br]

Comparison of homologue series

In order to further investigate the effect of the alkyl substituent methyl-imidazolium ILs substituted with four, six and eight carbon chains were compared. In this test KCl was used as it was previously shown to be indifferent electrolyte and was, therefore, unlikely to interfere.

The [OMIM][Cl] reversed zeta potential making it positive in whole pH range of measurement (Figure 45). With [HMIM][Cl] zeta potential was reversed only at low pH suggesting that it is in fact due to adsorption of protons. In suspensions containing [BMIM][Br] ζ potential of kaolinite remained negative. Two suspensions, having equal ionic strength (100 mM), were examined. In the first case only IL was present, and in the second case IL and an indifferent background electrolyte (KCl). The result showed that only [OMIM][Cl] contributed to changes in zeta potentials proving that it indeed is a PDI. In the same suspensions containing IL with shorter alkyl chains almost no difference between 'pure IL' and 'IL+background electrolyte' exists, suggesting that [BMIM]$^+$ behaves as indifferent ion.
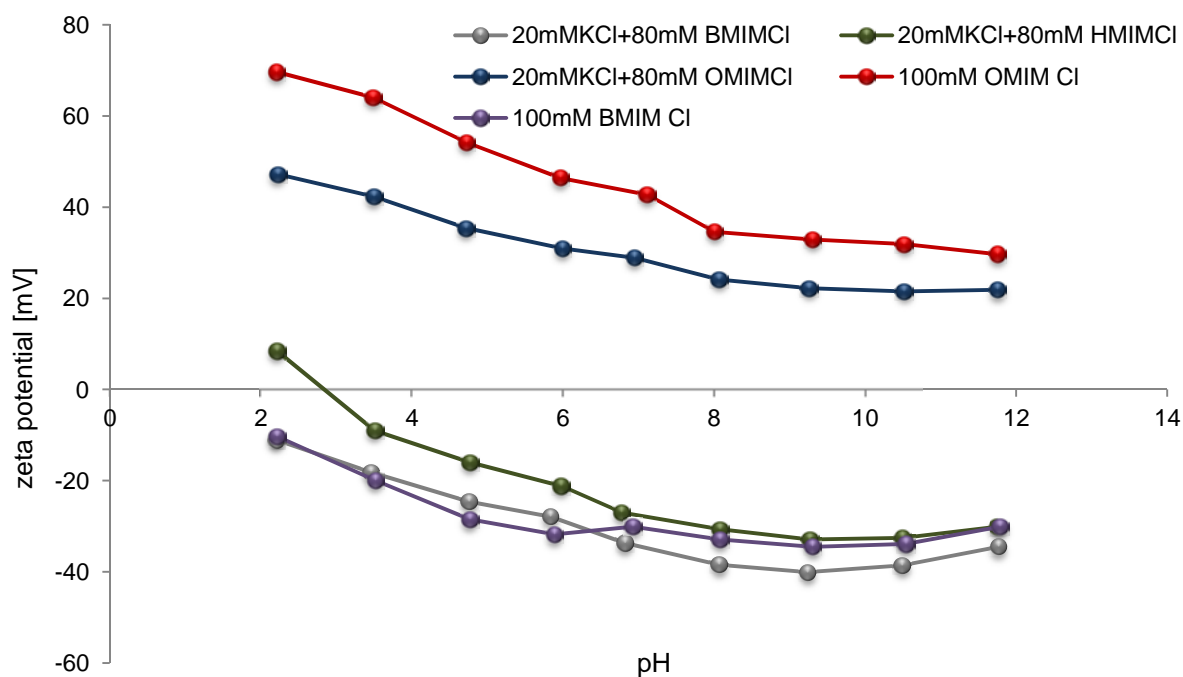
Figure 45. Influence of adsorption of 1-alkyl-3-methylimidazoliums on zeta potential of kaolinite

Vastly different influences of imidazolium homologues on the ζ potential were observed. If the electrostatic interaction would be the sole mode of interaction during the first layer formation, the changes in ζ potential would be independent of the alkyl chain length. Surprisingly, even though adsorption seems to rely on electrostatic attraction, at least in the initial stage, the hydrophobicity of molecules is equally important. Sanchez-Martinez *et al.* observed that adsorption of long-chained quaternary ammonium compounds (QACs) reversed the zeta potential of quartz and that the longer is the alkyl chain the lower concentration of QAC is needed to cause it. Yet no change is observed for ammonium cation suggesting that the mechanism is not solely based on electrostatic interactions [173]. Zadaka *et al.[174]* observed no reversal of montmorillonite charge for small, aromatic QACs but long chained QACs reversed sign of zeta potential for the studied mineral. A similar phenomenon was observed by Bauer *et al.* for adsorption of cationic polyelectrolyte on silica [175]. Double layer adsorption of [BMIM][Br] and [HMIM][Cl] on kaolinite was previously reported and a shift of the first plateau towards higher concentrations was observed within this homologue series [136] . Nevertheless, no reversal of zeta potentials for those ILs occurred in current study. All of these examples, suggest that it is not solely the electrostatic interaction between IL and sorbent that causes sorption, but rather the combination of it with the inability of ILs to interact with water molecules. The changes of electrical properties of mineral are the result of surface sorption/surface aggregation.

5.6.2. Solubilisation/precipitation of organic matter

Following analysis of chemistry of soils and ILs a series of experiments were made regarding the amenability of soil to extraction with ILs, evident by solution coluring. As presented in Figure 46 subsequent samples containing increasing concentration of [OMIM][Cl] were showing increasing turbidity after settling suggesting dissolution of organic matter. The possibility of OC release from soil was confirmed by total organic carbon measurements.

Figure 46. Lindum soil in solution containing increasing concentration of [OMIM][Cl]

TOC content was measured by dry combustion. Theoretical contribution of IL (TOC_{IL}) and PAH (TOC_{PAH}) to TOC value was subtracted from total TOC values for every sample to give a TOC_{DOM} according to equation 7

$$TOC = OC_{IL} + OC_{DOM} + OC_{PAH} \qquad (7)$$

TOC – total organic carbon content

OC_{IL} – organic carbon due to IL

OC_{PAH} – organic carbon due to PAH

OC_{DOM} – organic carbon due to soil dissolved organic matter
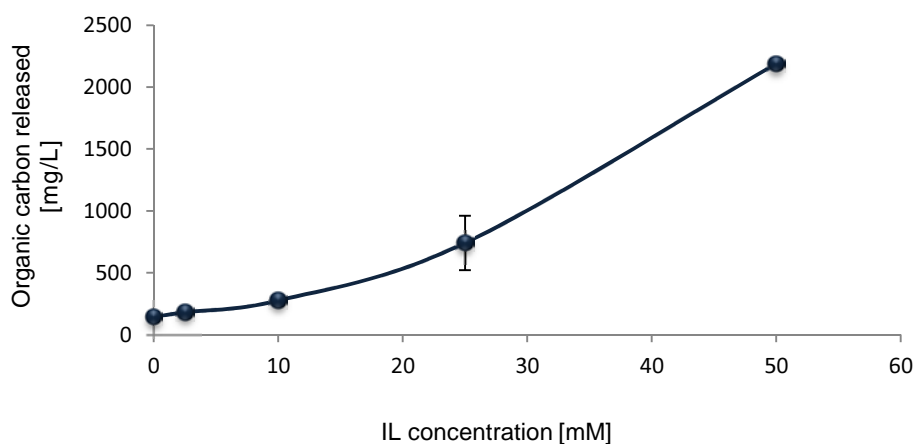
Figure 47. Release of organic carbon from soils under the influence of [OMIM][Cl]

Increased amounts of TOC_{DOM} released from soils to the aqueous phase were observed with increasing concentration of IL Figure 47. The increase could be fitted the best with an exponential line.

5.6.3. Solubilisation of HOC

It is believed that solubilisation of HOC by surfactants occurs mainly in the form of facilitated transport inside of surfactants micelles above the surfactants critical micelle concentration (CMC) in a given system. Therefore, it is necessary to exceed surfactant's CMC to achieve enhanced HOC removal [161, 176]. [OMIM][Cl] is the first in the imidazolium homologue series to show detectable CMC at around 220 mM in pure water, whereas in 10 mM $CaCl_2$ it is estimated to be 175 mM [177, 178]. The additional difficulty in determination of CMC in current system lies in the fact that part of [OMIM][Cl] as well as $CaCl_2$ was adsorbed on Lindum soil. For this reasons CMC of [OMIM][Cl] can only be estimated based on CMC in $CaCl_2$ solution and soil/water partition coefficient of [OMIM][Cl]. This estimation suggests that micellisation in current system should be expected in samples containing more than 100 mmol L^{-1} [OMIM][Cl].

Table 5. Common names, chemical structures, molecular weights (MW) and chemical formulas and octanol water partition coefficients (log $K_{o/w}$) of 16 PAHs used in the investigation
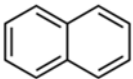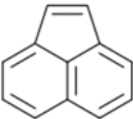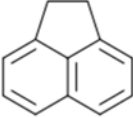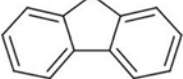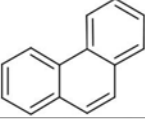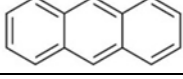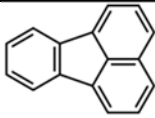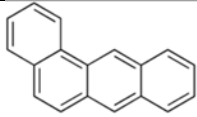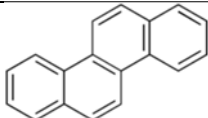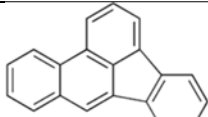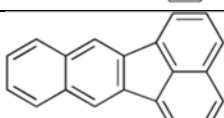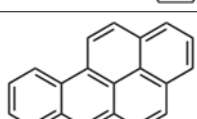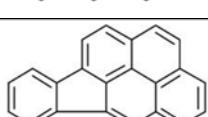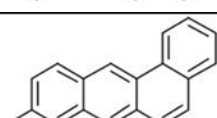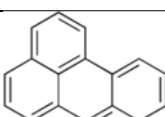
Name	Structure	MW [g/mol]	Chemical formula	Log $K_{o/w}$
Naphthalene		128	$C_{10}H_8$	3.30
Acenaphthylene		152	$C_{12}H_8$	3.94
Acenaphthene		154	$C_{12}H_{10}$	4.15
Fluorene		166	$C_{13}H_{10}$	4.01
Phenanthrene		178	$C_{14}H_{10}$	4.34
Anthracene		178	$C_{14}H_{10}$	4.34

Table 5. continued

Name	Structure	MW [g/mol]	Chemical formula	$K_{o/w}$
Fluoranthene		202	$C_{16}H_{10}$	4.93
Pyrene		202	$C_{16}H_{10}$	4.93
Benz(a)anthracene		228	$C_{18}H_{12}$	5.52
Chrysene		228	$C_{18}H_{12}$	5.52
Benzo(b)fluoranthene		252	$C_{20}H_{12}$	6.11
Benzo(k)fluoranthene		252	$C_{20}H_{12}$	6.11
Benzo(a)pyrene		252	$C_{20}H_{12}$	6.11
Indeno(123cd)pyren		276	$C_{22}H_{12}$	6.70
Dibenz(ah)anthracene		278	$C_{22}H_{14}$	6.70
Benzo(ghi)perylene		276	$C_{22}H_{12}$	6.70

The release of 16 PAHs (Table 5) from initial US Environmental Protection Agency (US EPA) *Priority Pollutants List* was studied in pre-contaminated soil. Figure 48 presents the amounts of small molecular mass PAHs released from soil in the presence of [OMIM][Cl] in submicellar, micellar and over micellar concentrations. Compounds having 2-3 fused rings are released at low IL's concentrations in extremely small and solubilisation becomes significant after ILs concentration approaches CMC.
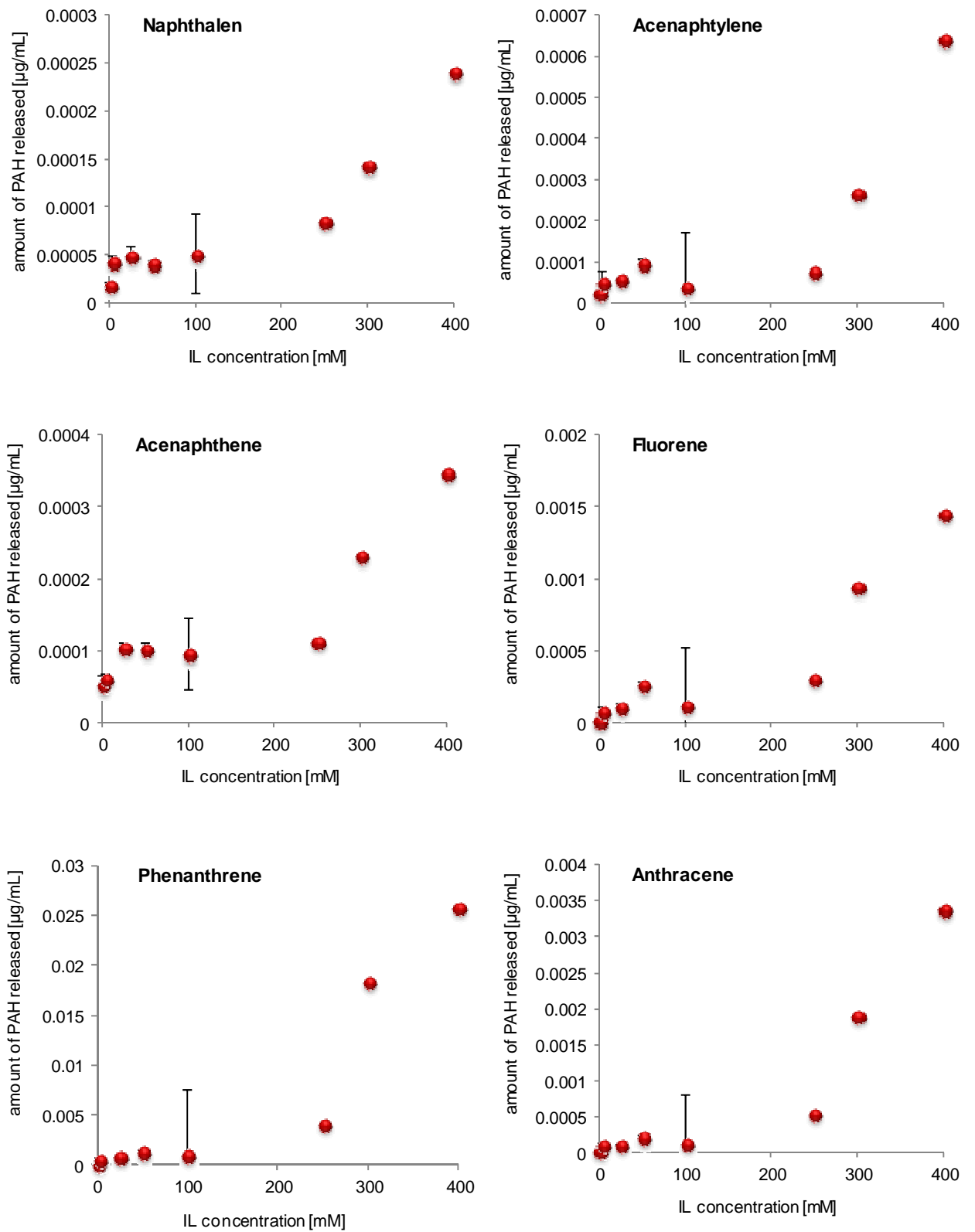
Figure 48. Amount of small molecular mass PAH released from soil

For compounds containing 4 fused rings enhanced release can be observed only above CMC in the system as shown in Figure 49 for fluoranthene, pyrene, benz(a)anthracene and chrysene.
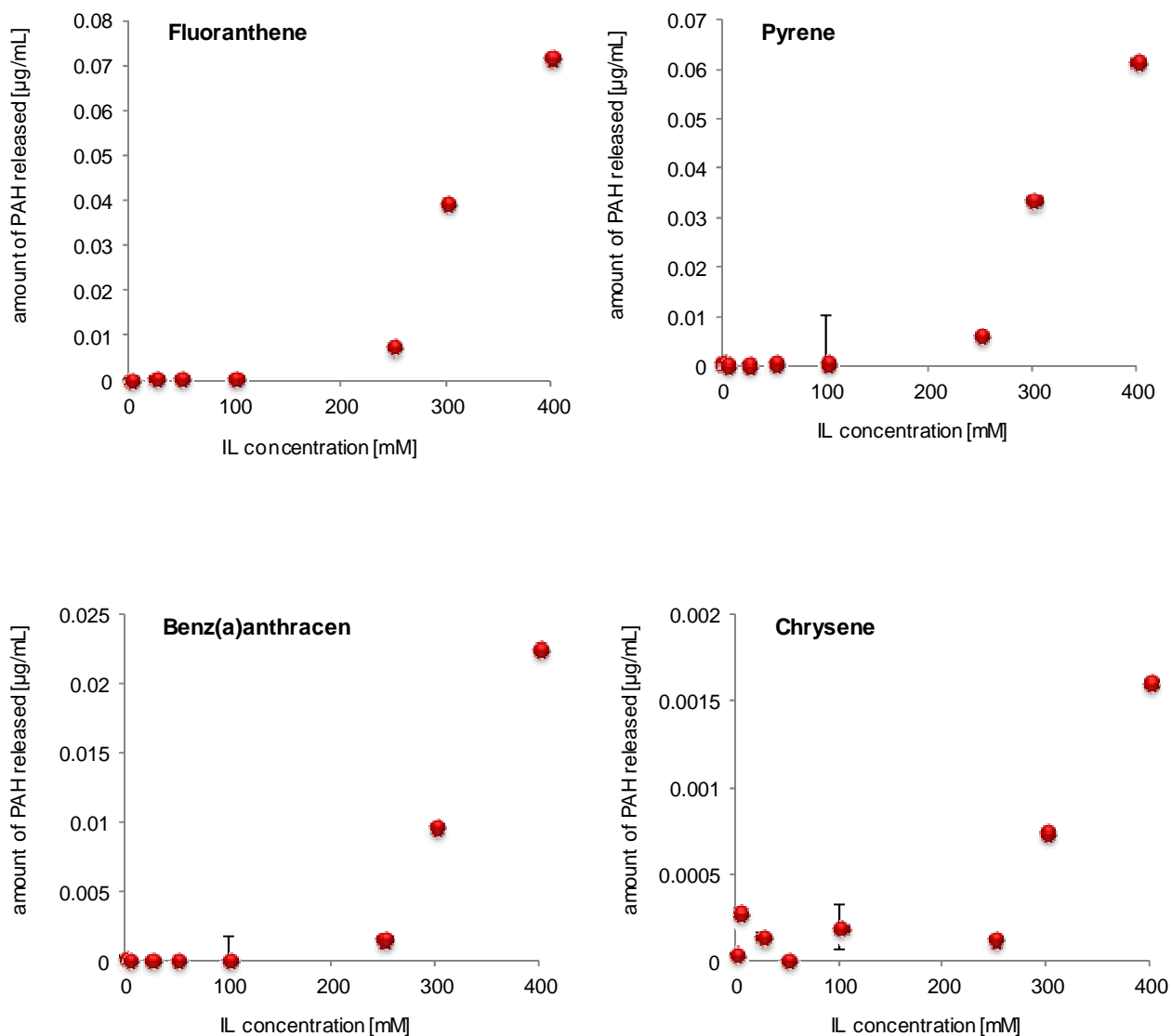
Figure 49. Release of PAH containing 4-5 rings from Lindum soil by [OMIM][Cl]

Figure 50 presents results of solubilisation experiments for bulky PAH containing 5-6 rings. As in the case of medium molecular mass compounds the release occurs only after CMC in the system is exceeded.
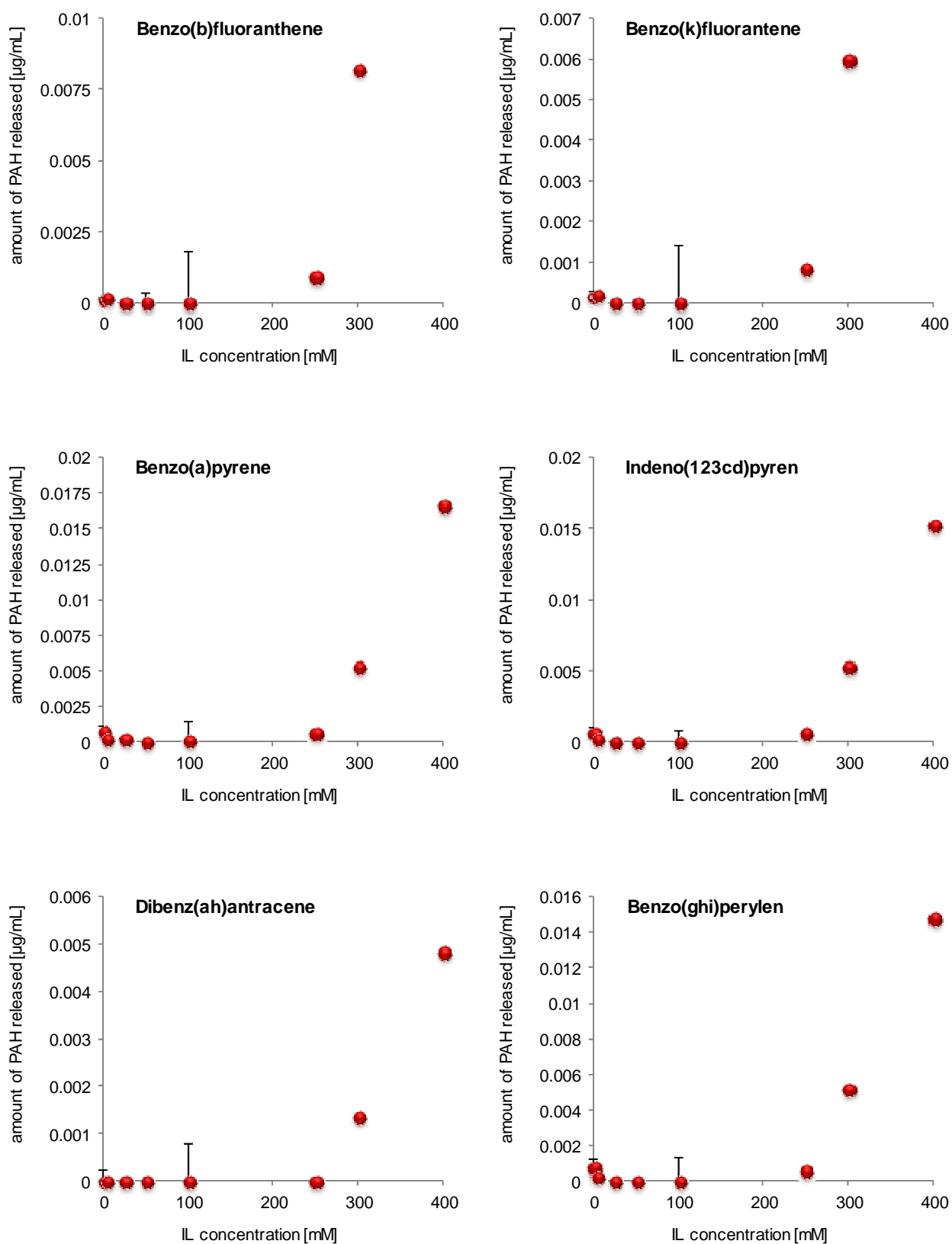
Figure 50. Amount of PAHs released from soil in presence of [OMIM][Cl]

Since Lindum soil used in this study was contaminated with native PAHs (and not spiked) the total content of PAH was measured by GC/MS preceded by organic solvent extraction as described in [179]. This value was taken as a total amount of PAH present in soil for calculation of per cent released due to IL solubilisation. The efficiency of release was substantially higher for high molecular mass PAH. For compounds containing 2-3 fused rings the amount released correlated well with octanol/water partition coefficient (Figure 51 – left). For more bulky structures, composed of 4-5 rings no such correlation exists (Figure 51 – right). This is in agreement with previously discussed findings by Liu *et al.* [162].

naphthalene (●), acenaphtylene (♦),
acenaphthene (▲), phenenthrene (■),
anthracene (●), fluoranthene (♦),
pyrene (▲),benz(a)anthracene (■)

benzo[b]fluoranthene(♦) , benzo[k]fluoranthene (●),
benzo(a)pyrene (▲), indeno(abc)pyrene (♦),
dibenzo(ah)anthracene (●), benzo(ghi)perylen (▲)
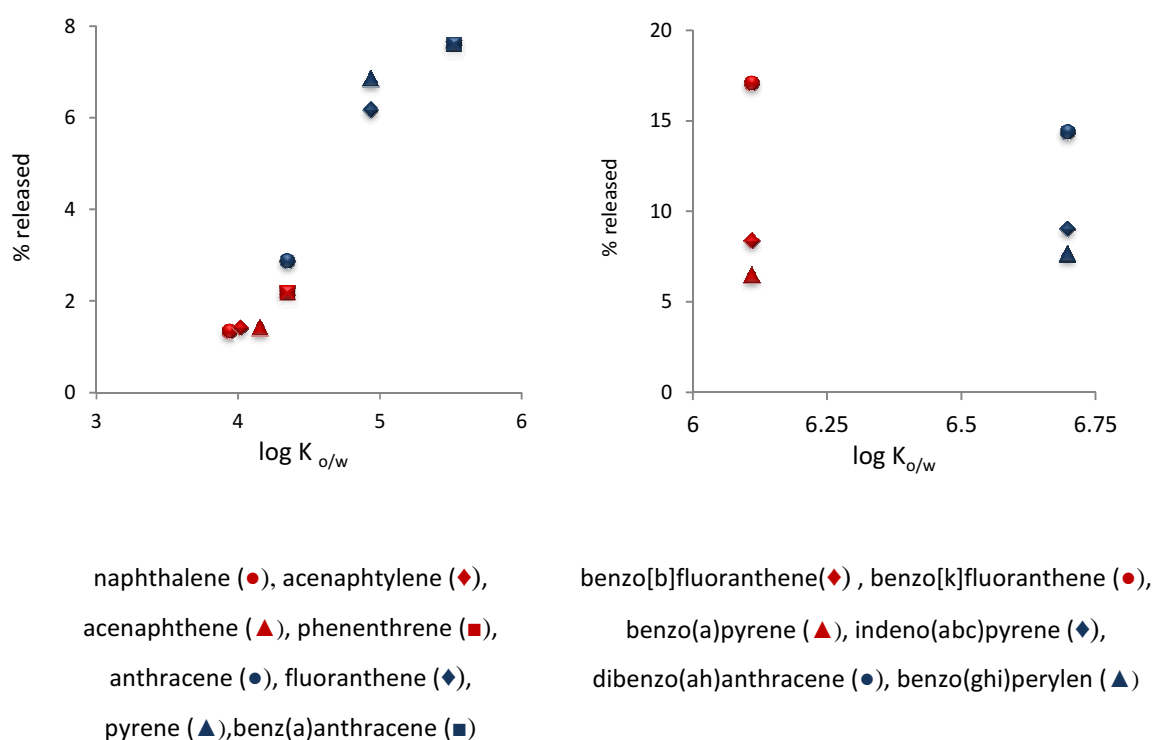
Figure 51. Relationship between maximum amounts of PAHs released from soil and octanol/water partition coefficient

Another mechanism of release below CMC is possible, namely formation of HOC-contaminated DOM complexes. The general scheme of interactions is presented in Figure 52. Initially certain amount of negatively charged DOM is present in soil solution. Negative charge causes DOM aggregates to repulse each other and stabilises the system (Figure 52. A). When small amounts of ILs are present more DOM (and PAH with it) is solubilised and transferred into solution (Figure 52. B). Increasing IL concentration changes the charge of DOM complexes (as

was shown before with zeta potential of minerals) and causes them to precipitate – this also means that less PAH is now transferred into solution (Figure 52.C).
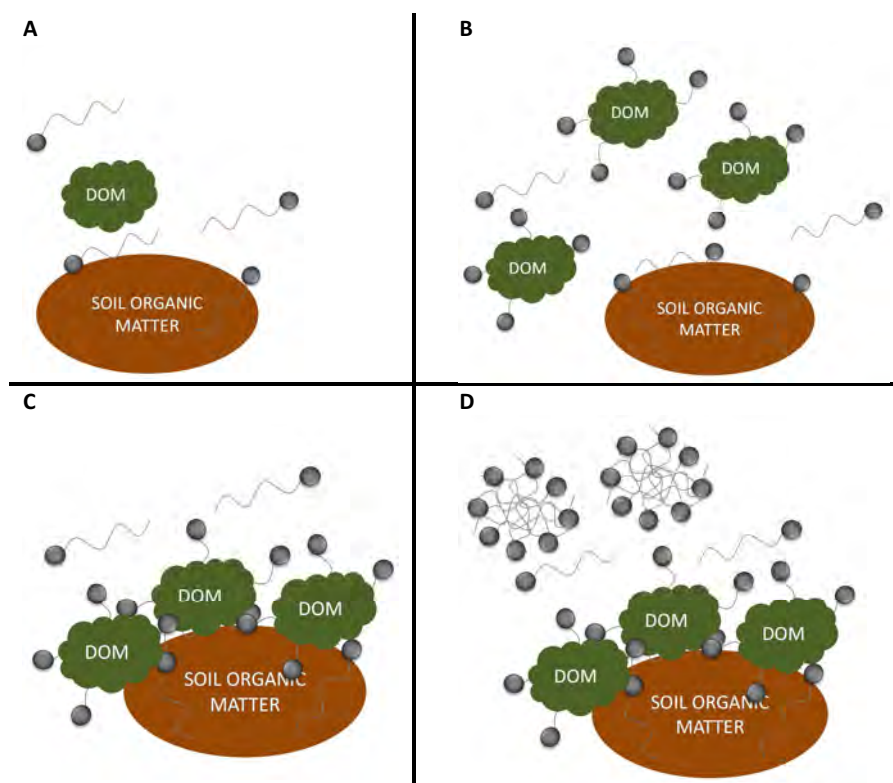

Figure 52. Schematic representation of ILs' interactions with soil particles and dissolved organic matter

At [OMIM][Cl] concentrations above CMC – well described in the literature – micellar solubilisation was observed. Release inside of ILs aggregates is far more significant and can be expected to be the main mechanism of HOC solubilisation by [OMIM][Cl].

5.7. Summary – scientific novelty

Organic cations have previously been shown to behave like potential determining ions for different minerals [122, 156, 174]. Within this work it was found that [OMIM][Cl] does behave liked PDI, however, [BMIM][Cl] does not.

Based on adsorption isotherms, charge reversal that mechanism of ILs sorption involves:

– adsorption of monomers with alkyl chains lying flat on the surface in order to minimize contact with water,

– formation of aggregates before completion of first layer (but above CEC) resulting from the presence of London dispersion forces between hydrophobic chains – orientation of

molecules is unknown but it is rather more probable that they do not adsorb head up as we do not observe increase in zeta potential in this region of isotherm,

− formation of double adsorption layer at sufficient surface coverage.

The possibility of secondary contamination of soils and water bodies by ILs' − enhanced PAH solubilisation from soils was reported for the first time. Release occurs most probably in the form of micellar solubilisation. Nevertheless other than previously speculated modes of PAH release from soils were suggested – namely mobilisation of IL-DOM-PAH.

Results presented in this chapter were submitted to:

M. Markiewicz, W. Mrozik, K. Rezwan, J. Thöming, J. Hupka, C. Jungnickel*, Changes in zeta potential of ionic liquids modified minerals − implications for determining mechanism of adsorption*, Chemosphere.

6. Practical aspects of results

It was shown within this work that results of standard biodegradation and toxicity test might be significantly different than results gathered in conditions resembling real environment. Enhanced biodegradability was observed for adapted microbial communities giving promising information on biodegradation of ILs in the activated sludge process where despite periodic removal of part of the microbial biomass in the form of excess sludge the adaptation of the remaining community is expected. Additionally, microbial flexibility, should be taken into account when evaluating the toxicity of ILs towards taxonomically complex communities like activated sludge. It was shown that significantly lower toxicity was observed for activated sludge than for single organisms (e.g. *Vibrio fischeri*). Furthermore, the presence of alternative nutrient sources should be taken into account when assessing or predicting biodegradation of biological half-life of ILs since it was proven to influence them to significant extend.

Moreover, sorption as a way of removing ILs from wastewater streams was shown hereby to be effective, not limited by concentration and much faster than biodegradation. On the basis of the obtained results, modification of wastewater treatment plant by incorporating a step of adsorption of pollutants using the excess sewage sludge was proposed (Figure 53).

The current work allowed for verification of suggested in the literature mechanisms of sorption of ILs on natural soils and minerals. The surface aggregate formation driven by water molecules forming hydrogen bonds from which ILs' molecules are excluded – and therefore are forced to aggregate on solid surfaces – was shown to be important sorption mechanism. When assessing ILs' potential to contaminate soil and water bodies the possibility of secondary pollution exists. The risk of release and mobilisation of xenobiotics previously sorbed on soil by micellar transport or formation of DOM-ILs-HOC complexes was shown in this work.
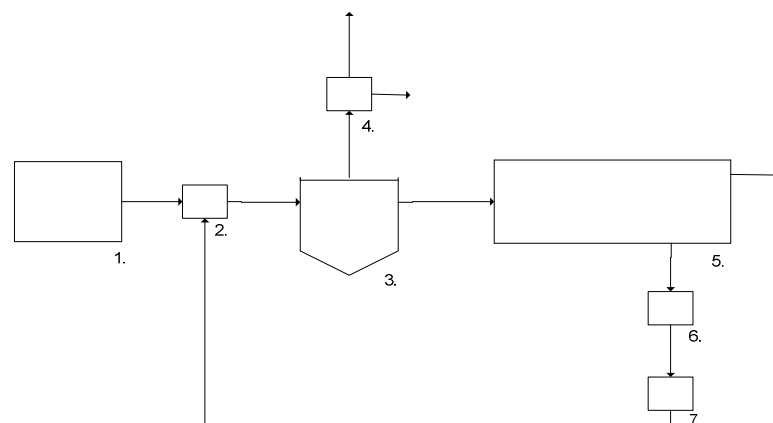

Figure 53. Schematic of WWTP modified by introduction of adsorption step (2)

7. Suggested further research

It has been shown that adaptation influences biodegradability, however, more research is required to elucidate the mechanisms as technologically important information.

Supplementation with nutrients worsens biodegradability of [OMIM][Cl] by providing source of carbon that is easier to degrade, which is the case encountered in an actual WWTP. Different influence of simple source of carbon (glucose) and complex source of carbon and nitrogen (synthetic feed) was observed but underlying mechanism is not well understood. Some concepts within this work need to be confirmed.

Both adaptation and supplementation influence biodegradability of ILs in real conditions (e.g. wastewater treatment plants, natural soils and water bodies etc.) require further thorough examination. Simulation tests would be particularly beneficial.

Sorption of ILs on excess activated sewage sludge should be investigated in simulation tests as well to confirm its usefulness in treatment of wastewaters containing poorly biodegradable compounds or well to moderately biodegradable compounds present in high concentrations. Moreover, the best way of treatment of excess sludge containing adsorbed xenobiotics should be investigated since some of conventionally used methods (e.g. composting) may not be suitable due to toxicity of ILs.

Adsorption on solid surfaces is an interplay between ILs structure and properties of the surface. It seems that for hydrophobic ILs organic matter content is decisive and it is more likely that 'hydrophobic partitioning' is responsible for this than cation exchange. The latter appears to be important for hydrophilic surfaces (e.g. minerals). Even though data concerning adsorption are present they should be unified, especially in terms of techniques used for measuring soil parameters, and modelled to give quantitative description.

Although first insights into modification of charge characteristics of minerals by adsorption of [BMIM][Cl], [HMIM][Cl] and [OMIM][Cl] were presented, many other variables than chain length need to be investigated. Further tests are necessary to elucidate mechanism of sorption of ILs in more details, since results presented hereby should be treated as preliminary.

There are still many unknowns regarding solubilsation of HOC by ILs. [OMIM][Cl] was previously shown in the literature to be the first IL in imidazolium homologue series that aggregates in water, since micellar release is the most significant, the ability to form aggregates seems to be prerequisite for solubilisation. Therefore, other ILs having shorter chain may not contribute to the release of PAH from soil. It is also possible that ILs having longer substituents will be retained in soils to higher extent requiring greater concentrations to achieve the same effect as [OMIM][Cl].

Using the SAR concept and data gathered earlier it is possible to make predictions with respect to fate of other ILs or even other xenobiotics in above mentioned processes. The accuracy of such approach should be, however, at least randomly confirmed before any general statements can be formulated. In biodegradation, sorption and solubilisation tests the behavior of cations has been investigated. The influence of the simple inorganic anions is supposed to be negligible nevertheless very hydrophobic moieties – such as $(C_2F_5)_3PF_3^-$ or $(CF_3SO_2)_2N^-$ - may change the sorption and mobility of the cation and should be investigated in more detail.

8. Conclusions

Imidazolium ILs are of great interest for industry and academia for over three decades and considerable amount of data concerning their chemical and physicochemical properties and interactions with various surfaces have been gathered. These data provide deeper understanding and broader knowledge of ILs chemistry and as such are extremely important for development of ILs-based industrial technologies. Interest in environmental impact of ILs started somewhat later, nevertheless it is gaining increasing attention as the importance of sustainable design is acknowledge and regulatory mechanisms such as REACh are set into motion.
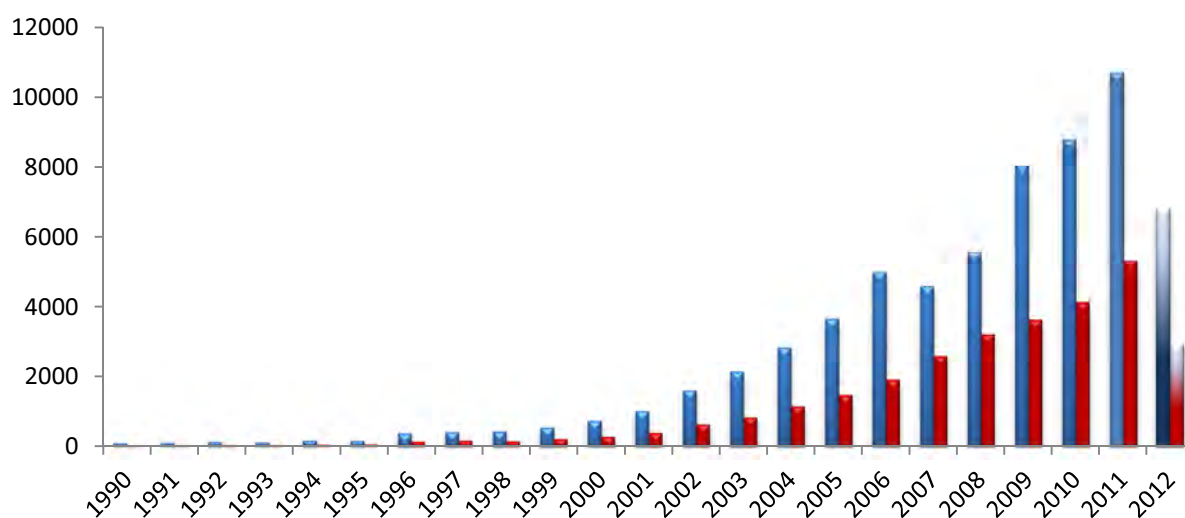
Figure 54. Increase in amount of scientific on-line publications pertaining to IL over last two decades; blue bars – total amount of publications on ILs (search term: "ionic liquids"), red bars – publications referring to environmental aspects of ILs (search term: "ionic liquids "+"environment"); source: Google Scholar accessed on 11.06.2012

Initial step of Ionic liquids environmental research inevitably was focused on filling gaps in basic knowledge regarding biodegradability, toxicity and mobility of IL in simple, well defined systems. As the knowledge is expanding a need for more holistic approach to ILs environmental science arises. This approach needs to take into account more developed, complex and interconnected testing systems, reflecting real environmental conditions.

Several aspects of ILs behaviour in aquatic environment and soil were investigated within this work. Removal of ILs from environmental matrices was the primary focus. Sorption and biodegradation were the main mechanisms. In wastewater treatment sorption was shown to be an effective measure of fast xenobiotic removal. As a result, a modification to wastewater treatment plant flowsheet and construction was suggested that may serve two functions. First,

allow rapid decrease of xenobiotic content even in concentrations significantly exceeding biodegradable concentration. Second, act as a buffer protecting activated sewage sludge bioreactor from unexpected fluctuations of xenobiotic concentration.

The influence of additional nutrients and pre-exposition on the rate and extent of biodegradation were examined aiming to elucidate natural environment clean-up scenario. It was shown that these are opposing processes with the former inhibiting and the latter promoting biodegradation. Adaptation allowed for increase of biodegradability threshold by an order of magnitude. Such situation can occur in e.g. WWTP continuously treating wastewaters containing ILs, like industrial WWTP. Supplementation increased time of biodegradation when simple source of carbon was added (glucose) or inhibited degradation, at least for the duration of the test, when a complex source of carbon and nitrogen was added. This scenario is possible in WWTP treating predominantly domestic wastewaters or any other biotopes which come into contact with ILs sporadically.

Whole biocenosis toxicity tests were also performed in order to compare the applicability of standard single organism toxicity tests in assessing the impact on the activated sludge. It was shown that single organism tests resulted in lower EC_{50} values meaning that activated sludge as a complex community of organisms shows higher resistance to IL.

All mentioned processes have profound meaning for wastewater treatment efficiency as they show that results of standard tests for biodegradation and toxicity usually do not reflect real environmental conditions well and that, in case of [OMIM][Cl] they would introduce considerable bias. Adapted activated sewage sludge community will probably be able to conduct biodegradation faster and in higher concentration than standard tests would suggest.

Sorption of ILs to soils was also investigated showing that this process might influence the surface properties of soil in at least two ways. First, by surface hydrophobisation resulting from adsorption of ILs on mineral and organic fraction of soil. Second – charge reversal which might change the amount of cations held by soil (e.g. inorganic cations important for plant growths thereby decreasing soil fertility or heavy metals present as pollutants thereby mobilising them) and destabilise soil colloids by eliminating repulsive forces between them. Moreover it was proven that ILs enhance the release of hydrophobic organic compounds like PAH from soil by at least two mechanisms. The first one, occurring at low concentrations of ILs (0 – 25 mM), is based on releasing IL-DOM-PAH complexes. The second one occurs at elevated ILs concentrations, above the critical micelle concentration in studied system, and results from solubilisation of PAH inside of ILs micelles. Both processes can contribute to a secondary pollution of soil and ground water not only by ILs themselves but also other compounds they might release from soil.

9. Bibliography

1. Wilkes, J.S., *A short history of ionic liquids—from molten salts to neoteric solvents*, Green Chemistry, 2002, 4: p. 73-80.
2. Earle, M.J. and K.R. Seddon, *Ionic liquids, green solvents for the future*, Pure and Applied Chemistry, 2000, 72 (7): p. 1391—1398.
3. Plechkova, N.V. and K.R. Seddon, *Applications of ionic liquids in the chemical industry*, Chemical Society Reviews, 2008, 37: p. 123-150.
4. Frank, E. and A.S.Z. El, *Air and water stable ionic liquids in physical chemistry*, Physical Chemistry Chemical Physics, 2006, 8: p. 2101–2116.
5. Holbrey, J.D. and K.R. Seddon, *Ionic Liquids*, Clean Products and Processes, 1999, 1: p. 223-236.
6. Markiewicz, M., J. Hupka, M. Joskowska, and C. Jungnickel, *Potential application of ionic liquids in aluminium production - economical and ecological assessment*, Physicochemical Problems of Mineral Processing, 2009, 43: p. 73-84.
7. Liu, Q.X., S.Z.E. Abedin, and F. Endres, *Electroplating of mild steel by aluminium in a first generation ionic liquid: A green alternative to commercial Al-plating in organic solvents*, Surface & Coatings Technology 2006, 201: p. 1352-1356.
8. Galiński, M., A. Lewandowski, and I. Stępniak, *Ionic liquids as electrolytes* Electrochimica Acta, 2006, 51 (26): p. 5567-5580
9. Predel, T. and E. Schlücker, *Ionic Liquids in Oxygen Compression*, Chemical Engineering Technology, 2009, 32 (8): p. 1183-1188.
10. Tempel, D.J., P.B. Henderson, J.R. Brzozowski, R.M. Pearlstein, and H. Cheng, *High Gas Storage Capacities for Ionic Liquids through Chemical Complexation*, Journal of the American Chemical Society, 2008, 130: p. 400-401.
11. Kawano, R., H. Matsui, C. Matsuyama, A. Sato, M.A.B.H. Susan, N. Tanabe, and M. Watanabe, *High performance dye-sensitized solar cells using ionic liquids as their electrolytes*, Journal of Photochemistry and Photobiology A: Chemistry, 2004, 164 (1-3): p. 87-92.
12. Weyershausen, B. and K. Lehmann, *Industrial application of ionic liquids as performance additives*, Green Chemistry, 2005, 7: p. 15-19.
13. Brennecke, J.F. and E.J. Maginn, *Ionic Liquids: Innovative Fluids for Chemical Processing*, American Institute of Chemical Engineers Journal, 2001, 47 (11): p. 2384-2389.
14. Beaulieu, J.J., J.L. Tank, and M. Kopacz, *Sorption of imidazolium-based ionic liquids to aquatic sediments*, Chemosphere 2008, 70 p. 1320-1328.
15. Stolte, S., S. Steudte, A. Igartua, and P. Stepnowski, *The Biodegradation of Ionic Liquids - the View from a Chemical Structure Perspective*, Current Organic Chemistry, 2011, 15 (12): p. 1946-1973.
16. Seddon, K.R., *Ionic liquids for clean technology*, Journal of Chemical Technology and Biotechnology, 1997, 68: p. 351-356.
17. Jhong, H.-R., H.-P. Cheng, S.-P. Feng, J.-L. Lan, C. Peng, T.-C. Wei, W.-C. Hzu, Y.-H. Chang, and W.-H. Chen, *Electrolyte composition and dye-sensitised solar cell (DSSC) comprising the same*, United States Patent and Trade Mark Office, 2010, US 2010/0101644A1.
18. Chauvin, Y., L. Magna, G.P. Niccolai, and J.-M. Basset, *Imidazolium salts and their use of these ionic liquids as a solvent*, United Nations Department of Economic and Social Affairs, 2011, US007915426B2.
19. Shiraishi, T. and K. Hirata, *Lubrication oil composition containing ionic liquid*, United Nations Department of Economic and Social Affairs, 2010, US 20100105581A1.
20. Liu, C., S. Kulprathipanja, M.E. Schott, and S.T. Wilson, *Ionic liquid-solid-polymer mixed matrix membranes for gas separations*, United Nations Department of Economic and Social Affairs, 2011, US 007943543B1.

21. Yoshimitsu, S., *Method for manufacturing a solid electrolytic capacitor*, United Nations Department of Economic and Social Affairs, 2009, US 20090242415A1.

22. Herzog, H., P. Schwab, and M. Naumann, *A process for producing antistatically treated artificial stone for flat structures*, United Nations Department of Economic and Social Affairs, 2010, US 2010/0192814 A1.

23. Coggan, J.A. and T.P. Bender, *Arylamine processes*, United Nations Department of Economic and Social Affairs, 2007, US 20070100164A1.

24. Olbert, G., T. Mattke, M. Fiene, O. Huttenloch, and U. Hammon, *Use of an ionic liquid as heat transfer medium for the indirect introduction or removal of heat into or from a reactor is described*, United Nations Department of Economic and Social Affairs, 2006, US20060251961.

25. Arlt, W., M. Seiler, C. Jork, and T. Schneider, *Ionic liquids as selective additives for separation of close-boiling or azeotropic mixtures* United Nations Department of Economic and Social Affairs, 2008, US 007435318B2.

26. BASF The Chemical Company. 2009. Available from: http://www.basf.com/group/corporate/en/.

27. *Water Framework Directive 2000/60/EC*. 23 October 2000, The European Parliament and the Council.

28. *Directive 2006/118/EC on the protection of groundwater against pollution and deterioration*. 12 December 2006, The European Parliament and the Council.

29. Kümmerer, K., A. Al-Ahmad, and A. Henninger, *Use of Chemotaxonomy To Study the Influence of Benzalkonium Chloride on Bacterial Populations in Biodegradation Testing*, Acta hydrochimica et hydrobiologica, 2002, 30 (4): p. 171-178.

30. W Kördel, D. Hennecke, and C. Franke, *Determination of the adsorption-coefficients of organic substances on sewage sludges*, Chemosphere, 1997, 35 (1-2): p. 107-119.

31. Harms, H. and T.N.P. Bosma, *Mass transfer limitation of microbial growth and pollutant degradation*, Journal of Industrial Microbiology & Biotechnology, 1997, 18: p. 97-105.

32. V. G. Khomenkov, A. B. Shevelev, V. G. Zhukov, N. A. Zagustina, A. M. Bezborodov, and V.O. Popov, *Organization of Metabolic Pathways and Molecular-Genetic Mechanisms of Xenobiotic Degradation in Microorganisms: A Review*, Applied Biochemistry and Microbiology, 2008, 44 (2): p. 117-135.

33. Grady, L.C.P.J., *Biodegradation: Its measurement and microbiological basis*, Biotechnology and Bioengineering, 1985, 27: p. 660-674.

34. Boethling, R.S., E. Sommer, and D. DiFiore, *Designing Small Molecules for Biodegradability*, Chemical Reviews, 2007, 107 (6): p. 2167-2820.

35. Boethling, R.S., E. Sommer, and D. DiFiore, *Designing Small Molecules for Biodegradability*, Chemical Reviews, 2007, 107 (6): p. 2207-2227.

36. Top, E.M. and D. Springaely, *The role of mobile genetic elements in bacterial adaptation to xenobiotic organic compounds*, Current Opinion in Biotechnology, 2003, 14: p. 262-269.

37. Prescott, L.M., J.P. Harley, and D.A. Klein, *Chapter 5: Microbial Nutrition*, in *Microbiology*, E.M. Sievers, Editor. 1996, Wm. C. Brown Publishers. p. 98-113.

38. Reineke, W., *Aerobic and Anaerobic Biodegradation Potentials of Microorganisms*, in *The Handbook of Environmental Chemistry*, B. Beek, Editor. 2001, Springer-Verlag: Berlin Heidelberg. p. 1-161.

39. Tyagi, M., M.M.R.d. Fonseca, and C.C.C.R.d. Carvalho, *Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes*, Biodegradation, 2011, 22: p. 231-241.

40. Loh, K.-C. and S.-J. Wang, *Enhancement of biodegradation of phenol and a nongrowth substrate 4-chlorophenol by medium augmentation with conventional carbon sources*, Biodegradation, 1998, 8: p. 329-338.

41. Goldstein, R.M., L.M. Mallory, and M. Alexander, *Reasons for Possible Failure of Inoculation to Enhance Biodegradation*, Applied and Environmental Microbiology, 1985, 50 (4): p. 977-983.

42. Lewis, D.L., H.P. Kollig, and R.E. Hodson, *Nutrient Limitation and Adaptation of Microbial Populations to Chemical Transformations*, Applied and Environmental Microbiology, 1986, 51 (3): p. 598-603.

43. Swindoll, C.M., C.M. Aelion, and F.K. Pfaender, *Influence of Inorganic and Organic Nutrients on Aerobic Biodegradation and on the Adaptation Response of Subsurface Microbial Communities*, Applied and Environmental Microbiology, 1987, 54 (1): p. 212-217.

44. Piekarska, K. and J. Pawlak, *Ocena skuteczności osadu czynnego w oczyszczaniu ścieków zaolejonych*, Ochrona Środowiska, 1994, 1 (52): p. 21-26.

45. Prescott, L.M., J.P. Harley, and D.A. Klein, *Chapter 6: Microbial Growth*, in *Microbiology*, E.M. Sievers, Editor. 1996, Wm. C. Brown Publishers. p. 114-135.

46. Dalzell, D.J.B., S. Alte, E. Aspichueta, A.d.l. Sota, J. Etxebarria, M. Gutierrez, C.C. Hoffmann, D. Sales, U. Obst, and N. Christofi, *A comparison of five rapid direct toxicity assessment methods to determine toxicity of pollutants to activated sludge*, Chemosphere, 2002, 47: p. 535-545.

47. Salyers, A.A. and D.D. Whitt, *Chapter 5: Genetyka bakterii I: replikacja DNA i inżynieria genetyczna*, in *Microbiology: Różnorodność, chorobotwórczość i środowisko*, K. Mostowiak, Editor. 2005, PWN: Warszawa. p. 73-101.

48. Spain, J.C. and P.A.V. Veld, *Adaptation of Natural Microbial Communities to Degradation of Xenobiotic Compounds: Effects of Concentration, Exposure Time, Inoculum, and Chemical Structure*, Applied and Environmental Microbiology, 1983, 45 (2): p. 428-435.

49. Aelion, M., M. Swindoll, and F.K. Pfaender, *Adaptation to and Biodegradation of Xenobiotic Compounds by Microbial Communities from a Pristine Aquifer*, Applied and Environmental Microbiology, 1987, 53 (9): p. 2212-2217.

50. Karahan, Ö., T. Olmez-Hanci, I. Arslan-Alaton, and D. Orhon, *Modelling biodegradation of nonylphenol ethoxylate in acclimated and non-acclimated microbial cultures*, Bioresource Technology, 2010, 10: p. 8058–8066.

51. Chipasa, K.B. and K. Mędrzycka, *Behavior of lipids in biological wastewater treatment processes*, Journal of Industrial Microbiology & Biotechnology, 2006, 33 (365): p. 635-645.

52. Jastorff, B., R. Störmann, and J. Ranke, *Thinking in Structure-Activity Relationships – A Way Forward Towards Sustainable Chemistry*, Clean 2007, 35 (5): p. 399 - 405.

53. Jastorff, B., R. Störmann, J. Ranke, K. Mölter, F. Stock, B. Oberheitmann, W. Hoffmann, J. Hoffmann, M. Nüchter, B. Ondruschka, and J. Filser, *How hazardous are ionic liquids? Structure–activity relationships and biological testing as important elements for sustainability evaluation*, Green Chemistry 2003, 5: p. 136-142.

54. Stolte, S., S. Abdulkarim, J. Arning, A.-K. Blomeyer-Nienstedt, U. Bottin-Weber, M. Matzke, J. Ranke, B. Jastorff, and J. Thöming, *Primary biodegradation of ionic liquid cations, identification of degradation products of 1-methyl-3-octyl -imidazolium chloride and electrochemical waste water treatment of poorly biodegradable compounds*, Green Chemistry, 2008, 10: p. 214–224.

55. Wells, A.S. and V.T. Coombe, *On the freshwater ecotoxicity and biodegradation properties of some common ionic liquids*, Organic Process Research and Development 2006, 10: p. 794-798.

56. Markiewicz, M., C. Jungnickel, A. Markowska, U. Szczepaniak, M. Paszkiewicz, and J. Hupka, *1-Methyl-3-octylimidazolium Chloride—Sorption and Primary Biodegradation Analysis in Activated Sewage Sludge*, Molecules, 2009, 14: p. 4396-4405.

57. Docherty, K.M., J.K. Dixon, and C.F.K. Jr, *Biodegradability of imidazolium and pyridinium ionic liquids by an activated sludge microbial community*, Biodegradation 2007, 18: p. 481-493.

58. Gathergood, N., P.J. Scammells, and M.T. Garcia, *Biodegradable ionic liquids Part III:The first readily biodegradable ionic liquids*, Green Chemistry, 2006, 8: p. 156-160.

59. Gathergood, N., M.T. Garcia, and P.J. Scammells, *Biodegradable ionic liquids: Part I. Concept, preliminary targets and evaluation*, Green Chemistry 2004, 6: p. 166-175.

60. Garcia, M.T., N. Gathergood, and P.J. Scammells, *Biodegradable ionic liquids Part II. Effect of the anion and toxicology*, Green Chemistry, 2005, 7: p. 9-14.

61. Coleman, D. and N. Gathergood, *Biodegradation studies of ionic liquids*, Chemical Society Reviews, 2010, 39: p. 600-637.

62. Pham, T.P.T., C.-W. Cho, and Y.-S. Yun, *Environmental fate and toxicity of ionic liquids: A review*, Water Research, 2010, 44 (2): p. 352-372.

63. Zhu, S., R. Chen, Y. Wu, Q. Chen, X. Zhang, and Z. Yu, *A Mini-Review on Greenness of Ionic Liquids*, Chemical and Biochemical Engineering Quarterly, 2009, 23 (2): p. 207-211.

64. E. Rorije, F. Germa, B. Philipp, B. Schink, and D.B. Beimborn, *Prediction of biodegradability from structure: Imidazoles*, SAR and QSAR in Environmental Research, 2002, 13 (1): p. 199-204.

65. Harjani, J.R., J. Farrell, M.T. Garcia, R.D. Singer, and P.J. Scammells, *Further investigation of the biodegradability of imidazolium ionic liquids*, Green Chemistry, 2009, 11: p. 821-829.

66. Yu, Y., X. Lu, Q. Zhou, K. Dong, H. Yao, and S. Zhang, *Biodegradable Naphthenic Acid Ionic Liquids: Synthesis, Characterization, and Quantitative Structure–Biodegradation Relationship*, Chemistry A European Journal, 2008, 14: p. 11174 - 11182.

67. OECD, *OECD Guideline for testing of chemicals 301 - Ready Biodegradability*. 1992.

68. Docherty, K.M., M.V. Joyce, K.J. Kulacki, and C.F. Kulpa, *Microbial biodegradation and metabolite toxicity of three pyridinium-based cation ionic liquids.*, Green Chemistry, 2010, 12: p. 701-712.

69. Pham, T.P.T., C.-W. Cho, C.-O. Jeon, Y.-J. Chung, M.-W. Lee, and Y.-S. Yun, *Identification of Metabolites Involved in the Biodegradation of the Ionic Liquid 1-Butyl-3-methylpyridinium Bromide by Activated Sludge Microorganisms*, Environmental Science and Technology, 2009, 43 (2): p. 516-521.

70. Abrusci, C., J. Palomar, J.L. Pablos, F. Rodriguez, and F. Catalina, *Efficient biodegradation of common ionic liquids by Sphingomonas paucimobilis bacterium*, Green Chemistry, 2011, 13 (3): p. 709-717.

71. Michal, G., *Chapter 4: Amino acids and derivatives*, in *Biochemical pathways: an atlas of biochemistry and molecular biology*, G. Michal, Editor. 1999, Willey-Blackwell. p. 47-67.

72. Mehler, A.H., *Amino acid metabolism II: Metabolism of individual amino acids*, in *Textbook of biochemistry*, T.M. Devlin, Editor. 1992, Wiley-Liss: New York. p. 491-528.

73. Romero, A., A. Santos, J. Tojo, and A. Rodrıguez, *Toxicity and biodegradability of imidazolium ionic liquids*, Journal of Hazardous Materials, 2008, 151: p. 268-273.

74. Błaszczyk, M.K., *Rola bakterii w obiegu pierwiasktów w przyrodzie*, in *Mikrobiologia środowisk*, K. Mostowik, Editor. 2010, PWN: Warszawa.

75. Neumann, J., O. Grundmann, J. Thöming, M. Schulte, and S. Stolte, *Anaerobic biodegradability of ionic liquid cations under denitrifying conditions.*, Green Chemistry, 2010, 20 (4): p. 620-627.

76. Modelli, A., A. Sali, P. Galletti, and C. Samori, *Biodegradation of oxygenated and non-oxygenated imidazolium-based ionic liquids in soil*, Chemosphere, 2008, 73 (8): p. 1322-1327.

77. Tian, Y., *Behaviour of bacterial extracellular polymeric substances from activated sludge: a review*, International Journal of Environment and Pollution, 2008, 32 (1): p. 78-89.

78.   Vijayaraghavan, K., T. Phuong, T. Pham, C.-W. Cho, S.W. Won, S.B. Choi, M. Juan, S. Kim, Y.-R. Kim, B.-W. Chung, and Y.-S. Yun, *An assessment on the interaction of a hydrophilic ionic liquid with different sorbents*, Industrial and Engineering Chemistry Research, 2009, 48: p. 7283–7288.

79.   Stepnowski, P., A.C. Składanowski, A. Ludwiczak, and E. Laczynska, *Evaluating the cytotoxicity of ionic liquids using human cell line HeLa*, Human & Experimental Toxicology, 2004, 23 (11): p. 513-517.

80.   Pernak, J., K. Sobaszkiewicz, and I. Mirskab, *Anti-microbial activities of ionic liquids*, Green Chemistry, 2003, 5: p. 52-56.

81.   Matzke, M., S. Stolte, K. Thiele, T. Juffernholz, J. Arning, J. Ranke, U. Welz-Biermannd, and B. Jastorff, *The influence of anion species on the toxicity of 1-alkyl-3-methylimidazolium ionic liquids observed in an (eco)toxicological test battery*, Green Chemistry, 2007, 9 (11): p. 1198-1207

82.   Łuczak, J., C. Jungnickel, I. Łącka, S. Stolte, and J. Hupka, *Antimicrobial and surface activity of 1-alkyl-3-methylimidazolium derivatives*, Green Chemistry, 2010, 12: p. 593-601.

83.   Petkovic, M., J. Ferguson, A. Bohn, J. Trindade, I. Martins, M.B. Carvalho, M.C. Leitão, C. Rodrigues, H. Garcia, R. Ferreira, K.R. Seddon, L.P.N. Rebelo, and C.S. Pereira, *Exploring fungal activity in the presence of ionic liquids*, Green Chemistry, 2009, 11 (6): p. 889-894.

84.   Kulacki, K.J. and G.A. Lamberti, *Toxicity of imidazolium ionic liquids to freshwater algae*, Green Chemistry, 2008, 10: p. 104-110.

85.   Latała, A., P. Stepnowski, M. Nędzi, and W. Mrozik, *Marine toxicity assessment of imidazolium ionic liquids: Acute effects on the Baltic algae Oocystis submarina and Cyclotella meneghiniana*, Aquatic Zoology, 2005, 73 (1): p. 91-98.

86.   Bernot, R.J., M.A. Brueseke, M.A.E.-. White, and G.A. Lamberti, *Acute and chronic toxicity of imidazolium-based ionic liquids on Daphnia magna*, Environmental Toxicology and Chemistry, 2005, 24 (1): p. 87-92.

87.   Marianne Matzke, Stefan Stolte, Andrea Böschen, and Juliane Filser, *Mixture effects and predictability of combination effects of imidazolium based ionic liquids as well as imidazolium based ionic liquids and cadmium on terrestrial plants (Triticum aestivum) and limnic green algae (Scenedesmus vacuolatus)*, Green Chemistry, 2008, 10: p. 784-792.

88.   Swatloski, R.P., J.D. Holbrey, S.B. Memon, G.A. Caldwell, K.A. Caldwell, and R.D. Rogers, *Using Caenorhabditis elegans to probe toxicity of 1-alkyl-3-methylimidazolium chloride based ionic liquids*, Chemical Communications, 2004, 6: p. 668 - 669.

89.   Pretti, C., C. Chiappe, D. Pieraccini, M. Gregori, F. Abramo, G. Monni, and L. Intorre, *Acute toxicity of ionic liquids to the zebrafish*, Green Chemistry, 2006, 8 (3): p. 238-240.

90.   Grabińska-Sota, E. and J. Kalka, *An assessment of the toxicity of pyridinium chlorides and their biodegradation intermediates*, Environment International 2003, 28: p. 687-690.

91.   Ranke, J., K. Mölter, F. Stock, U. Bottin-Weber, J. Poczobutt, J. Hoffmann, B. Ondruschka, J. Filser, and B. Jastorff, *Biological effects of imidazolium ionic liquids with varying chain lengths in acute Vibrio fischeri and WST-1 cell viability assays*, Ecotoxicology and Environmental Safety, 2004, 58: p. 396-404.

92.   Stolte, S., J. Arning, U. Bottin-Weber, A. Muller, W.-R. Pitner, U. Welz-Biermann, B. Jastorff, and J. Ranke, *Effects of different head groups and functionalised side chains on the cytotoxicity of ionic liquids*, Green Chemistry, 2007, 9: p. 760-767.

93.   Stolte, S., M. Matzke, J. Arning, A. Boschen, W.-R. Pitner, U. Welz-Biermann, B. Jastorff, and J. Ranke, *Effects of different head groups and functionalised side chains on the aquatic toxicity of ionic liquids*, Green Chemistry, 2007, 9: p. 1170-1179.

94.   Farre, M. and D. Barcelo, *Toxicity testing of wastewater and sewage sludge by biosensors, bioassays and chemical analysis.*, Trends in Analytical Chemistry, 2003, 22 (5): p. 299-310.

95.   Ren, S., *Assessing wastewater toxicity to activated sludge: recent research and developments*, Environment International, 2004, 30: p. 1151- 1164.

96.   Gutierrez, M., J. Etxebarria, and L.d.l. Fuentes, *Evaluation of wastewater toxicity: comparative study between Microtox and activated sludge oxygen uptake inhibition*, Water Research, 2002, 36: p. 919-924.

97.   Gray, N.F. and C. O'Neill, *Acid mine-drainage toxicity testing*, Environmental Geochemistry and Health, 1997, 19: p. 165-171.

98.   Stepnowski, P., A. Müller, P. Behrend, J. Ranke, J. Hoffmann, and B. Jastorff, *Reversed-phase liquid chromatography of selected room-temperature ionic cations*, Journal of Chromatography A, 2003, 993: p. 173-178.

99.   *OECD Guidelines for Testing of Chemicals - 209 Activated Sludge, Respiration Inhibition Test*, 1984.

100.   Gorman-Lewis, D.J. and J.B. Fein, *Experimental Study of the Adsorption of an Ionic Liquid onto Bacterial and Mineral Surfaces*, Environmental Science and Technology, 2004, 38: p. 2491-2495.

101.   Huang, H.-C., J.-F. Lee, H.-P. Chao, P.-W. Yeh, Y.-F. Yang, and W.-L. Liao, *The influences of solid-phase organic constituents on the partition of aliphatic and aromatic organic contaminants*, Journal of Colloid and Interface Science, 2005, 286: p. 127-133.

102.   William F. Loomis Jr and B. Magasanik, *Glucose-Lactose Diauxie in Escherichia coli*, Journal of Bacteriology, 1967, 93: p. 1397-1401.

103.   Tchobanoglous, G., F.L. Burtob, and H.D. Stensel, *Chapter 7: Fundamentals of Biological Treatment*, in *Wastewater engineering: treatment and reuse*. 2003, McGraw-Hill Companies Inc. p. 545-658.

104.   Markiewicz, M., J. Hupka, and C. Jungnickel, *Sposób oczyszczania ścieków zawierających ksenobiotyki*, Nr wewnętrzny PG 53/2009.

105.   Haigh, S.D., *A review of the interaction of surfactants with organic contaminants in soil*, The Science of the Total Environment, 1996, 185: p. 161-170.

106.   Providenti, M.A., H. Lee, and J.T. Trevors, *Selected factors limiting the microbial degradation of recalcitrant compounds*, Journal of Industrial Microbiology, 1993, 12: p. 379-395.

107.   Jungnickel, C., W. Mrozik, M. Markiewicz, and J. Łuczak, *Fate of Ionic Liquids in Soils and Sediments*, Current Organic Chemistry, 2011, 15: p. 1928-1945.

108.   Gaterman, R., H. Huhnerfuss, G. Rimkus, M. Wolf, and S. Franke, *The distribution of nitrobenzene and other nitroaromatic compounds in the North Sea* Marine Pollution Bulletin, 1995, 30 (3): p. 221-227.

109.   Voice, T.C. and W.J. Weber Jr., *Sorption of hydrophylic compounds by sediments, soils and suspended solids. I: Theory and background.*, Water Resources 1983, 17: p. 1433-1441.

110.   Bailey, G.W. and J.L. White, *Factors influencing the adsorption, desorption, and movement of pesticides in soils.* , Residue Reviews *1970*, 32: p. 29-92.

111.   Senesi, N., *Binding mechanisms of pesticides to soil humic substances.*, Science of the Total Environment, 1992, 123/ 124: p. 63-76.

112.   Pignatello, J.J. and B. Xing, *Mechanisms of slow sorption of organic chemicals to natural particles*, Environmental Science and Technology 1996, 30: p. 1- 11.

113.   A.T.Aboul-Kassim, T. and B.R.T. Simoneit, *Interaction Mechanisms Between Organic Pollutants and Solid Phase Systems*, in *The Handbook of Environmental Chemistry*. 2001. p. 107-167.

114.   Jungnickel, C., M. Markiewicz, U. Preiss, W. Mrozik, and P. Stepnowski, *Interaction of imidazolium type ionic liquids with the solid phase*, Journal of Optoelectronics and Advanced Materials - Symposia, 2009, 1 (1): p. 82-87.

115.   Stevenson, F.J., *Organic Matter Reactions Involving Pesticides in Soil*, in *Humus Chemistry: Genesis, Composition, Reactions*. 1994, Wiley. p. 453-472.

116.   Tan, K.H., *Chapter 8: Cation exchange*, in *Principles of soil chemistry*. 2011, CRC Press. p. 205-224.

117. Hance, R.J., *Observations on the relationship between the adsorption of diuron and the nature of the adsorbent*, Weed Research, 1965, 5: p. 108-114.

118. Frissel M. J. and G.H. Bolt, *Interaction Between Certain Ionizable Organic Compounds (Herbicides) and Clay Minerals*, Soil Science, 1965, 94: p. 284-291.

119. Tan, K.H., *Chapter 12: Chemistry of Soil - Organic Matter Interactions*, in *Principles of Soil Chemistry*. 2011. p. 293-309.

120. Chorom, M. and P. Rengasamy, *Dispersion and Zeta Potential of Pure Clays as Related to Net Particle Charge under Varying Ph, Electrolyte Concentration and Cation Type*, European Journal of Soil Science, 1995, 46: p. 657-665.

121. Rupprecht, H. and T. Gu, *Structure of adsorption layers of ionic surfactants at the solid/liquid interface*, Colloid and Polymer Science, 1991, 269: p. 506-522.

122. Paria, S. and K.C. Khilar, *A review on experimental studies of surfactant adsorption at the hydrophilic solid–water interface*, Advances in Colloid and Interface Science, 2004, 110: p. 75- 95.

123. Gu, T., B.-Y. Zhu, and H. Rupprecht, *Surfactant adsorption and surface micellization*, Progress in Colloid and Polymer Science, 1992, 88: p. 74-85.

124. Rosen, M.J., *Micelle formation by surfactants*, in *Surfactants and interfacial phenomena*. 2004, Wiley-Interscience. p. 123-200.

125. Johnson, R.A. and R. Nagarajan, *Modeling self-assembly of surfactants at solid–liquid interfaces. II. hydrophilic surfaces*, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2000, 167: p. 21-30.

126. Tiberg, F., J. Brinck, and L. Grant, *Adsorption and surface-induced self-assembly of surfactants at the solid-aqueous interface*, Current Opinion in Colloid & Interface Science, 2000, 4: p. 411-419.

127. Manne, S., J.P. Cleveland, H.E. Gaub, G.D. Stucky, and P.K. Hansma, *Direct Visualization of Surfactant Hemimicelles by Force Microscopy of the Electrical Double Layer*, Langmuir, 1994, 10: p. 4409-4413.

128. Fuerstenau, D.W. and R. Jia, *The adsorption of alkylpyridinium chlorides and their effect on the interfacial behavior of quartz*, Colloids and Surfaces A: Physicochemical Engineering Aspects, 2004, 250: p. 223-231.

129. Stepnowski, P., *Sorption, Lipophiilicity and Partitioning phenomena of ionic liquids in environmental systems*. Thermodynamics, Solubility and Environmental Issues,, ed. T.M. Letcher. 2007: Elsevier B.V.

130. Berry, D.F. and S.A. Boyd, *Decontamination of soil through enhanced formation of bound residues.* , Environmental Science and Technology 1985, 19: p. 1132- 1133.

131. Calderbank, A., *The occurrence and significance of bound pesticide residues in soil*, Environmental Contamination and Toxicology, 1989, 108: p. 71-103.

132. Bollag, J., *Decontaminating soil with enzymes: An in situ method using phenolic and anilinic compounds.*, Environmental Science and Technology 1992, 26: p. 1876- 1881.

133. Dec, J. and J. Bollag, *Determination of covalent and non-covalent binding interactions between xenobiotic chemicals and soil.*, Soil Science 1997, 162: p. 858- 874.

134. Stepnowski, P., *Preliminary Assessment of the Sorption of some Alkyl Imidazolium Cations as used in Ionic Liquids to Soils and Sediments*, Australian Journal of Chemistry, 2005, 58: p. 170-173.

135. Stepnowski, P., W. Mrozik, and J. Nichthauser, *Adsorption of Alkylimidazolium and Alkylpyridinium Ionic Liquids onto Natural Soils*, Environmental Science and Technology, 2007, 41: p. 511-516.

136. Mrozik, W., C. Jungnickel, M. Skup, P. Urbaszek, and P. Stepnowski, *Determination of the adsorption mechanism of imidazolium-type ionic liquids onto kaolinite*, Environmental Chemistry, 2008, 5 (4): p. 299-306.

137. Matzke, M., K. Thiele, A. Müller, and J. Filser, *Sorption and desorption of imidazolium based ionic liquids in different soil types*, Chemosphere, 2009, 74: p. 568–574.

138. Mrozik, W., C. Jungnickel, T. Ciborowski, W.R. Pitner, J. Kumirska, Z. Kaczyński, and P. Stepnowski, *Predicting mobility of alkylimidazolium ionic liquids in soils*, Journal of Soils and Sediments, 2009, 9: p. 237-245.

139. Mrozik, W., A. Kotłowska, W. Kamysz, and P. Stepnowski, *Sorption of ionic liquids onto soils: Experimental and chemometric studies*, Chemosphere, 2012.

140. Zhang, Z., J.-f. Liu, X.-q. Cai, W.-w. Jiang, W.-r. Luo, and G.-b. Jiang, *Sorption to Dissolved Humic Acid and Its Impacts on the Toxicity of Imidazolium Based Ionic Liquids*, Environmental Science & Technology, 2011, 45: p. 1688–1694.

141. Palomar, J., J. Lemus, M.A. Gilarranz, and J.J. Rodriguez, *Adsorption of ionic liquids from aqueous effluents by activated carbon*, Carbon, 2009, 47: p. 1846-1856.

142. Lungwitz, R. and S. Spange, *Structure and Polarity of the Phase Boundary of N-Methylimidazolium Chloride/Silica*, The Journal of Physical Chemistry C, 2008, 112 (49): p. 19443-19448.

143. Atkin, R., V.S.J. Craig, E.J. Wanless, and S. Biggs, *Mechanism of cationic surfactant adsorption at the solid–aqueous interface*, Advances in Colloid and Interface Science, 2003, 103: p. 219-304.

144. Alila, S., S. Boufi, M.N. Belgacem, and D. Beneventi, *Adsorption of a Cationic Surfactant onto Cellulosic Fibers I. Surface Charge Effects*, Langmuir 2005, 21: p. 8106-8113.

145. Grathwohl, P. and B. Susset, *Comparison of percolation to batch and sequential leaching tests: Theory and data*, Waste Management, 2009, 29: p. 2681-2688.

146. Studzinska, S., T. Kowalkowski, and B. Buszewski, *Study of ionic liquid cations transport in soil*, Journal of Hazardous Materials, 2009, 168: p. 1542–1547.

147. Studzinska, S., M. Sprynskyy, and B. Buszewski, *Study of sorption kinetics of some ionic liquids on different soil types*, Chemosphere 2008, 71: p. 2121-2128.

148. Modelli, A., A. Sali, P. Galletti, and C. Samorì, *Biodegradation of oxygenated and non-oxygenated imidazolium-based ionic liquids in soil*, Chemosphere, 2008, 73 (8): p. 1322-1327

149. OECD, *OECD Guideline for testing of chemicals 106 - Adsorption/desorption using a batch equilibrium method*. 2000.

150. Marta Markiewicz, Stefan Stolte, Zofia Lustig, Justyna Łuczak, Michał Skup, Jan Hupka, and C. Jungnickel, *Influence of microbial adaption and supplementation of nutrients on the biodegradation of ionic liquids in sewage sludge treatment processes*, Journal of Hazardous Materials, 2011, 195 (15): p. 378-382.

151. Wang, P. and A.A. Keller, *Particle-Size Dependent Sorption and Desorption of Pesticides within a Water-Soil Nonionic Surfactant System*, Environmental Science and Technology, 2008, 42: p. 3381-3387.

152. Jung-Won Moon, Mark N. Goltz, Kyu-Hong Ahn, and J.-W. Park, *Dissolved organic matter effects on the performance of a barrier to polycyclic aromatic hydrocarbon transport by groundwater*, Journal of Contaminant Hydrology, 2003, 60: p. 307- 326.

153. Finkel, M., R. Liedl, and G. Teutsch, *Modelling surfactant-enhanced remediation of polycyclic aromatic hydrocarbons*, Environmental Modelling & Software 1999, 14: p. 203-211.

154. Lippold, H., U. Gottschalch, and H. Kupsch, *Joint influence of surfactants and humic matter on PAH solubility. Are mixed micelles formed?* , Chemosphere 2008, 70: p. 1979-1986.

155. Schramm, L.L., *Surfactants - Fundamentals and Application in Petroleum Industry*. 2000: Cambridge University Press.

156. Alkan, M., M. Karadas, M. Dogan, and O. Demirbas, *Zeta potentials of perlite samples in various electrolyte and surfactant media*, Colloids and Surfaces A, 2005, 259: p. 155-166.

157. Fuerstenau, D.W. and Pradip, *Zeta potentials in the flotation of oxide and silicate minerals*, Advances in Colloid and Interface Science, 2005, 114-115: p. 9-26.

158. Koopal, L.K., T.P. Goloub, and T.A. Davis, *Binding of ionic surfactants to purified humic acid*, Journal of Colloid and Interface Science, 2004, 275 (2): p. 360-367.

159. Brown, M.J. and D.R. Burris, *Enhanced Organic Contaminant Sorption on Soil Treated with Cationic Surfactants*, 1996, 34 (4): p. 734-744.

160. Ou, Z., A. Yediler, Y. He, A. Kettrup, and T. Sun, *Effects of linear alkybenzene sulfonate (LAS) on the adsorption behaviour of phenathrene on soils*, Chemosphere, 1995, 30 (2): p. 313-325.

161. Prak, D.J.L. and P.H. Pritchard, *Solubilization of polycyclic aromatic hydrocarbon mixtures in micellar nonionic surfactant solutions*, Water Research, 2002, 36: p. 3463-3472.

162. Liu, J.-f., Y.-g. Chi, J.-f. Peng, G.-b. Jiang, and J.A. Jonsson, *Ionic Liquids/Water Distribution Ratios of Some Polycyclic Aromatic Hydrocarbons*, Journal of Chemical and Engineering Data, 2004, 49 (5): p. 1422-1424.

163. Łuczak, J., P. Jaśkiewicz, C. Jungnickel, M. Markiewicz, R. Aranowski, and J. Hupka. *Effect of amphiphilic ionic liquids on solubility of hydrocarbons in the aqueous environment*. in *EUCHEM Conference on Molten Salts and Ionic Liquids 2010*. 2010. Bamberg, Germany.

164. Dukhin, A.S. and P.J. Goetz, *Acoustic and Electroacoustic Spectroscopy*, Langmuire, 1996, 12: p. 4336-4344.

165. O'Brien, R.W., W.N. Rowlands, and R.J. Hunter, *Determining Charge and Size with the Acoustosizer*, NIST Special Publication, 1993, 856: p. 1-22.

166. Ross, D., *Recommended Methods for Determining Soil Cation Exchange Capacity*, in *Recommended Soil Testing Procedures for the Northeastern United States*. 1995.

167. Alkan, M., O. Demirbas, and M. Dogan, *Electrokinetic properties of kaolinite in mono- and multivalent electrolyte solutions*, Microporous and Mesoporous Materials, 2005, 83: p. 51-59.

168. Fuerstenau, D.W., *Streaming potential studies on quartz in solutions of aminium acetates in relation to the formation of hemimicelle at the quartz-solution interface*, The Journal of Phisical Chemistry, 1956, 60: p. 981-985.

169. Chorom, M. and P. Rengasamy, *Dispersion and zeta potential of pure clays as related to net particle charge under varying pH, electrolyte concentration and cation type*, European Journal of Soil Science, 1995, 46: p. 657-665.

170. Duman, O. and S. Tunç, *Electrokinetic and rheological properties of Na-bentonite in some electrolyte solutions*, Microporous and Mesoporous Materials, 2009, 117: p. 331-338.

171. Kaya, A. and Y. Yukselen, *Zeta potential of clay minerals and quartz contaminated by heavy metals*, Canadian Geotechnical Journal, 2005, 42: p. 1280–1289.

172. Yukselen, Y. and A. Kaya, *Zeta Potential of Kaolinite in the Presence of Alkali, Alkaline Earth and Hydrolyzable Metal Ions*, Water, Air, and Soil Pollution, 2003, 145 (1-4): p. 155-168.

173. Sanchez-Martın, M.J., M.C. Dorado, C.d. Hoyo, and M.S. Rodrıguez-Cruz, *Influence of clay mineral structure and surfactant nature on the adsorption capacity of surfactants by clays*, Journal of Hazardous Materials, 2008, 150: p. 115-123.

174. Zadaka, D., A. Radian, and Y.G. Mishael, *Applying zeta potential measurements to characterize the adsorption on montmorillonite of organic cations as monomers, micelles, or polymers*, Journal of Colloid and Interface Science, 2010, 352: p. 171-177.

175. Bauer, D., H. Buchhammer, A. Fuchs, W. Jaeger, E. Killmann, K. Lunkwitz, R. Rehmet, and S. Schwarz, *Stability of colloidal silica, sikron and polystyrene latex influenced by the adsorption of polycations of different charge density*, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 1999, 156 p. 291-305.

176. Laha, S., B. Tansel, and A. Ussawarujikulchai, *Surfactant-soil interactions during surfactant-amended remediation ofcontaminated soils by hydrophobic organic compounds: A review*, Journal of Environmental Management, 2009, 90: p. 95-100.

177. Łuczak, J., M. Markiewicz, J. Thöming, J. Hupka, and C. Jungnickel, *Influence of the Hofmeister anions on self-organization of 1-decyl-3-methylimidazolium chloride in aqueous solutions*, Journal of Colloid and Interface Science, 2011, 362 (2): p. 415-422.

178. Jungnickel, C., J. Łuczak, J. Ranke, J.F. Fernández, A. Müller, and J. Thöming, *Micelle formation of imidazolium ionic liquids in aqueous solution*, Colloids and Surfaces A, 2008, 316 (1-3): p. 278-284.

179. Brändli, R.C., T. Hartnik, T. Henriksen, and G. Cornelissen, *Sorption of native polyaromatic hydrocarbons (PAH) to black carbon and amended activated carbon in soil*, Chemosphere, 2008, 73 (11): p. 1805-1810.

List of figures

List of tables

Curriculum Vitae

Personal data

Name   Marta Markiewicz

Address   Bonin 48,76-009 Bonin, Poland

E-mail   martam@chem.pg.gda.pl

Born   27.11.1983, Koszalin, Poland

Education

Since 2007   Doctoral studies in the Department of Chemical Technology, Faculty of Chemistry, Gdansk University of Technology in Removal of xenobiotics, especially ionic liquids from environmental matrices.

2002-2007   Master degree studies in biotechnological engineering in Department of Biochemistry and Biotechnology, Faculty of Mechanics, Koszalin University of Technology Diploma direction: Protein separation from industrial wastewaters using HPLC, Master thesis : *Analysis of installation for separation of a protein fraction from potato industry wastewater* - under the supervision of prof. dr hab J. Lewosz

2005-2007   Pharmacy College. Diploma exam In 2007.

List of publications

1. Markiewicz M., Z. Lustig, S. Stolte, J. Łuczak, M. Skup, J. Hupka, C. Jungnickel, *Influence of microbial adaption and supplementation of nutrients on the biodegradation of ionic liquids in sewage sludge treatment processes,* Journal of Hazardous Materials, 2011, 195, p: 378-382

2. Łuczak J., M. Markiewicz, J. Thöming, J. Hupka, C. Jungnickel, *Influence of the Hofmeister anions on self-organization of 1-decyl-3-methylimidazolium chloride in aqueous solutions,* Journal of Colloid and Interface Science, 2011, 362 (2), p: 415,422,

3. Jungnickel C., W. Mrozik, M. Markiewicz, J. Łuczak, *Fate of ionic liquids in soils and sediments,* Current Organic Chemistry, 2011, 15(12), p: 1928-1945

4. Dębski B., M. Joskowska, J. Łuczak, M. Markiewicz, R. Aranowski, J. Hupka, *Wyznaczanie współczynników dyfuzji tlenku węgla (IV) przez membrany wysycane cieczą jonową,* Przemysł Chemiczny, 89/11, p.1189-1193, 2010

5. Markiewicz M., Jungnickel C., Markowska A., Szczepaniak U., Hupka J., *1-methyl-3-octylimidazolium chloride – sorption and primary biodegradation analysis in activated sewage sludge.* Molecules, 2009, 14:p. 4396-4405

6. Markiewicz M., Hupka J., Joskowska M., Jungnickel C., *Potential application of ionic liquids in aluminium production – economical and ecological assessment,* Physicochemical Problems of Mineral Processing, 2009, 4, 73-84

7. Jungnickel C., Markiewicz M., Preiss U., Mrozik W., Stepnowski P., I*nteraction of imidazolium type ionic liquids with the solid phase,* Journal of Optoelectronics and Advanced Materials – Symposia, 2009, 1, (1), 82-87

8. <u>Markiewicz M.</u>, Jungnickel Ch., Hupka J., *Sorption of ionic liquids*, Environment Protection Engineering, 2009, 3, 53-64

Conference oral presentations

1. Markiewicz M., Jungnickel C., Arp H.P.: Release of polycyclic aromatic hydrocarbons from soil caused by the presence of submicellar and micellar ionic liquids solutions, International Conference on Materials and Technologies for Green Chemistry: SustainChem2011, 5-9 September 2011, Tallinn, Estonia,
2. <u>Markiewicz M.</u>, Lustig Z., Henke J., Markowska A., Jungnickel C., *Influence of additional carbon/nitrogen sources on biodegradability of ionic liquids*, Science and Technology for Environment Protection SETAC 20th European Meeting, 23-27 May 2010, Seville, Spain
3. <u>Markiewicz M.</u>, Łuczak J., Henke J., Joskowska M. and Jungnickel C., *Ionic liquids – greener solvents of environmental threat?,* 13th International Symposium of Students and Young Mechanical Engineers Advances in Chemical and Mechanical Engineering Gdansk, 20th – 22th May 2010, Poland
4. <u>Markiewicz M.</u>, Jungnickel C., Hupka J., *Biodegradation study of 1-methyl-3-octylimidazoilium chloride.* Interfacial phenomena in theory and practice, Sudomie, Poland, 29.05-04.06.2009.
5. <u>Markiewicz M.</u>, Jungnickel C., Hupka J., *Ionic liquids mobility and biodegradation tests for contamination risk assessment,* 3rd Spring School on Functional interfaces in technology, Sudomie 2008, p. 14

Fellowships and training

1. Centre for Environmental research and Sustainable Technology (UFT), University of Bremen, Germany, 09.2011-12.2011, *Sorption of ionic liquids anions on soils and minerals,*
2. Norwegian Geotechnical Institute, Oslo, Norway, Department of Environmental Engineering, 01.07.2010-30.09.2010 *Enhanced removal of PAH/PCB from soils by ionic liquids,*
3. New European Chemicals Regulation (REACh) and the Globally Harmonized System, 21-22.04.2009, Łódź, Polska

Scholarships and grants

1. INNOdoktorant II edition scholarship awarded by Pomorskie Voivodship's Marshal Office in year 2009/2010
2. Scholarship in program *The development of interdisciplinary doctoral studies at the Gdansk University of Technology in modern technologies* in academic year 2010/2011
3. Scholarship of the Rector of Gdansk University of Technology for scientific achievements in academic years: 2010/2011, 2009/2010 i 2008/2009
4. DAAD (Deutscher Akademischer Austausch Dienst) scholarship for cooperation between Gdansk University of Technology and University of Bremen, 2008-2009
5. Scholarship of the Dean of Faculty of Chemistry, Gdansk University of Technology for scientific achievements in academic year 2008/2009
6. Grant of Polish Ministry of Science and Higher Education: *Bioremediation of soils contaminated with neoteric solvents,* 2009-20011 – co-investigator
7. Grant of Polish Ministry of Science and Higher Education: *Characterization of the mechanism of interaction of ionic liquids with minerals,* 2010-2011 – principal investigator
8. Grant of Polish Ministry of Science and Higher Education: *Influence of ionic liquids on physicochemical and biological parameters of activated sewage sludge,* 2010-2011 co-investigator.