Joana Saraiva Rocha de Almeida
Licenciada em Ciências de Engenharia do Ambiente

Electrodialytic recovery of phosphorus and organic contaminants removal from sewage sludge

Dissertação para obtenção do Grau de Mestre em Engenharia do Ambiente, Perfil de Engenharia Sanitária

Orientadora: Mestre Paula Alexandra Rodrigues e Araújo Guedes, CENSE, FCT-UNL

Co-orientadora: Professora Doutora Alexandra de Jesus Branco Ribeiro, CENSE, DCEA, FCT-UNL

Júri:

Presidente: Professora Doutora Maria Teresa Calvão Rodrigues
Arguente: Doutor Eduardo Manuel Hipólito Pires Mateus
Vogal: Mestre Paula Alexandra Rodrigues e Araújo Guedes

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

Setembro de 2015

Joana Saraiva Rocha de Almeida
Licenciada em Ciências de Engenharia do Ambiente

Electrodialytic recovery of phosphorus and organic contaminants removal from sewage sludge

Dissertação para obtenção do Grau de Mestre em Engenharia do Ambiente, Perfil de Engenharia Sanitária

Orientadora: Mestre Paula Alexandra Rodrigues e Araújo Guedes, CENSE, FCT-UNL

Co-orientadora: Professora Doutora Alexandra de Jesus Branco Ribeiro, CENSE, DCEA, FCT-UNL

Júri:

Presidente: Professora Doutora Maria Teresa Calvão Rodrigues
Arguente: Doutor Eduardo Manuel Hipólito Pires Mateus
Vogal: Mestre Paula Alexandra Rodrigues e Araújo Guedes

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

Setembro de 2015

Electrodialytic recovery of phosphorus and organic contaminants removal from sewage sludge

Acknowledgements

First of all, I express my gratitude towards Professor Alexandra Ribeiro, for receiving me and letting me collaborate with CENSE in this work. Thank you for trust in me and believe in my skills.

I am thankful to Master Paula Guedes. This work was not possible without your help, kindness, availability and constant presence over these six months. Thank you for the encouragement and for pushing me further than I thought I could go.

Additionally, I want to thank *Águas de Lisboa e Vale do Tejo*, namely *Quinta do Conde* wastewater treatment plant and Dr. Cristina Santos for providing the sewage sludge samples to do the experiments for the present dissertation, and also Dr. Olga Paredes and the Laboratory of Control and Processes for the samples characterization.

I would like to acknowledge Professor António Rodrigues for his availability to help in the microbiological part. I want to thank Doctor Eduardo Mateus for the support with the analytical methodologies. Professor Marco Silva is also acknowledged for granting me access to his laboratory and equipment whenever it was needed. I am also thankful to Professor Conceição Santos and Maria Luísa Caldeira for her assistance in the laboratory during colorimetric phosphorus determinations. In addition, thanks to Dr. Maria do Rosário Duarte from the FCT/UNL library for her availability to review the references chapter.

Moreover, thanks to my laboratory colleagues Joana Pinto, Ana Rita Ferreira and Doctor Nazaré Couto. Thank you for your company and good mood during this period as well as for helping me when I needed. Thanks also to Cátia Magro for sharing her personal experience in this matter and for helping me in the laboratory.

A special thank goes to my friends and family. Thanks for always believing in me and put up with me.

Resumo

O fósforo é um macronutriente essencial à vida que provem da fosforite, um recurso não renovável. As lamas de estações de tratamento de águas residuais (ETAR) são um recurso secundário rico em fósforo que pode ser valorizado. Contudo, as lamas podem conter compostos orgânicos, devido ao seu caráter não-polar e hidrofóbico, o que representa um risco ambiental. A presente dissertação pretende estudar a eficiência do processo eletrodialítico (ED) quando aplicado a lamas de ETAR para recuperação de fósforo e remoção de contaminantes orgânicos. Analisaram-se quatro compostos orgânicos: 17*α*-etinilestradiol (EE2), bisfenol A (BPA), cafeína (Caf) e oxibenzona (MBPh). Os ensaios realizaram-se em células com dois compartimentos e uma membrana de troca aniónica, colocando a lama no compartimento do cátodo. Os ensaios realizaram-se durante três dias com lama dopada (seis experiências). Realizou-se um ensaio controlo sem corrente, três ensaios aplicando correntes constantes de 50, 75 e 100 mA e dois ensaios aplicando correntes sequenciais: 50 mA, 75 mA e 100 mA e o inverso (100-75-50 mA). As análises qualitativa e quantitativa dos organismos existentes nas amostras foram também realizadas. No final observou-se um aumento do pH que favoreceu a recuperação do fósforo. Em termos de fósforo, a maior recuperação verificou-se na experiência com 100 mA, onde 70.3±2.0% do fósforo total foi recuperado no eletrólito. Em geral, a degradação dos compostos foi favorecida pela aplicação de corrente. A Caf e o MBPh atingiram percentagens de degradação de 96.2±0.2% e 84.8±1.3%, respetivamente, no ensaio de 100 mA. A degradação do EE2 (83.1±1.7%) e do BPA (91.8±4.6%) foi favorecida pela corrente de 50 mA. Foram identificadas 35 taxa de quatro grupos, contabilizando-se entre 81 600-273 000 indivíduos por grama nas lamas iniciais. Após ED, verificaram-se decréscimos populacionais entre 47-98%. A *Arcella gibbosa* representa 61% do total de organismos observados, revelando-se também a mais tolerante às alterações do meio.

Palavras-chave: Processo eletrodialítico; Lamas de ETAR; Recuperação de fósforo, Contaminantes orgânicos; Comunidade microbiana.

Abstract

Phosphorus is a macronutrient essential to life which comes from phosphate rock, a non-renewable resource. Sewage sludge from wastewater treatment plants (WWTP) is a secondary resource rich in phosphorus that can be valorized. However, organic compounds are detected in sewage sludge, due to its non-polar and hydrophobic character, being considered an environmental risk. The present dissertation aims to study the efficiency of the electrodialytic process (ED) when applied to sewage sludge aiming phosphorus recovery and organic contaminants removal. Four organic compounds were analyzed: 17*α*-ethynylestradiol (EE2), bisphenol A (BPA), caffeine (Caf) and oxybenzone (MBPh). The experiments took place in an ED cell with two compartments and an anion exchange membrane, with the sludge in the cathode compartment. The experiments were carried out for three days with spiked sewage sludge (six assays). One control experiment was done without current, three experiments were carried out applying a constant current of 50, 75, and 100 mA and two experiments were carried out applying sequential currents: 50 mA, 75 mA and 100 mA and the opposite (100-75-50 mA). A qualitative and quantitative analysis of microorganisms existing in the samples was also done. At the end, the pH increased in the sewage sludge favoring phosphorus recovery. In terms of phosphorus, the highest recovery was achieved in the experiment run with 100 mA, where 70.3±2.0% of total phosphorus was recovered in the electrolyte. Generally, compounds degradation was favored by the current. Caf and MBPh achieved degradation percentages of 96.2±0.2% and 84.8±1.3%, respectively, in 100 mA assay. EE2 (83.1±1.7%) and BPA (91.8±4.6%) degradations were favored by 50 mA current. A total of 35 taxa from four different groups were identified, totalizing between 81,600-273,000 individuals *per* gram of initial sludges. After ED, microbial community population decreased between 47-98%. *Arcella gibbosa* represented 61% of the total observed organisms and revealed to be more tolerant to medium changes.

Key words: Electrodialytic process; Sewage sludge; Phosphorus recovery; Organic contaminants, Microbial community.

Table of contents

Page

List of figures

List of tables

Abbreviations and symbols

AC: Activated Carbon

ACN: Acetonitrile

ADLVT: *Águas de Lisboa e Vale do Tejo*

AN: Anion Exchange Membrane

BOD: Biological Oxygen Demand

BPA: Bisphenol A

Caf: Caffeine

CAO: Chemical Advanced Oxidation

CAT: Cation Exchange Membrane

BOD: Biochemical Oxygen Demand

CO_2: Carbon Dioxide

COD: Chemical Oxygen Demand

dSPE: Dispersive Solid Phase Extraction

e^-: Electrons

EE2: 17*α*-Ethinylestradiol

ED: Electrodialytic Process

EPA: Environmental Protection Agency

$FePO_4^{3}$-: Iron Phosphate

GAC: Granular Activated Carbon

H^+: Proton

HNO_3: Nitric acid

$H_2PO_4^-$: Dihydrogen phosphate ion

HPO_4^{2-}: Hydrogen Phosphate ion

H_3PO_4: Orthophosphoric Acid

HPLC: High-Performance Liquid Chromatography

H_2SO_4: Sulfuric Acid

ICP-AES: Inductively Coupled Plasma-Atomic Emission Spectroscopy

K_a: Acid Dissociation Constant

KEPRO: Kemwater Recycling Process

K_{ow}: Octanol-Water Partition Coefficient

LAS: Linear Alkylbenzene Sulfonates

LD: Limit of Detection

LOPROX: Low Pressure Oxidation

LQ: Limit of Quantification

MBPh: Oxybenzone (2-Hydroxy-4- Methoxybenzophenone)

MeOH: Methanol

$MgSO_4$: Magnesium Sulfate

MLSS: Mixed Liquor Suspended Solids

Mw: Microwave

$NaNO_3$: Sodium Nitrate

NaOAC: Sodium Acetate

$(NH_4)_2S_2O_8$: Ammonium persulfate

NP: Nonylphenol

NPE: Nonylphenol Ethoxylates

DO: Dissolved Oxygen

OH^-: Hydroxyl ion

$^{\bullet}OH$: Hydroxyl radical

PAC: Powder Activated Carbon

PAH: Polycyclic Aromatic Hydrocarbons

PCA: Polychlorinated alkanes

PCB: Polychlorinated Biphenyls

PCDD: Polychlorinated Dibenzodioxins

PCDF: Polychlorinated Dibenzofurans

PCN: Chlorinated Naphtalenes

p.e: Population Equivalents

PO_4^{3-}: Phosphate

ppm: Parts Per Million

PSA: Primary and Secondary Amine

R^2: Determination coefficient

Rpm: rotations per minute

RSD: Relative Standard Deviation,

SPE: Solid Phase Extraction

SVI: Sludge Volume Index

TSS: Total Suspended Solids

UV: Ultra-Violet

WWTP: Wastewater Treatment Plant

1 Introduction

Phosphorus (P) is a macronutrient essential to life which primary source is the non-renewable phosphate rock. Recently, phosphate rock was included in the European Union list of 20 Critical Raw Materials (2014). For these, it makes sense to search for secondary sources of phosphorus.

Sewage sludge is a product of wastewater treatment plants (WWTP). Sludge can exhibit different compositions, depending on the type of systems applied and on the main characteristics of the wastewater being treated (Metcalf and Eddy, 2003).

Sewage sludge can be regarded as a P-source as it is rich in phosphorus, an element usually removed from wastewater due to the possible eutrophication of water resources (Cornel and Schaum, 2009). According to Portuguese law (D.L. 348/98 of 9 November), treated water must have a phosphorus concentration under 2 mg/L or a phosphorus removal efficiency over 80%. The techniques to recover phosphorus from WWTPs, namely sewage sludge, have been gaining increasing importance (Cornel and Schaum, 2009) .

However, some types of sludge can be considered hazardous due to the presence of contaminants, such as organic compounds, heavy metals and pathogenic organisms. Since sludge from WWTP is commonly valorized in agriculture and used to make compost, organic compounds can be a problem, not only for the environment but also for human's health, due to their bioaccumulation (Sørensen et al., 2015). The EC Directive (86/278/EEC) limits the concentration values of heavy metals in sewage sludge to be applied to soil, whereas the Portuguese law (D.L. 276/2009 of 2 October) also expands the limitations to organic compounds in the sludge, namely linear alkylbenzene sulfonates (LAS), nonylphenol ethoxylates (NPE), polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), polychlorinated dibenzodioxins (PCDD) and polychlorinated dibenzofurans (PCDF). Still, there is no specific legislation for emerging contaminants.

Electrodialytic remediation (ED) is a technique used to remove contaminants from polluted matrices. This process is based on a combination of the electrokinetic movement of ions with the principle of electrodialysis (Ribeiro and Rodríguez-Maroto, 2006). Recently studies have shown that ED can be applied to recover phosphorus (Guedes et al., 2015; Ebbers et al., 2015) and degrade organic contaminants (Guedes et al., 2015) or remove heavy metals present in sewage sludge (Ebbers et al., 2015).

The influence of the ED in microbial community's behavior was also assessed in the sludge compartment. Microbiological populations are an important parameter during the wastewater treatment and its characteristics can report information about sludge quality (Metcalf and Eddy, 2003). Some species, commonly called "bio indicators", appear in specific circumstances,

providing information about medium conditions. Also, microbiological populations present in wastewater treatment systems can help to improve biodegradation of organic compounds, what drives studies to equilibrate technical processes and appropriate life conditions for microorganisms (Guedes et al., 2015).

1.1 Objectives and research

The present dissertation aims to address the following issues:

a) Is the electrodialytic process efficient for phosphorus recovery from sewage sludge?
b) Can the electrodialytic process help to improve organic contaminants degradation in sewage sludge?
c) How does the electric field affect the microbiological community of the sewage sludge?

In order to find answers to these questions, experimental tests with sewage sludge, collected in a WWTP from *Águas de Lisboa e Vale do Tejo* (ADLVT) located in *Quinta do Conde*, Sesimbra, Portugal, were carried out to evaluate phosphorus recovery and organic contaminants removal. The analysis presented herein is focused on four compounds considered as emerging contaminants known to be endocrine disrupters: 17*α*-ethynylestradiol (EE2), bisphenol A (BPA), caffeine (Caf) and oxybenzone (MBPh) (Peysson and Vulliet, 2013). During all experiments, an evaluation of the microbiological community was also carried out. Laboratory tests were conducted with fresh sewage sludge from the secondary settling tank. Experiments were started on the sampling day in order to minimize physical, chemical and microbiological changes of the sewage sludge.

Six ED experiments (in duplicate) were performed using laboratorial cells with two compartments and an anion exchange membrane. Sewage sludge was spiked with 8 mg/L of each organic compound under study. One control experiment was done without current for three days. Three experiments were carried out during three days with a constant current of 50 mA, 75 mA, and 100 mA. Two experiments were done applying different currents during the three days: one with 50 mA in the first day, 75 mA in the second day and 100 mA in the last day of the experiment and the second with 100-75-50 mA.

First, to achieve the answer for question a), total phosphorus content was determined by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) after daily collecting electrolyte from the cell. The same was done with a sample of the effluent from the first and the last day of the assay. Membranes and electrodes were dipped in acid after each experiment, and the liquid was also analyzed. In addition, when the assay was over, sludge extraction was done by Microwave (Mw) assisted acid digestion. After extraction, sludge was filtered and, the acid used in this process, which contains phosphorus, was also analyzed by ICP-AES for total phosphorus content. Therefore, in order to quantify organic and inorganic phosphorus colorimetric method was applied to final electrolyte, effluent and sludge samples.

Secondly, to answer question b), after ED process has been conducted for three days, sludge, effluent and electrolyte were analyzed. Some steps were done in order to obtain compatible samples with High-Performance Liquid Chromatography (HPLC) system, such as Solid Phase Extraction (SPE), QuEChERS extraction (Quick Easy Cheap Effective Rugged Safe), centrifugation, filtration and samples concentration under nitrogen stream. HPLC allows the analysis of each organic compound under study at specific wave length absorbencies. The correspondent peak is integrated and the area used to determinate the concentration of each organic compound, through a calibration curve primarily calculated.

Finally, in order to find the answer to question c), a microbiological control was done once a day, except when a constant current was applied where in the first day control was done twice, along experiments. Sewage sludge samples were collected daily from each cell (three/four per experiment) and microorganisms were identified and quantified. The results provided an overview of life inside the cell and it was useful to understand the evolution of it over the time.

1.2Dissertation structure

The present dissertation is organized in the following eight chapters:

1. Introduction - work scope and relevance, main objectives and structure;
2. Literature review - description of the central theme, relevant terminology and previous work developed;
3. Materials and methods - description of materials used, characterization analysis, identification and data treatment methods;
4. Results and discussion - presentation of results, hypotheses formulation and their discussion;
5. Conclusions – main outcomes;
6. Future developments;
7. References;
8. Appendix.

2 Literature review

2.1Wastewater treatment evolution in Europe

Over the past years, sanitation conditions have improved due to the direct effects in human's health and in the environment. Water resources in Europe are plenty when compared to other regions of the world. However, this idea has changed, since during the last decades water stress has been growing, not only related to water scarcity but also to quality deterioration. Almost 70% of European population is facing water stress issues today (Bixio et al., 2006). WWTP not only decrease environmental impacts in water resources but also can incentivize the reduction of water consumption and promote its reuse such as in agriculture (irrigation) and for washing equipment inside WWTP (European Comission, 2013). Urban Wastewater Directive defines five requirements to manage wastewater, described in **Table 2.1** (European Commission, 2015).

Table 2.1: Requirements to manage wastewater in Europe

Requirement	Purpose
1	Collection and treatment of wastewater in all agglomerations of >2000 p.e;
2	Secondary treatment of all discharges from agglomerations of > 2000 p.e., and more advanced treatment for agglomerations >10 000 p.e in designated sensitive areas and their catchments
3	A requirement for pre-authorization of all discharges of urban wastewater, of discharges from the food-processing industry and of industrial discharges into urban wastewater collection systems
4	Monitoring of the performance of treatment plants and receiving waters
5	Controls of sewage sludge disposal and re-use, and treated wastewater re-use whenever it is appropriate

In order to guarantee these five requirements, WWTP are built in the main municipalities, to serve inhabitants and offer a good life quality. Different systems and organs can be applied to treat wastewater, depending on region characteristics, such as population equivalents (p.e.) to serve, available area to build infrastructures, wastewater quality and the budget. However, the aim is the same, independently of the main characteristics: WWTP should reduce suspended solids, biodegradable organics, pathogenic bacteria and nutrients (including nitrates and phosphates) before discharging the effluent in municipal sewers or water courses (Metcalf and Eddy, 2003; World Bank Group, 2015).

Due to the improvement in wastewater treatment, sludge production increased over the years, as seen in **Figure 2.1**. Until 1988, solids from wastewater were only removed and disposed by

land application and ocean discharging. However, since the total amount of sludge has increased, during 1988 ocean dumping was banned. Biochemical oxygen demand (BOD) also started to be reduced from the wastewater by this time. In 1993, wastewater treatment plants started to focus on nutrients removal (nitrogenous and phosphorus). It was developed a new regulation by the Environmental Protection Agency (EPA) in order to protect public health and the environment from any adverse effects caused by certain pollutants that might be present in sewage sludge biosolids. This document is known as “The Standards for the Use or Disposal of Sewage Sludge” and refers to the regulation as “the Part 503 rule” or “Part 503”, which encourages sludge land applications (EPA, 2002; Peccia and Westerhoff, 2015).

In 2000, health concerns were intensified with research and inspection being done in this matter, in order to understand and develop ways to protect the environment and human’s health against hazardous biosolids. Now, the challenge is to find a social, economic and environmental sustainability for sewage sludge. In other words, sludge should be converted from a cost to an asset and the reduction of sludge and exploitation of sludge chemical and energy content must be pursued (Peccia and Westerhoff, 2015).

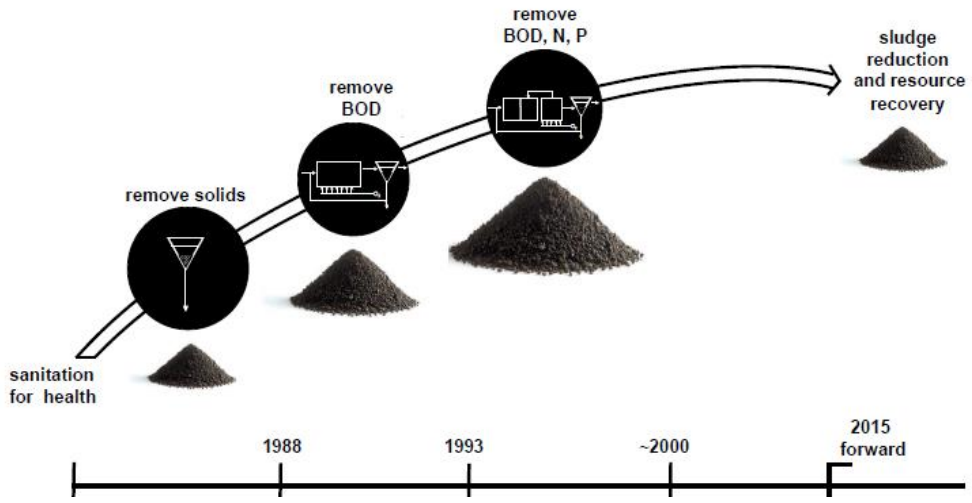


Figure 2.1: Sewage sludge increasing over the years (adaptaded from Peccia and Westerhoff, 2015)

2.1.1 Wastewater treatment stages

Primary treatment is generally the first phase of wastewater treatment. Industrialized countries often start with primary treatment and add other treatment stages as wastewater load grows, as the need for treatment has increased, and as resources become available. Primary treatment is designed to remove gross, suspended and floating solids from raw sewage. It usually includes screening to trap solid objects and sedimentation by gravity to remove suspended solids. It is frequently referred as mechanical treatment, although chemicals may be used to accelerate the sedimentation process. Primary treatment can reduce the biodegradable organics of the

incoming wastewater by 20-30% and the total suspended solids by some 50-60% (Metcalf and Eddy, 2003; World Bank Group, 2015).

After, a secondary treatment is usually applied. This process aims to remove dissolved organic matter that escapes from primary treatment. It is based on biological processes, where microorganisms consume organic matter and convert it into carbon dioxide, water, and energy for their own growth and reproduction. The biological process is often followed by an additional settling tank to remove more suspended solids. Around 85% of the suspended solids and biodegradable organics can be eliminated in this step. Secondary treatment technologies include the basic activated sludge process, the variants of pond and constructed wetland systems, trickling filters and other treatments which use biological activity to break down organic matter (Metcalf and Eddy, 2003; World Bank Group, 2015).

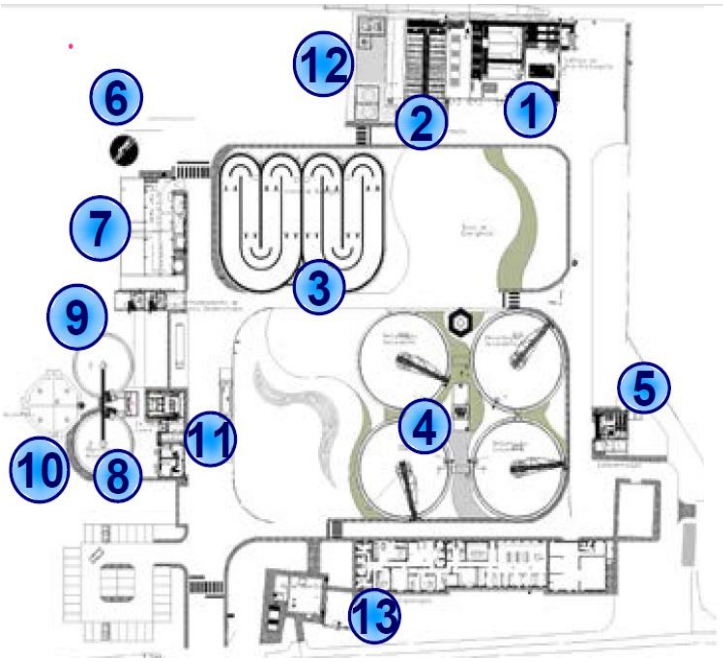
Finally, tertiary treatment usually takes place. In this stage, 99% of all the impurities can be removed, producing an effluent of almost drinking-water quality. The related technology can be very expensive, requiring a high level of technical know-how and well trained treatment plant operators, a steady energy supply, chemicals and specific equipment which may not be readily available. Disinfection, typically with chlorine, can be the final step before discharge of the effluent. However, some environmental authorities are concerned that chlorine residuals in the effluent can be a problem in their own right. As an option, ultra-violet (UV) lights can be applied to disinfect the water, although it is more expensive and needs more maintenance. Disinfection is frequently built but not successfully practiced, because of the high cost of chlorine, or the reduced effectiveness of UV radiation where the water is not sufficiently clear or free of particles (Metcalf and Eddy, 2003; World Bank Group, 2015).

At the same time, when solid phase (sludge) is separated from the liquid phase, in primary and/or secondary treatment, a row of processes are applied to treat the sewage sludge. It is common to drive sludge to a thickening process in order to increase its solids concentration. After, dehydration of the sludge occurs. Here, water percentage of the sludge is reduced and a polyelectrolyte (a reagent) is commonly applied to improve the efficiency of this process. In the end, it is common to add calcium oxide (known as quicklime or burnt lime) in order to sanitize the produced sludge and prepare it to be transported. Additionally, if the treatment plant is dealing with toxic and reactive sludge, a stabilization process is often applied (Metcalf and Eddy, 2003).

2.1.2 Quinta do Conde wastewater treatment plant

Sewage sludge analyzed in the present dissertation was collected in a WWTP located *in Quinta do Conde*, Sesimbra, Portugal (38º34'13'' N, 9º2'7'' W). This WWTP has about 3 years and has capacity to treat a water flow of 19,300 m^3/day, corresponding to 94,000 p.e. Adopted treatment system is based on activated sludge process (medium loading) characterized for two treatment

lines with primary clarification in the beginning and UV disinfection in the end. This WWTP has sludge anaerobic digestion with a cogeneration system (402 kW) and ensures a deodorization system in the infrastructures. There is also a reuse system for service water, with a reservoir and a pressurizing system of the disinfected effluent, which guarantees the quality of the water to be used inside the WWTP (Simarsul, 2012). **Figure 2.2** shows the infrastructures to treat liquid and solid phase, inside *Quinta do Conde* WWTP.



Legend:

1-Mechanical pre-treatment building
2-Lamellar primary settling tank
3-Biological reactors (oxidation ditch)
4-Secondary settling tank
5-UV disinfection
6-Gravity thickener
7-Sludge treatment building
8-Sludge digestion
9- Dehydrated sludge silos
10- Gasometer
11-Cogeneration
12- Deodorization
13- Exploration building (laboratory and atelier)

Figure 2.2: *Quinta do Conde* wastewater treatment plant infrastructures and design (adaptated from Simarsul, 2012)

The infrastructures for the liquid treatment phase are: two sieves and two grit chambers (to remove fat and sand), one receptive compact equipment and treatment of the content from septic tanks cleaning (**Figure 2.2**, 1); two intermediate pumping stations and one more pump group; two primary settling lamellar tanks (**Figure 2.2**, 2); two biological reactors with aeration by bubble diffusers (**Figure 2.2**, 3); four secondary settling tanks (**Figure 2.2**, 4) and two UV disinfection systems (**Figure 2.2**, 5) (Simarsul, 2012).

Pre-treatment and primary treatment are two steps which take place inside buildings or closed tanks, in order to decrease odor emissions and also to improve landscaping. The treatment applied is based on sieving to remove solid particles followed by compact equipment which promote sand and fat removal. Primary treatment occurs in covered lamellar settling tanks. Materials collected as a result of screening operation and sand are conducted to containers and sent to an appropriate final destination. Fats can be sent to anaerobic digestion to be biologically degraded (Simarsul, 2012).

Secondary treatment takes place inside aerobic reactors with suspended biomass, designated oxidation ditch. Here, there are adequate conditions to develop microorganism's populations which ensure biological purification of wastewater. Effluent from these reactors is forwarded to secondary settling tank where phase separation happens. Finally, tertiary treatment is based on UV radiation for disinfection (Simarsul, 2012).

The infrastructures (**Figure 2.2**, 7) provided to treat the solid phase are: one gravity thickener (**Figure 2.2**, 6); two gravity tables sludge thickeners and one storage tank for mix sludge ; two anaerobic digesters (**Figure 2.2**, 8); one digested sludge tank; two horizontal centrifugal decanters ; two silos for sludge storage (**Figure 2.2**, 9) and one silo for quicklime . In addition, associated to the solid phase there is one gasometer (**Figure 2.2**, 10), one biogas treatment system and two cogeneration engines (**Figure 2.2**, 11) that also can work as an emergency system, if electricity from the public network is with problems, for instance (Simarsul, 2012).

Solid phase is treated and biogas produced is valorized. Sludge from primary and biological treatment goes to a thickener and stabilization process is done in anaerobic digesters. Then mechanical dehydration and sludge storage takes place, before it goes to an adequate final destination. During anaerobic digestion biogas produced is collected, stored and, after being treated, it is energetically valorized through a cogeneration system, with a total power of 402 kW. In fact, it is possible to produce electric energy and use thermic energy to warm the digesters. Therefore, emissions of green houses are reduced (Simarsul, 2012).

This WWTP was supposed to serve four municipalities (*Seixal, Sesimbra, Setúbal* and *Barreiro*) although is serving one more (Azeitão). Actually, *Quinta do Conde* WWTP is oversized since the expected flow to be reached during the first year of its exploration was not achieved (Simarsul, 2012). *Quinta do Conde* appeared in 1970, due to clandestine subdivision of one rural property. As a consequence lands were sold, where owners built dwelling houses. It was an illegal genesis as a result of the huge housing crisis which Portugal faced that time. Water streams were commonly conducted to cesspools, decreasing costs for the owners related to sewage management (C.M. de Sesimbra, 2015). *Quinta do Conde* is a region with many cesspools so it is possible that the calculated flow to be treated in the present WWTP has some nonconformities from the real flow. Therefore, there is equipment not working since the flow is not enough to guarantee the parameters such as sewage sludge age, hydraulic loading and adequate microbiology densities inside biological reactor. These parameters have to be guaranteed since they are responsible for the success of wastewater treatment. Now, one oxidation ditch and two secondary settling tanks are stopped. In addition, biogas system production is not rental with the present flow and does not working frequently (it is just working occasionally to keep the equipment active in order to reduce the probability to damage the system) (Simarsul, 2012).

2.2Phosphorus

Commonly, a person obtains 1-1.5 g of phosphorus *per* day from food, and excretes the same amount in urine and feces (Vaccari, 2011). However, this is only about 17% of the amount obtained from mining of phosphate rock. Most of the balance, plus much of the excreted waste, finds its way into the environment, causing excessive growth and eutrophication. Awareness has been growing in specialized circles that phosphorus is not only an environmental pollution challenge, but also a resource challenge (Vaccari, 2011).

Phosphorus is obtained on an industrial scale mainly from rich sedimentary deposits of phosphate rock. Unfortunately, phosphate rock deposits are unequally distributed around the world. This happens due to the requirement for a certain amount of geological and oceanographic circumstances which just can be found in some places. About 20% of all the worlds phosphate rock mined to date came from a small area in central Florida near Tampa, and up to 85% of the remaining available reserves may be under the control of a single country, that of Morocco (which also controls most of neighboring Western Sahara) (Vaccari, 2011).

About 95% of mined phosphorus is used in agriculture, mostly as fertilizer, and there is no alternative. Concentrated phosphate rock resources, like fossil fuels, are nonrenewable on a human time-scale. However, there is a little appreciated renewable source of phosphorus which is the erosion of low-grade rocks that was the pre-industrial source for agriculture and, indeed, for the entire biosphere (Vaccari, 2011).

Phosphorus exists in soils, minerals, living organisms and water, although phosphorus is not found by itself in its elemental form in nature. **Figure 2.3** illustrates phosphorus cycle. The global phosphorus cycle has four relevant components. One is the tectonic uplift and exposure of phosphorus bearing rocks to the forces of weathering. The other is physical erosion and chemical weathering of rocks producing soils and providing dissolved and particulate phosphorus to rivers. Also, riverine transport of phosphorus to flood plains, lakes and the ocean. Finally, sedimentation of phosphorus associated with organic and mineral matter and buried sediments. The cycle is constantly beginning with the uplift of sediments into the weathering regime (Guedes et al., 2014b).

Human activities increased the global phosphorus cycle. Phosphorus inputs from non-point and point sources in the freshwater bodies may lead into an excessive algal growth (eutrophication) in lakes and rivers, decreasing water quality through undesirable color, odor and taste, and possible toxicity to humans, livestock and wildlife (Guedes et al., 2014b).

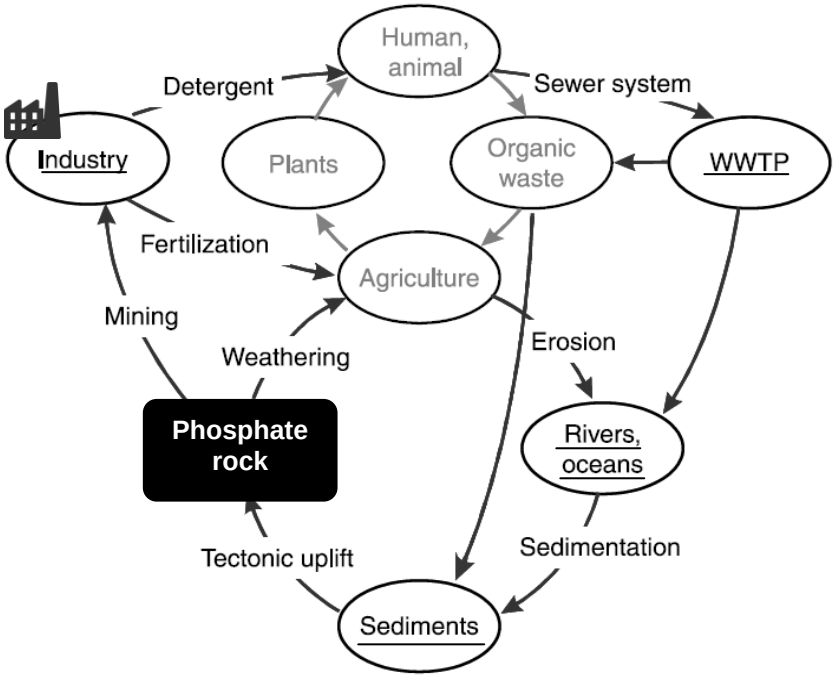


Figure 2.3: Geological and organic phosphorus cycles including human impacts (adapted from Cornel and Schaum, 2009)

The challenge to remove and recover phosphorus from WWTP is nowadays a relevant matter. Examples of applied technologies are chemical precipitation, biological processes or adsorption (Couto et al., 2013). In WWTP without phosphorus removal, 90-95% of the incoming phosphorus load is contained in the sewage sludge. Therefore, concepts to recover phosphorus within the wastewater treatment scheme are attractive, if they promise to obtain a product which is free from contaminants and of a high quality as fertilizer (Blöcher et al., 2012). Phosphorous recovery potential from sewage sludge is significantly higher than with separation processes from the aqueous phase (Cornel and Schaum, 2009).

2.2.1 Phosphoric acid speciation and bioavailability

Aqueous solutions of phosphoric acid contain numerous species in chemical equilibria with each other (acidic dissociations and associations). Orthophosphoric acid, H_3PO_4, is a polyprotic weak acid, which is conversing into different species while environmental pH is changing, as shown in **Figure 2.4** (Cherif et al., 2000).

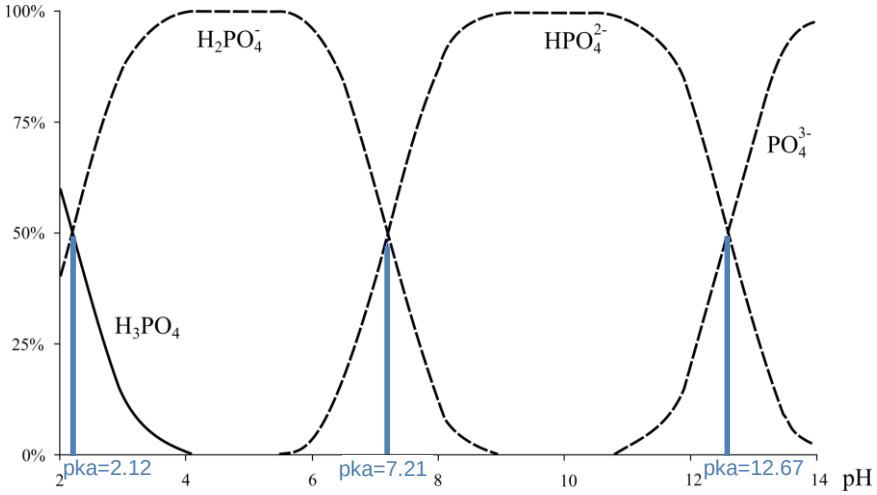


Figure 2.4: Phosphoric acid speciation diagram (adapated from Cherif et al., 2000)

Its stepwise dissociation involves three equilibrium reactions described by the following equations, (2.1), (2.2) and (2.3) (Cherif et al., 2000):

pka= 2.12 $H_3PO_4 \rightleftharpoons H^+ + H_2PO_4^-$ **(2.1)**

pka= 7.21 $H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-}$ **(2.2)**

pka= 12.67 $HPO_4^{2-} \rightleftharpoons H^+ + PO_4^{3-}$ **(2.3)**

Sewage sludge is often used as a fertilizer on farmland due to its considerable phosphorus content. However, just bioavailable phosphorus contributes for nutrient absorbency. Bioavailable phosphorus is defined as the sum of immediately available phosphorus and the phosphorus that can be transformed into an available form by naturally occurring processes: physical (desorption), chemical (dissolution) and biological (enzymatic degradation). Orthophosphate (H_2PO^{4-}, HPO_4^{2-} or PO_4^{3-}) is the only directly available phosphorus source for planktonic algae and bacteria, for example. Still, direct uptake of some organic phosphates cannot be excluded. The rate of orthophosphate mobilization from different phosphorus compounds is highly variable depending on the type of mobilization mechanism involved. Factors like pH, redox conditions, occurrence of chelators and degree of dilution might affect both mobilization and the subsequent uptake of phosphorus (Boström et al., 1988).

2.2.2 Phosphorus recovery procedures

Recovery of phosphorus from sewage sludge has been the focus of many studies, in order to determine the process efficiencies, costs, benefits and the potential for an environmentally beneficial recovery. There are many technological advances for the recycling of phosphorus from the liquid phase, from sewage sludge or from sewage sludge ash. Recovery from the liquid phase is based mostly on precipitation or crystallization processes (Jaffer et al., 2002; Sørensen et al., 2015). Recovery from sludge or ash requires a prior hydrolysis, disintegration and

dissolution (Muller et al., 2008). The phosphorus recovery rate from the liquid phase is around 40 to 50%, while recovery rates from sewage sludge and sewage sludge ash can reach up to 90% (Cornel and Schaum, 2009).

In 1995, a pilot study in Sweden started to develop one of the most known technologies of phosphorus compounds recovery from sewage sludge. The KEPRO process (KEmwater REcycling PROcess) was developed to create organic sludge with a high dry solids content, which can be used as a biofuel; inorganic sludge with a high phosphorus content, which can be used for agricultural fertilizer; and liquid-containing soluble organic matter and the precipitation chemical used in phosphorus removal. The KREPRO process depends on thermal hydrolysis of sewage sludge in a sulphuric acid environment. Sewage sludge thickened to 5/7% of dry mass is mixed with sulphuric acid to a pH between one and three. The acidified suspension is heated in the autoclave (140°C). As a result of the process nearly 40% of organic matter is hydrolyzed into liquid form, easily biodegradable, which can be used as a carbon source in the denitrification processes. Most of the inorganic compounds from sewage sludge are also dissolved. The solution after centrifugation is directed into the reactor, where the pH is raised to the range needed for orthophosphate precipitation in the form of $FePO_4$ (Wzorek and Gorazda, 2007; Stina, 1998).

Another process, PHOXNAN, was also developed and combines a low pressure wet oxidation (LOPROX) with two membrane filtration steps. In the low pressure wet oxidation increased temperature (160-220 ºC) and pressure (12-28 bar) at acidic conditions (addition of sulphuric acid to adjust pH 1.5) are applied for the sludge oxidation with pure oxygen. Under these conditions the content of organic components decreased and PAHs pharmaceuticals or other micropollutants are oxidized. Due to the low pH, phosphate is existent in the solution mainly as H_3PO_4 and $H_2PO_4^-$ (Holzer and Horak, 1994).

The first membrane filtration step uses ultrafiltration membranes to separate the remaining solids from the LOPROX effluent. Permeate from the ultrafiltration contains dissolved phosphate and other dissolved components like ammonium and metal cations (but is free from solids). This effluent enters the second membrane filtration step, which is a nanofiltration membrane. The low pH and therefore high concentration of OH^- ions lead to a high retention for other, especially multivalent cations (Niewersch et al., 2010; Yaroshchuk et al., 2011). At the same time, these conditions result in a relatively low retention for phosphorus, which appears almost exclusively as the neutral phosphoric acid and the monovalent $H_2PO_4^-$. The nanofiltration permeate contains the recovered phosphate while the concentrate is recycled into the wastewater treatment plant after a heavy metal precipitation (Blöcher et al., 2012).

Phosphorus recovery from sewage sludge by wet chemical technology is achieved dissolving sewage sludge in acid or base, in combination with temperature if necessary. Thereby, in most cases (heavy) metals are re-dissolved as well. After the insoluble compounds have been

removed, phosphates can be separated from the phosphorus-rich water, e.g. via precipitation, ion exchange, nanofiltration, or reactive liquid–liquid extraction (Cornel and Schaum, 2009).

2.3Organic contaminants: endocrine disruptors

The quantity of substances frequently introduced into the environment by industrial, agricultural and domestic actions and that have been the topic of human or environmental toxicological studies are present at very low amounts (trace level). In addition, only a small list of these compounds is subjected to regulations. The adverse effects (including endocrine disruption and antibiotic resistance) of some compounds, called “emerging”, have been observed in humans, animals and other organisms (Peysson and Vulliet, 2013).

Endocrine disruptors are any compound that interacts with and disturbs the endocrine system, which means production, release, transport, metabolism, binding, action, or elimination of the natural hormones in the body responsible for the maintenance of homeostasis and the regulation of development process. This occurs through various mechanisms, such as mimicking natural hormones or blocking their receptors (Plotan et al., 2014; Lima et al., 2011). Its effects on human’s health are mostly correlated to breast cancer in women and a decrease reproductive capacity in men (Safe, 2000).

These compounds are released through urban wastewater streams and many of them can go through the water cycle and reach drinking water systems, due to their hydrophilic character and low removal at WWTP. Sewage sludge applied in agricultural land can in fact lead to the contamination by 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) of surface and groundwaters (Stumpe and Marschner, 2009; Lima et al., 2012). Thus, emerging compounds need to be monitored in the environment. The number of families of emerging pollutants increases year by year and includes pharmaceuticals, synthetic musks, preservatives and bactericides, UV filters, polar plasticizers, flame retardants and illicit drugs (Rodil et al., 2012).

The transport and fate of hydrophobic organic contaminants in the environment involve complex phenomena influenced by sorption by soil components, uptake by plants, transport via runoff and leaching, biodegradation, photodegradation, volatilization and chemical degradation (Lima et al., 2010). Adsorption is the most important process that can affect the fate of pollutants in soils and control their distribution in the soil/water environment (Kah and Brown, 2007; Lima et al., 2012).

K_{ow} coefficient consists in the division between the substance not ionized between octanol and water, and it was adopted to measure the hydrophobicity of a chemical compound. When a compound is extremely hydrophobic, it is strongly linked to organic matter and does not dissolve in interstitial water. The list of priority substances of the Water Framework Directive includes substances which have a low solubility in water, a corresponding high log K_{ow} and a high

potential for bioaccumulation and bioconcentration (Miège et al., 2012; Reinhold et al., 2010; Pilon-Smits, 2005).

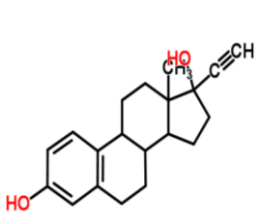
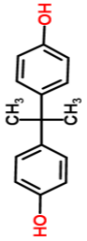
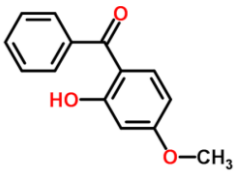
Bioaccumulation occurs within an organism, where a concentration of a substance builds up in the tissues and is absorbed faster than its removal. Bioaccumulation may occur in two ways: by eating contaminated food and/or by directly absorption from water. This second case is referred as bioconcentration. In addition, very often these persistent pollutants are transferred up the food chain faster than they are broken down or excreted. This situation illustrates bioamplification (or biomagnification), when an increase in the concentration of a compound moves up the food chain and could even reach humans (Vermeulen et al., 2010).

A study showed that by 2020 around 43% of the sludge treated in WWTP should be directly used in agriculture activities (European Commission, 2010). However, this practice may be complicated due to public awareness, odors or difficulties of transport/storage and, in other cases it is not possible due to the presence of emerging contaminants. During wastewater treatments, degradation and attenuation remove significant amounts of organic contaminants. However, many of these compounds are transferred to sewage sludge and may be present in residual concentrations in the dry solids, depending on their initial amounts, their lipophilicity and the extent of destruction during treatment (Clarke et al., 2011).

Several organic compounds have already been detected in sludge: organochlorine pesticides (McIntyre and Lester, 1984; Clarke et al., 2010), PCBs (Alcock and Jones, 1993; Wilson et al., 1997), dioxin-like compounds (Sewart et al., 1995; Stevens et al., 2001); and more recently: chlorinated napthalenes (PCNs), PAHs, polychlorinated alkanes (PCAs), synthetic musks (Stevens et al., 2003), oestrogens (Gomes et al., 2009), organotin compounds (Voulvoulis et al., 2004; Voulvoulis and Lester, 2006) and nonylphenol (NP) (Sjöström et al., 2008). The concentrations of PAHs, PCBs and PCDD/Fs in sludge have declined substantially due to effective source control (Wild et al., 1990; Clarke et al., 2010). These compounds are risky to human health and the environment from biosolids land application due to their persistence, potential to bioaccumulate up foodwebs and toxicity (Chaney et al., 1996).

The present dissertation will focus on four specific emerging compounds considered endocrine disruptors: 17α-ethynylestradiol (EE2), bisphenol A (BPA), Caffeine (Caf) and Oxybenzone (MBPh). **Table 2.2** summarizes the chemical, physical and structural properties related to these persistent pollutants, since their destiny and effects are depending on its characteristics.

Table 2.2: Chemical and physical properties of the endocrine disruptores being studied (Sigma-aldrich, 2015; Pubchem, 2015;Chemspider, 2015)

Compound	*17α-ethynylestradiol (EE2)*	*Bisphenol A (BPA)*	*Caffeine (Caf)*	*Oxybenzone (MBPh)*
Structure				
CAS	57-63-6	80-05-7	58-08-2	131-57-7
IUPAC designation	(8R,9S,13S,14S,17R)-17-ethynyl-13-methyl-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthrene-3,17-diol	4-[2-(4-hydroxyphenyl)propan-2-yl]phenol	1,3,7-trimethylpurine-2,6-dione	2-Hydroxy-4-methoxybenzophenone
Formula	$C_{20}H_{24}O_2$	$C_{15}H_{16}O_2$	$C_8H_{10}N_4O_2$	$C_{14}H_{12}O_3$
Molecular weight (g/mol)	296.40	228.29	194.19	228.24
Category	Estrogen	Plasticizer	Stimulant	Sunscreen agent
Water solubility (mg/L)	11.3 (27ºC)	120 (25ºC)	$2.16x10^4$ (25ºC)	69 (25ºC)
pKa (25ºC)	10.33	9.59/11.3	10.4	7.6
Henry's law constant (atm-cu m/mol) (25ºC)	$7.90x10^{-12}$	$1.00x10^{-11}$	$3.58x10^{-11}$	$1.5x10^{-8}$
Log Kow	3.67	3.32	-0.07	3.80
UV light absorption wavelengths (nm)	281	280	273	288

2.3.1 17α-ethynylestradiol

This is one of the most common estrogenic compounds found in the aquatic environment. EE2 is used as a synthetic estrogen in birth control pills, medical treatments of cancer, hormonal imbalance, osteoporosis, and other ailments. EE2 is released from conjugated glucuronides or sulphate complexes present in urine (Feng et al., 2010). Its estrogenic potency is about ten times more than natural hormones (Stumpe and Marschner, 2009).

EE2 is not completely removed by wastewater treatment and persists in treated effluents, due to its resistance to be biodegrade (Meina et al., 2013). The potential for bioconcentration in aquatic organisms is high, since this synthetic hormone is not metabolized by the organisms (Meina et al., 2013; Lima et al., 2011). EE2 interferes with fish endocrine system when concentrations are

over 1 ng/L and can be found in the environment with concentrations of µg/L to ng/L. This compound is also associated to endometrial cancer, in women (Bila and Dezotti, 2007; Daniel and Lima, 2014).

Recently, Directive 2013/39/EU, published that EE2 shall be included in the first watch list, in order to gather monitoring data for the purpose of facilitating the determination of appropriate measures to address the risk posed by the substances.

2.3.2 Bisphenol A

BPA is one of the highest production-volume chemicals over the world. It is commonly used to manufacture polycarbonate plastic and epoxy resins, which can be used as part of impact-resistant safety equipment, sports equipment, as protective coatings in metal food containers, and as composites and sealants in dentistry. In addition, BPA can also be used in the processing of eyeglass lenses, CDs and DVDs, household electronics and thermal paper. Baby bottles were frequently made with BPA but the European Union banned its use in baby bottles. Due to the extensive use of BPA in consumer products, human exposure to BPA is widespread (Miège et al., 2012; Ye et al., 2011).

BPA is an endocrine disruptor which production and use in the manufacturing of materials may result in its release to the environment through several waste streams (Pubchem, 2015). BPA is considered a weak estrogen being toxic when its concentration is over 1000 ppm. Bioconcentration risk in aquatic organisms is low to moderate. Freshwater and saltwater algae, invertebrates (daphnids) and fish are the organisms which can be more affected by BPA concentrations (endpoints of survival, growth and reproductive fitness) (Bisphenol A, 2015). Also, BPA is suspected to induce biochemical changes in brain, immune-modulatory effects and enhanced susceptibility to breast tumors (Miège et al., 2012).

2.3.3 Caffeine

Caf is an alkaloid that acts as a central nervous system stimulant, temporarily warding off drowsiness and restoring alertness. It can be found in drinks (cola drinks, coffee, tea, alcoholic drinks and energy drinks) food (chocolate, pastries, dairy desserts) and medicines (painkillers). This drug is the psychoactive substance more consumed in the world and in high-developed countries, 90% of adults consume Caf daily (Lovett, 2005; Buerge et al., 2003). However, contrasting with other psychoactive substances, Caf is legal. Therefore, there is an important occurrence of this pollutant not only into some industrial effluents but also into domestic wastewaters (Indermuhle et al., 2013).

Due to its high solubility (21.7 g/L) and volatility, Caf is susceptible to be persistent in the environment, and it was detected frequently in treated wastewaters, surface waters and ground waters, in the range of µg/L (Ternes et al., 2001; Buerge et al., 2003). The presence of Caf in surface and groundwater is a proof of the anthropogenic contamination, since Caf is not

consumed by animals or present in fertilizers. In addition, it was demonstrated that Caf is an anthropogenic indicator of surface water contamination by domestic wastewater (Buerge et al., 2003). The potential for bioconcentration in aquatic organisms is low (Pubchem, 2015).

2.3.4 Oxybenzone

MBPh is a high sun protection factor, present in cosmetics such as creams and sunscreens. This compound is able to protect against to UV radiation, UV-B and UV-A. MBPh penetrates the skin, and it can be found in urine, feces, and blood (Magi et al., 2012).

The potential for bioconcentration in aquatic organisms is moderate to high. Coronado et al., 2008, showed that the UV-filter MBPh alters endocrine or reproduction endpoints in two fish species, but at concentrations significantly higher than those measured in the environment. There are different opinions if MBPh is risky to population, as an endocrine disruptor. However, nowadays there are many countries that already regulate its utilization (Jansen et al., 2013).

2.3.5 Organic emerging contaminants removal procedures

Since emerging organic contaminants are often poorly removed in conventional WWTP, advanced water reclamation technologies have been studied. Emerging pollutants removal methods fall into three categories; physical removal, biodegradation and chemical advanced oxidation (CAO). Removal efficiencies of individual emerging compounds vary depending on unit operations and processes commonly used in WWTP. The proper removal process for an individual target compound needs to be carefully selected in accordance with the characteristic property of each persistent pollutant (Chang et al., 2009; Liu et al., 2009).

Physical methods

Activated carbon (AC) is a process for removing several organic contaminants. AC is most frequently applied as a powder activated carbon (PAC), or in a granular activated carbon (GAC), in packed bed filters. Several authors have demonstrated the efficiency of AC (PAC and GAC), for the removal of trace organic pollutants from water (Matsui et al., 2002; Asada et al., 2004; Westerhoff et al., 2005; Zhou et al., 2007). AC also has a strong capability of removing a broad range of representative emerging compounds for artificial and real wastewater in the laboratory, pilot and full-scale plants (Nakanishi et al., 2002; Iwasaki et al., 2001; Zha and Wang, 2005; Choi et al., 2005; Fukuhara et al., 2006; Tsai et al., 2006; Snyder et al., 2007).

The membrane process is also applied for contaminant removal in advanced water and wastewater treatment. Its advantage is the high quality of effluent, including extremely low organic concentration, and removal of microbes and viruses without chemical disinfection (Van der et al., 1998; Snyder et al., 2007). Studies have discovered that the rejection efficiency of emerging pollutants by membranes strongly depended on compounds physicochemical properties (molecule weight, K_{ow}, water solubility, electrostatic property). Emerging compounds

retention by the membrane processes is mainly due to size exclusion, charge repulsion and adsorption. Comparing different membrane types, emerging compounds rejection rate by reverse osmosis is the highest, followed by nano-membrane types, then ultra-membranes, with the rejection of micro-membranes as the lowest (Liu et al., 2009).

Biodegradation methods

The objective of WWTP is to remove organic substances, phosphorus and nitrogen from wastewater. In addition, research has discovered that emerging compounds can also be reduced by wastewater treatment systems. The activated sludge process is the most used in the world, and as the proportion of removal by primary settling, chemical precipitation, aerating volatilization and sludge absorption was small, the majority of the emerging compounds in wastewater is regarded as removed by biodegradation (Svenson et al., 2003; Andersen et al., 2003; Braga et al.,2005). Studies showed that the activated sludge process got the highest estrogenic removal, which can achieve 81% (Svenson et al., 2003).

In addition, a recently study with high-rate algal ponds showed the removal of a wide range of emerging pollutants from urban wastewater with efficiencies over 90%. Results suggest that biodegradation and photodegradation are the most important removal pathway (Matamoros et al., 2015).

Chemical advanced oxidation

There are several studies on the removal of emerging compounds through the use of different chemical oxidants, known as chemical advanced oxidation (CAO). The essential mechanisms of CAO are mineralization of pollutants in wastewater to carbon dioxide (CO_2) or transference of pollutants to some other metabolite products by some strong oxidizers through oxidation–reduction reactions. Therefore, the key for CAO is the choice of the oxidizer. These methods are characterized by the generation of the hydroxyl radical ($^{\bullet}OH$), where the higher redox potential can give better results (Liu et al., 2009).

2.4Microbiology in sewage sludge

Diversity in a WWTP derives principally from activated sludge process, which is essentially characterized by primarily bacteria, ciliated protozoa and metazoa. These organisms are responsible for decomposing biodegradable organic components and remove most of the inorganic elements, such as nitrates and phosphates, from the wastewater (Kocerba-Soroka et al., 2013). Bacteria are the most abundant microorganisms in the activated sludge process (95% of total biomass) and are directly responsible for the mineralization of pollutant matter (Nsabimana et al.,1996).

Protozoa are single-celled microscopic organisms, several hundred times larger than bacteria. They are associated with the age of the activated sludge. There are four types of protozoa

commonly found in WWTP, which can be identified through their method of movement in wastewater environment. The four types are flagellates, amoebae, ciliates (free-swimming, crawling and stalked) and suctoreans (Kerry et al., 1989).

Flagellates have one only nucleus and one or more flagella. In activated sludge process, small heterotrophic flagellates are common and numerous. They can be the only protozoa present in a sludge with loadings over 0.9 kg BOD/kg MLSS*day. Flagellates with bigger dimensions can be observed in sludge from aerated tank, which receive diluted affluent or present low loadings (Kerry et al., 1989).

Amoebae are protozoa without cellular membrane and which movement is based on pseudopods (cytoplasm prominences). This group is abundant in low organic loading systems, where nitrogen removal takes place. On the other hand, when organic loading is high, it is common to find stalked ciliates instead of amoebae (Kerry et al., 1989).

Free-swimming ciliates predominate in initial treatment phases of aeration tanks. While flocculation is increasing, this group is gradually replaced for stalked ciliates. In addition, if ciliates are the dominant group, the quality of the effluent is low (related to extremely high organic loadings). Small ciliates predominate when oxygenation of the aeration tank is not efficient. They are resistant to low quantities of oxygen and toxic compounds, although they need wide bacteria densities to survive. Sometimes, these microorganisms dominate microfauna (Kerry et al., 1989).

Crawling ciliates, jointly with stalked ciliates, commonly share the microfauna dominance in activated sludge. When the organic loading is high there is a decrease in the crawling ciliates densities (over 0.6 kg BOD/kg MLSS*day) while stalked ciliates are more resistant. Some species tolerate low concentrations of metals, such as copper (Kerry et al., 1989).

Stalked ciliates are almost exclusively from subclass *Peritrichia*. They are associated to flocs since they are stuck to them through a peduncle. Some species have a myoneme (contractile helical fibril) in the stalk that allows contraction of the microorganism. When transitory conditions of weak cleaning efficiencies are observed, these ciliates can increase quickly and achieve densities around 80% of the total microfauna (Kerry et al., 1989).

In addition, other ciliates can be found in WWTP, which are not inserted in the groups described above. There are carnivores and omnivores ciliates and they can be free-swimming or stalked. They feed on others ciliates or they capture microorganisms from several groups (Kerry et al., 1989).

Metazoa are multicellular life beings where the several cells form tissues and organs. Since metazoa have a complex organization, their reproduction is slower than in protozoa. Therefore, their presence in activated sludge is limited to some species where the time of generation is

lower. In systems with total oxidation, metazoa are very common to find whereas in conventional systems they are more sporadic (Kerry et al., 1989).

Rotifers are multi-celled (metazoa) organisms also commonly found in activated sludge systems. The most frequently species belong to the genera *Rotaria*. It has a rotary device which provides water filtration and presents chewing mouthparts that works continuously. The relative predominance of these microorganisms is commonly associated with the age of the activated sludge (Kerry et al., 1989).

Nematodes also belong to metazoa group and are vermiforms that develop from eggs. They can be very common in old sludge from activated sludge systems. Nematodes also secrete a sticky substance in order to anchor themselves to a substrate or floc particles so that they can feed without interference by currents or turbulence. A lack of nematode activity or dead and hollow nematodes can be one bio-indicator of a toxic condition that may be developing in the treatment process (Environmental Leverage, 2003).

Filamentous microorganisms, like bacteria and fungi, are important for floc structure. Organic and inorganic particles, together with floc-forming bacteria bound to the structure, create sludge flocs often populated by ciliates and other microorganisms. However, when filamentous microorganisms proliferate excessively, they modify floc structure and deteriorate sludge sedimentation. This occurrence, known as “bulking of activated sludge” being a biological problem in WWTP (Kocerba-Soroka et al. 2013).

Figure 2.5 summarizes and illustrates the most common microorganisms to find depended on sludge age. Additionally, the figure shows the microorganisms that provide better conditions to settler and avoid bulking sludge.

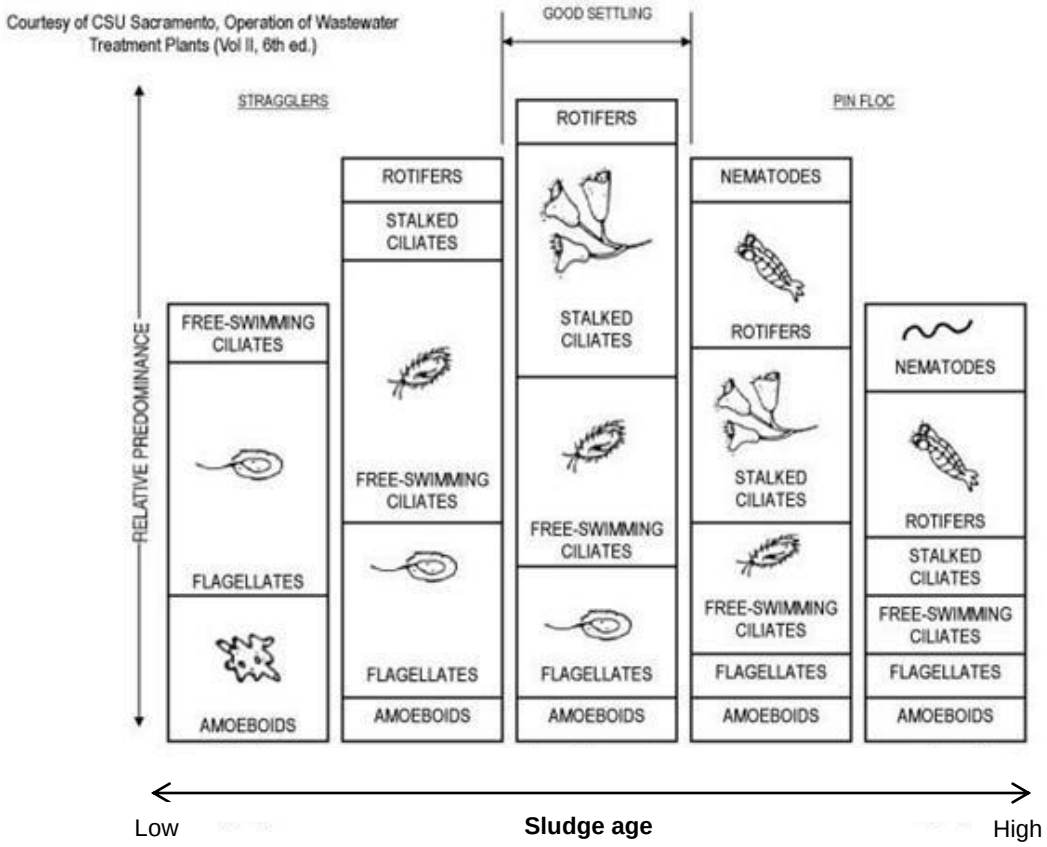


Figure 2.5: Relative predominance of microorganisms in WWTP in function of sludge age (adaptaded from Kerry et al., 1989)

Microalgae are also find in WWTP. Algal systems have traditionally been employed as a tertiary process. They also have been proposed as a potential secondary treatment due to the ability of microalgae to use inorganic nitrogen and phosphorus for their growth. Another reason is justified for their capacity to remove heavy metals and some toxic organic compounds. They produce oxygen and have a disinfecting effect due to increase in pH during photosynthesis. During the last three decades several investigations have described the algal bioassays in response to environmental perturbations and their use as indicator organisms of water quality (Abdel-Raouf et al., 2012).

2.5Electrodialytic process

Electrokinetic remediation uses an electric current density of the order of milliamps per square centimeter applied to the cross-sectional area of a matrix between the electrodes, producing electric potential drops of the order of volts per centimeter. The main goal of this process is to concentrate and confine contaminants close to an electrode and remove them if possible (Ribeiro and Rodríguez-Maroto, 2006).

ED remediation is a technique used to remove contaminants from polluted substrates. This process is based on a combination of the electrokinetic movement of ions with the principle of

electrodialysis. The general principle of the ED process is similar to the electrokinetic remediation process, except from the ion exchange membranes (instead of passive membranes), which are used to separate the contaminated matrix volume (central cell compartment) from the two electrode compartments at the extremes (Ribeiro and Rodríguez-Maroto, 2006).

The first ED remediation experiments were made with soil in 1991 at the Technical University of Denmark (patent PCT/DK95/00209). **Figure 2.6** shows a scheme of a remediation cell with three compartments with an anion exchange membrane (AN) and cation exchange membrane (CAT). Together, with developing the soil remediation, the ED process was also used to remediate other porous materials, such as fly ash (Pedersen, 2002), impregnated wood waste (Ribeiro et al., 2000) and sewage sludge (Jakobsen et al., 2004).

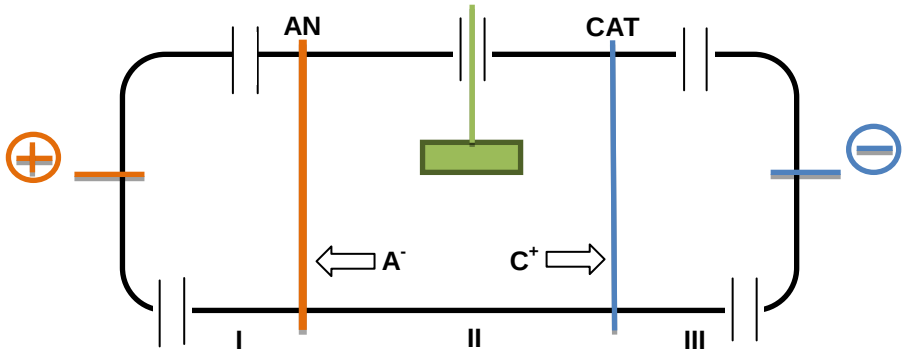


Figure 2.6: Three compartments ED remediation cell with stirrer

In the ED process, the use of selective membranes increases the efficiency of the removal procedure. In an ED cell for remediation purposes, ion exchange membranes should have good conducting properties, to decrease power consumption, and good chemical stability over an extensive pH range and in the presence of oxidizing agents. Also, it must have low degree of swelling in order to avoid problems due to changes in shape and size. Membranes should be characterized by good mechanical stability and the possibility that, after regeneration, the membranes can be reused in the cell in reproducible conditions (Ribeiro and Rodríguez-Maroto, 2006).

An AN, interposed between the anode compartment and the matrix, will prevent cations from passing from this electrode compartment into the matrix. Some authors report that H^+ can be an exception, which explains the acidification of the central compartment environment (Ribeiro and Rodríguez-Maroto, 2006). However, other authors also mentioned that water splitting (water separation into oxygen and hydrogen) at the AN can cause the same effect (Ottosen et al., 2000). Similarly, a CAT, interposed between the cathode compartment and the matrix, will prevent the passage of negatively charged ions into the matrix and will allow the cations to pass from the matrix into the cathode compartment (Ribeiro and Rodríguez-Maroto, 2006).

Figure 2.7 shows a scheme of the remediation cell with two compartments. In order to recover phosphorus through ED process, another cell set-up was recently developed and patented, patent PCT/EP2014/068956 (**Figure 2.7** (A)). This cell set up was created to separate heavy metals from a suspension comprising heavy metal containing particulate material, where heavy metals are removed by use of the ED process. In addition, this cell may also be used for phosphorous recovery when particulate material also comprises phosphorous (Ottosen et al., 2014).

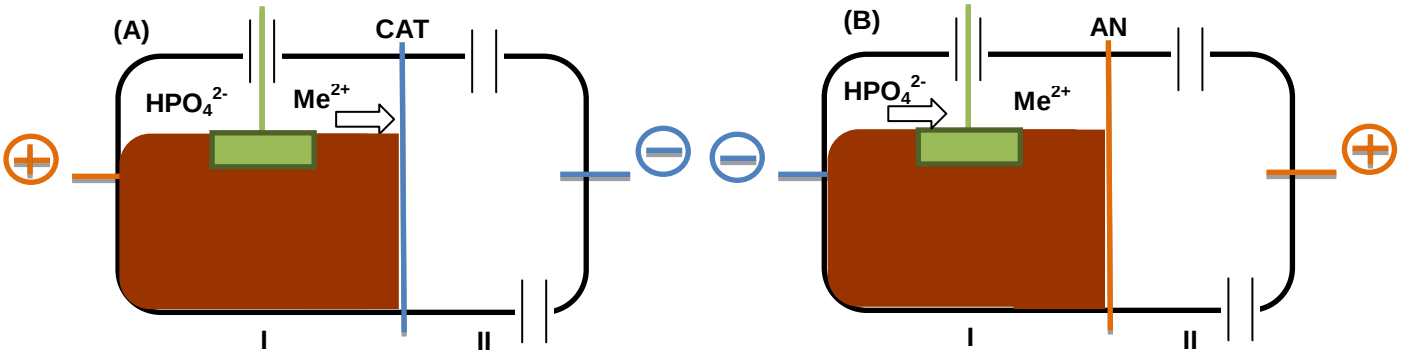


Figure 2.7: Two compartment ED remediation cell

Recently, several ED experiments using different set-ups of a two compartment cell were tested aiming phosphorus recovery and organic contaminants degradation (Guedes et al., 2015). Phosphorus removal and recovery from the studied sewage sludge seems to be more efficient when the matrix is placed in the cathode end and the compartment separation is done by an AN instead of a CAT (**Figure 2.7** (B)). Due to the pH increase, phosphorus is solubilized and electromigrates to the anode end from where it can be recovered. Experimental studies showed that around 80% of phosphorus can be recovered in the anolyte in five days. In addition, this cell design also shows the highest potential for contaminants degradation, since experimental studies show that, in the final sludge, BPA, EE2 and Caf were below limit of quantification (LQ) and limit of detection (LD) after the process takes place. Only MBPh was above the limits (Guedes et al., 2015). Therefore, the experimental assays for the present dissertation were done with the sewage sludge in the cathode end and AN.

Additionally, another study tested a three compartment cell (with a CAT and AN) against to a two compartment cell (with an AN) aiming phosphorus recovery and heavy metal removal from WWTP matrices (Ebbers et al., 2015). Phosphorus extraction was most effective when cell setup had three compartments, where 68% was extracted from raw wastewater after 48 hours. Phosphorus extraction from rejected water in the three compartment cell achieved 95% of removal. In the two compartment cell phosphorus recovery leveled out around 24 hours, with only 67% being extracted after 48 hours. The significant increase of pH provides an explanation of the lower extraction of phosphorus in the second experiment. A high pH is favorable for phosphorous precipitation as calcium phosphate, a form of chemical precipitation also used in

WWTP to remove this nutrient. Furthermore, at a high pH, more OH^- will carry current instead of phosphorus, decreasing extraction efficiency (Ebbers et al., 2015).

2.5.1 Transport of species

Electrokinetic remediation occurs through an electric current density of the order of milliamps *per* square centimeter applied to the cross sectional area of a porous matrix mass between the electrodes. Here, electric potential drops of the order of volts *per* centimeter are produced. Due to the electric field present, when a low-level direct current is passed between a pair of electrodes positioned in a system containing charged particles, the contaminants are driven towards one of the electrodes, from where they may be removed. Three main mass-transports are responsible for this movement: electromigration, electroosmosis and electrophoresis. Diffusion and hydraulic convention are also usually present in some extension (Ribeiro and Rodríguez-Maroto, 2006).

Electromigration

Electromigration is the movement of ions under an applied electric field. In fact, this transport mechanism is dominant in soils with soluble charged species, such as heavy metals cations. Positive ions are driven towards cathode while negative ions are conducted towards anode (Ribeiro et al., 1999).

The migration flux, J_m, is given by:

$$J_m = u^* c \Phi_e \qquad \textbf{(2.4)}$$

where u^* and c represent the ionic mobility and concentration of species and Φ_e the gradient of electric potential.

The current efficiency of electromigration of a specific ionic species is expressed as the proportion of electrical charge carried by the species of interest, relative to the amount of charge carried by all charged species in solution (Ribeiro et al., 1998). Electromigration is the most important transport mechanism for ions in porous matrices and the effective ionic mobility of a specific ion in a soil is a function of its molecular diffusion coefficient, soil porosity, tortuosity factor and charge (Acar and Alshawabkeh, 1993).

Electroosmosis

Electroosmosis describes the mass flux of pore fluid relative to soil particles under the influence of an electric potential gradient. It is the major mechanism in removing uncharged and/or weakly dissociated organic contaminants, such as phenols (Ribeiro et al., 1999). The negative charge at the surface of most soil particles causes an accumulation of positively charged cations near the surface. Under the electric field, these cations will generate a net flow of ions in the direction

of the cathode and water molecules are dragged/pushed toward the cathode by these cations (Acar and Alshawabkeh, 1993). However, at a low range of pH, charge reversal of the soil may occur, and change electroosmotic flow direction (Jensen, 2005).

Soil porosity, texture and macrostructure affect fluid flow under hydraulic gradients. However, electroosmotic flow under electric potential differences depends on the porosity and the zeta potential. It is also dependent on the pore structure and the pore size distribution or the presence of macropores. Electroosmosis is an efficient way to generate a uniform fluid and mass transport in fine-grained deposits (Acar and Alshawabkeh, 1993).

The development of a net charge at the particle surface affects the distribution of ions in the surrounding interfacial region, resulting in an increased concentration of counter ions, ions of opposite charge to that of the particle, close to the surface. Thus an electrical double layer exists round each particle. The liquid layer surrounding the particle exists as two parts; an inner region (stern layer) where the ions are strongly bound and an outer (diffuse) region where they are less firmly associated. Within the diffuse layer there is a notional boundary inside which the ions and particles form a stable entity. When a particle moves, ions within the boundary move it. Those ions beyond the boundary stay with the bulk dispersant. The potential at this boundary (surface of hydrodynamic shear) is the zeta potential (Malvern, 2005).

The electroosmotic flux, J_{eo}, is obtained from the following expression:

$$J_{eo} = -k_e c \Phi_e \qquad \textbf{(2.5)}$$

where k_e is the electroosmotic permeability of soil, c the concentration of species and Φ_e the gradient of electric potential .

The electroosmotic transport of water should be low in order to ensure the matrix volume to maintain a wet interface between the soil and the membrane, to guarantee contact between the soil and the membrane (Ribeiro and Rodríguez-Maroto, 2006).

Electrophoresis

Electrophoresis is the movement of charged colloids under an applied electric field. Charged particles are electrostatically attracted to one of the electrodes and repelled from the other (Ribeiro et al., 1999). For electrochemical soil remediation, electrophoretic transport of negative clay particles is significant only when soil slurries are processed (Acar and Alshawabkeh, 1993).

Diffusion

Diffusion is a transport mechanism that occurs under a chemical concentration gradient. In free solutions and porous media, is usually expressed by Fick's law. In the latter case, the effective diffusion coefficient must be obtained correcting the diffusive coefficient, considering the

porosity and the tortuosity effects, which can decline this movement in more than one order of magnitude (Ribeiro and Rodríguez-Maroto, 2006).

The diffusive flux in soils, J_d, can be calculated by:

$$J_d = -D^* \nabla_C \qquad \textbf{(2.6)}$$

where D^* is the effective diffusion coefficient and ∇c is the concentration gradient.

Generally, this is a secondary transport and can be important only in some areas of soil where gradients are especially high (areas where acid and basic fronts, or metal cations and hydroxyl ions, are to meet) (Ribeiro and Rodríguez-Maroto, 2006).

Electrodialysis

Electrodialysis is a movement derivate from the presence of exchange membranes. This transport mechanism promotes some improvements related to electrokinetics simple process. As CAT and AN are permselective, their use increases transport efficiency of species for the cathode or anode end. Second, there is no flux of the electrolytes between the two electrodes compartments, if membranes are ideally permselective. Third, the matrix is continuously emptied of anions and cations, until there are no ions to be transported, which is indicated by a substantial increase in the volume of the matrix resistance. Forth, the resistance will increase until a certain level and then it will become constant (Ribeiro et al., 1999).

Thus, membranes should have a high exchange capacity to maximize the fluxes of counter-ions and minimize electrical resistance, high selectivity for opposite charged ions and high permeability, not to waste electricity in the transport of co-ions through the membranes and into the matrix volume. The selective membrane bounding the cathode compartment must be resistant to strong bases. On the other hand, the anion exchange membrane must resist to strong oxidant and acidic conditions (Ribeiro and Rodríguez-Maroto, 2006).

2.5.2 Reactions in the electrode compartments

The ED cell has the electrolyte in compartment II (according to the **Figure 2.7**) and this solution is kept re-circulating with a peristaltic pump. Therefore, the build-up of concentration gradients in the vicinity of the electrode is avoided as well as gasses produced by the electrode reactions (Lima, 2008). In fact, electrode reactions are responsible for the variation of pH values during the assays. The electrodes have to be inert in order to guarantee that they will not take part in the electrode reaction. Thus, electrodes are commonly made of carbon, platinum or titanium (Nystrøm, 2001).

The applied electric current normally leads to electrolysis of water at the electrodes, generating an acidic medium at the anode and an alkaline medium at the cathode. Water electrolysis is given by the follow equations (Ribeiro and Rodríguez-Maroto, 2006).

$$\text{Anode: } 2H_2O \rightarrow O_2 + 4H^+ + 4e^- \qquad \textbf{(2.7)}$$

$$\text{Cathode: } 4H_2O + 4e^- \rightarrow 2H_2 + 4OH^- \qquad \textbf{(2.8)}$$

These reactions are the principal reason for the pH changes in the matrix during electrokinetics, when the technique is applied without conditioning the process fluid at the electrodes. The development of the acid and the basic fronts can have a major consequence on the magnitude of electroosmosis, solubility, ionic state and charge, and level of adsorption of the contaminants (Denisov et al., 1996).

If chlorides occur in the solution (in the anolyte), chlorine gas can be produced:

$$\text{Anode: } 2Cl^- \rightarrow Cl_2 \uparrow + 2e^- \qquad \textbf{(2.9)}$$

Additionally, in porous matrices which are rich in phosphorus (such as sewage sludge), phosphorus speciation takes place in the cathode end (compartment I, according to the **Figure 2.7**) (Ribeiro and Rodríguez-Maroto, 2006).

2.5.3 Vantages and limitations of electrodialytic remediation

ED process may be a suitable remediation procedure in solid matrix. Nowadays, there are no other viable *in-situ* methods for treating inorganic and organic compounds in porous media simultaneously. Ionic contaminants are absorbed to sediment particles and are frequently not available for removal by the simple flushing action of water. The pH shift produced by the electrolysis of the water effectively desorbs contaminating ions. This process is an effective method of inducing movement of water, ions, and colloids through fine-grained sediment. Finally, the process is competitive in cost and remediation effectiveness to other methods currently in use (Gardner, 2005).

However, some limitations are associated to ED process. The process is limited by the solubility of the contaminant and desorption of contaminants from the soil matrix. Organic compounds may be tightly bound to natural organic matter. The process is also not efficient when the target ion concentration is low and non-target ion concentration is high. Acidic conditions and corrosion of the anode may create difficulties in *in-situ* efforts. Precipitation of species close to the electrode is an impediment to the process (Gardner, 2005).

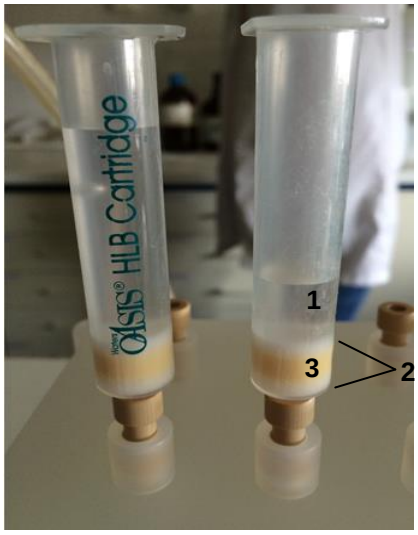
2.6Analytical Techniques

2.6.1 Organic contaminants determination

Solid Phase Extraction

Solid phase extraction, SPE, is commonly used to extract, concentrate and clean up liquid samples. Therefore, SPE is an important tool to prepare liquid samples and extract semivolatile or nonvolatile analytes, although it can also be used with solids that are pre-extracted into solvents. SPE is more efficient than liquid/liquid extraction and can prevent incomplete phase separations, less-than-quantitative recoveries, use of expensive breakable specialty glassware and disposal of large quantities of organic solvents (Sigma-Aldrich Co., 1998).

The SPE is based on disposable cartridges containing silica based chemically bonded sorbents of a suitable size for sample processing by gentle suction (Sigma-Aldrich Co., 1998). **Figure 2.8** shows SPE tubes from OASIS, which are used in the experiments for this work.



Legend:

1-Sample
2-Filter
3-Packing

Figure 2.8: SPE OASIS tubes of 200 mg, 6ml

"QuEChERs"

"QuEChERS" is an acronym that stands for Quick, Easy, Cheap, Effective, Rugged, and Safe. It appeared to aim pesticides removal from food, such as fruits and vegetables, coupled with a cleanup method that removes sugars, lipids, organic acids, sterols, proteins, pigments, and excess water. However, nowadays this technique can be applied to another type of samples, such as sewage sludge (Peysson and Vulliet, 2013).

"QuEChERs" provides the optimization of sludge extraction since it is done an extraction with "salting-out" followed by a cleaning extract step with dispersive solid phase extraction (dSPE). Extraction process is done with an organic solvent, usually acidified acetonitrile (with acetic acid), and salts, such as magnesium sulfate and sodium acetate/ citrate acetate). Additionally,

a propylene tube containing, e.g., primary and secondary amines (PSA), magnesium sulfate and sodium hydroxide is used to remove excess water and unwanted contaminants from the sample extracts (Peysson and Vulliet, 2013).

High-Performance Liquid Chromatography

HPLC is commonly applied to quantify analytes in a sample/mixture by physically separating its components. This method is based on two phases: mobile and stationary. Mobile phase corresponds to an eluent, usually made of water-based solvent, organic solvent or a mixture of both, which drag the several components of the sample. Stationary phase corresponds to the column, where the sample migrates. This separation depends on three main factors: compounds retention time, selectivity or differential migration of analytes in the column and separation power or column efficiency (Dong, 2013).

This method can be applied to diverse analytes or sample types. It is precise, flexible, customizable, automatic and has highly reproducible quantitative analyses and separation power with sensitive detection (through UV lamps, for example) (Dong, 2013).

2.6.2 Phosphorus determination

Microwave Assisted Acid digestion

This microwave extraction method is designed to mimic extraction using conventional heating with nitric acid, or alternatively, nitric acid and hydrochloric acid. It is intended to provide a rapid multi-element acid extraction or dissolution prior to analysis. Digests produced by the method are suitable for analysis by spectroscopic methods, such as inductively coupled plasma atomic emission spectrometry (ICP-AES) (EPA, 2012).

This analytical technique has some advantages. It is easy to carry out, allows simultaneous extractions of several samples, only small quantities of solvents are required and extraction time is short. On the other hand, disadvantages related to this process are the insufficient selectivity of extraction, extract must be separated from post-extraction residue, cleanup step is needed, thermolabile compounds cannot be used and the time for the vessel to cool down can be very long (Wilkowska and Biziuk, 2011).

Inductively Coupled Plasma-Atomic Emission Spectrometry

Its high specificity, multi-element capability and good detection limits result in the use of the technique in a large variety of applications. All kinds of dissolved samples can be analyzed, varying from solutions containing high salt concentrations to diluted acids. A plasma source is used to dissociate the sample into its constituent atoms or ions, exciting them to a higher energy level. They return to their ground state by emitting photons of a characteristic wavelength depending on the element present. This light is recorded by an optical

spectrometer. When calibrated against standards the technique provides a quantitative analysis of the original sample (Phillips, 2013).

Colorimetric Method

Phosphorus, specifically in phosphate form, can be quantitatively determinate by volumetric, gravimetric and colorimetric methods. Colorimetric method is based on a combination of phosphate ions with ammonium molibdate, under acid conditions, in order to produce a complex compound (ammonium phosphomolibdate). Molybdenum present in phosphomolibdate can be rapidly reduced, generating a blue color solution (proportional to the concentration of phosphate in the sample). Absorbencies are read in a spectrophotometer to achieve phosphorus concentrations in the sample (through calibration curves previously determined). Total and inorganic phosphorus can be converted in phosphate form acidifying and boiling the samples. Hydrolysis can be faster by promoting the heating of the samples in autoclave (Sawyer and McCarty, 1978).

3 Material and methods

3.1Sewage sludge sampling procedure

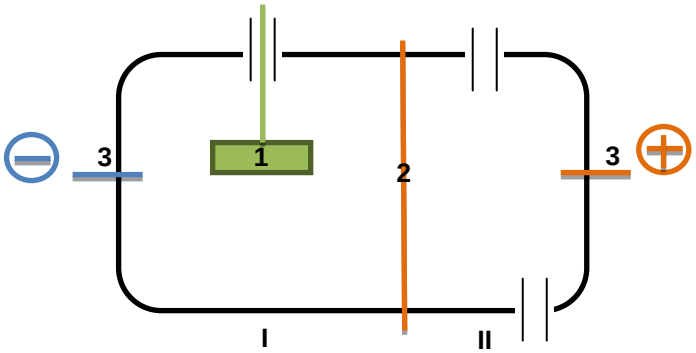
The sewage sludge samples were collected at a WWTP from ADLVT (described in section 2.1.2), located in *Quinta do Conde*, Sesimbra, Portugal (38º34'13'' N, 9º2'7'' W). The sampling of the sewage sludge was carried at the secondary settling tank (**Figure 2.2**, 4) between March and July 2015, following the recommendations of the norm NF EN ISO 5667-15 (October 2009) on the conservation and treatment of sludge and sediment samples.

3.2pH desorption tests

To determine the pH dependent P desorption, 4 g of filtered sewage sludge were suspended in 20 ml of different concentrations of HNO_3 and NaOH as well as in deionized H_2O, in order to have solutions with pH between 1 and 14. The suspensions were placed at an agitating table for 24 hours at room temperature. At the end of the experiment, pH was measured, suspensions were filtered by vacuum using 0.45 µm filters and P concentrations measured by ICP-AES.

3.3Electrodialytic laboratory cell

The experiments were carried out in a two compartment laboratorial cell. **Figure 3.1** shows ED laboratory cell used in the experiments. The two compartments had an internal diameter of 8 cm, an electrode each, and were separated by a commercial anion exchange membrane (AR204 SZRA B02249), from Ionics. The cathode compartment, where the sludge was placed, had a length of 10 cm and was equipped with a stirrer, whereas the electrolyte compartment had a length of 5 cm. The electrodes were platinized titanium bars, with a 3 mm diameter and a length of 5 cm (Bergsøe Anti Corrosion A/S, Herfoelge, Denmark).

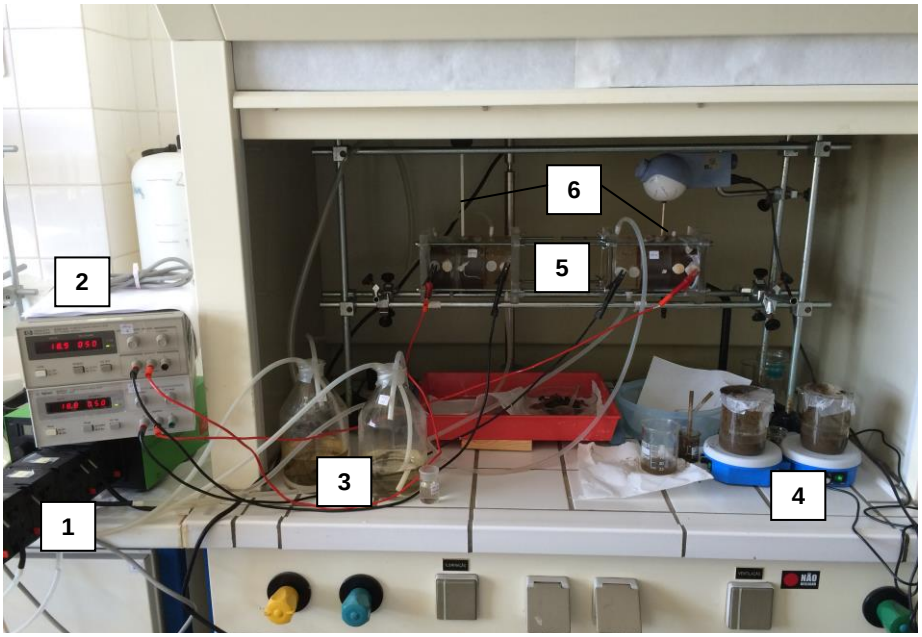


Legend: **1-**Agitator; **2-** Anion exchange membrane; **3-**Electrodes; **I-**Cathode compartment (sludge) and **II-**Anode compartment (electrolyte).

Figure 3.1: Laboratory cell scheme

A power supply (Hewlett Packard E3612A, Palo Alto, USA) was used to maintain a constant current and the voltage drop was monitored (Kiotto KT 1000H multimeter). The fresh electrolyte

was a 10^{-2} M $NaNO_3$ solution with pH 7, being circulated by means of a peristaltic pump (Watson-Marlow 503 U/R, Watson-Marlow Pumps Group, Falmouth, Cornwall, UK), with one head and two extensions. In addition, to do microbiological control, sludge was distributed into two beakers of 200 ml, which were placed in magnetic agitators. **Figure 3.2** shows the set-up of the several assays.



Legend: **1-**Peristaltic pump; **2-** Power supply; **3-**Electrolyte bottles; **4-**Control beakers; **5-**two compartment ED cells and **6**-agitators.

Figure 3.2: Experimental set-up

3.4Electrodialytic experimental conditions

The sludge cell compartment (cathode end) was filled with around 350 g of sewage sludge (90–95% water). For this, a funnel was placed in the agitator hole and the sludge was poured through it and, since the sludge was extremely thick, 50 mL of deionized water were used to help the passage through the funnel. Prior to the beginning of the experiments, the sludge was spiked with a mixture of the contaminants under study. In order to assess the contaminants' mobilization and remediation/degradation under the influence of ED (one of the study goals), the sewage sludge was spiked to have a concentration of approximately 8 mg/L of each analyte in 1:1 MeOH:Acetone. This was done to assure that all the contaminants would be detected or quantified in all cell compartments at the end of the experiments (sludge, effluent and electrolyte), even in cases of high degradation efficiencies and to test the limits of the technique by using a highly contaminated matrix.

In addition, two beakers, for microbiological control, were filled with approximately 100 g of sewage sludge and 18 mL of deionized water were added. One beaker was spiked with the mixture of contaminants under study (8 mg/L) and the other was not doped.

Six ED experiments (in duplicate) were performed during three days. All the assays were performed in a fume hood protected from the sunlight. One control experiment was done without current for three days. Three experiments were carried out during three days with a constant current of 50 mA, 75 mA, and 100 mA. Two experiments were done applying different currents during the three days: one with 50 mA in the first day, 75 mA in the second day and 100 mA in the last day of the experiment and the second with 100-75-50 mA.

During the experiments voltage drop, conductivity and pH were measured twice a day (morning and at the end of the afternoon). To measure the pH and conductivity, samples from the sewage sludge and electrolyte were collected to a small beaker. After doing the measurements, samples were returned to the cell. At the end of the experiments the electrolyte samples (anolyte) were collected and their pH and volume registered. The sewage sludge in the cell was collected and pH and volume were registered too. Sludge was centrifuged during five minutes with 10,000 rpm and also filtrated under vacuum to separate the solid (sludge) from the liquid phase (effluent).

The filtrated sludge was then extracted (two replicates) for organic contaminants determination (according to section 3.5.5). The remaining sludge sample was then dried in an oven at 50 ºC during two days (until constant weight). Finally, for phosphorus determination, the dried sludge sample was extracted as described in section 3.5.2.

The pH of the liquid samples, effluent and electrolyte, was manually adjusted to pH 2, when applicable, and then filtered through a filter (pore size 0.45 µm) prior to extraction as described in section 3.5.5 for organic contaminants and 3.5.2 for phosphorus determination. Membranes and electrodes were soaked in HNO_3 (1 and 5 M, respectively) for 24 hours and the liquid was used for further phosphorus analysis.

For microbiological analysis (described in section 3.6), sludge samples were collected and prepared once a day (T0, T1, T2 and T3) during the time that the ED process was running (three days) except when a constant current was applied where in the first day control was done after 8 hours of experiment (T½).

3.5Analytical methodologies

3.5.1 Chemicals and standards

Chemicals used to develop the present dissertation were caffeine (≥90%), BPA (≥99%), EE2 (≥98%), which were purchased from Aldrich (Steinheim, Germany), and MBPh (≥98), purchased from Alfa Aesar. All used solvents were from sigma-Aldrich (Steinheim, Germany), Panreac (Barcelona, Spain) and Merk (Darmstadt, Germany). Acetonitrile (ACN), methanol (MeOH) and acetone were gradient grade. Water used for analyte extraction and their analytical determinations was deionized and purified with a Mili-Q plus system from Millipore (Bedford, MA, USA), formic acid (LC-MS grade), acetic acid (LC-MS grade) and sodium hydroxide were

purchased from sigma-Aldrich. Sodium nitrate was reagent grade from Panreac. Individual stock solutions for calibration purposes were prepared at 400 mg/L in methanol and stored at -18ºC. Working solutions were prepared by the adequate mixture and dilution of the stock solutions.

3.5.2 Total phosphorus extraction and measurement

Liquid samples (effluent and electrolyte) were directly filtered through a filter (pore size 0.45 µm) and stored until analysis. The phosphorus content was measured at 178.229 nm in an ICP–AES, Varian 720-ES. Solid samples were pre-treated to be converted into a liquid sample appropriate to be analyzed in ICP-AES, which is explaining in section 3.5.3.

3.5.3 Microwave-assisted extraction

The concentration of phosphorus in the solid phase of the sludge was determined after a pre-treatment of the sludge in accordance to EPA3051 (EPA, 2012): 0.5 g of dry sludge and 10 mL of HNO_3 (65%) were placed in a vessel and extracted in a Mw from Milestone Ethos (Bergamo, Italy). The Mw program was set to reach 175ºC in 15 minutes and then keep the temperature for another 15 minutes. The samples were then collected, filtered through a filter (pore size 0.45 µm), diluted to 50 mL and stored in the fridge (-18ºC) until analysis.

3.5.4 Colorimetric method

In order to evaluate the quantity of organic and inorganic phosphorus, the colorimetric method (according to Sawyer and McCarty, 1978) was applied to the effluent, electrolyte and sludge (liquid solution) after being filtered through filter (pore size 0.45 µm). In addition, it was done a control using a blank (deionized water) and a standard solution (2.5 µg $P\text{-}PO_4^{3-}$ in 50 mL). Concentrations of the several phosphorous species in the samples were obtained according to calibration curves shown in **Table 3.1**.

Table 3.1: Calibration curves for phosphorous species

Phosphorous	Calibration curve*	R^2
Total	y = 0.0621x - 0,.0031	0.9997
Inorganic	y = 0.0633x – 0.0008	1.000
Orthophosphates	y = 0.0631x - 0.0015	1.000

*where *y* is the absorbency (nm) and *x* the concentration (µg/L) of phosphorous in the sample

In order to quantify orthophosphates, samples were dilute in water till 50 mL (to provide values inside the calibration curve, between 2 µL and 30 µL). The solutions were transferred to 100 mL Erlenmeyer flasks. A colorimetric reagent (50 ml of H_2SO_4 5 N, 5 mL of potassium antimony tartrate solution, 15 mL ammonium molybdate solution and 30 mL of acid ascorbic solution) was prepared and 8 mL were added to each solution. After 10 minutes, the absorbance of the

developed color was measured in a Pharmacia LKB Ultrospec Plus Spectrophotometer (880 nm wavelength). A blank with reagent was used as reference.

In order to quantify inorganic and total phosphorus from the samples, samples were pipetted to different flasks (between 4 µL and 200 µL). For inorganic phosphorus determination, 75 ml of deionized water and 1 mL of an acid solution (nitric and sulfuric acid) were added to the sample to promote acid hydrolysis and flasks were covered. On the other hand, for total phosphorus, 75 ml of deionized water, 1 mL of H_2SO_4 solution and 0.4 g of solid $(NH_4)_2S_2O_8$ were added.

Samples were then placed in autoclave, with a pressure between 98 and 137 kPa, for 30 minutes. After cooling down, one phenolphthalein drop was added to the samples. The mixture was neutralized with NaOH 6 N, until light pink and the samples transferred to a 100 mL volumetric flask, and deionized water was added. With a volumetric pipette, 50 mL of each sample were transferred to 100 mL Erlenmeyer flasks and 8 mL of colorimetric reagent were added. After 10 minutes, the absorbance of the developed color was measured in the spectrophotometer (880 nm wavelength), using a blank with reagent as a reference.

3.5.5 Organic contaminants determination

Aqueous samples

The extraction of the analytes present in the electrolytes and effluent (aqueous phase of the sewage sludge) was performed by SPE, using Oasis HLB (200 mg, 6 ml) from Waters (Saint-Quentin En Yvelines Cedex, France). The SPE cartridges were conditioned by washing with 3x 6 mL of MeOH, followed by re-equilibrium with 3 x 6 mL of Milli-Q water. For organic compounds enrichment, the samples were acidified to pH 2 before extraction, using nitric acid. The aqueous samples, 200 mL, were passed through the cartridge at a flow-rate of approximately 10 mL/min by applying a moderate vacuum. After that, the cartridges were dried for approximately 2 minutes under vacuum. The retained analytes were eluted sequentially with 2 x 3 mL of MeOH. All the extracts were collected as one and concentrated under a gentle stream of nitrogen till 0.5 mL. Samples were transferred to a vial and kept at -18 ºC until analysis.

Solid samples

Sludge (solid phase of the sewage sludge) extraction was performed using QuEChERS extraction (adapted from Peysson and Vulliet, 2013). Extract tubes were obtained from Waters (Dublin, Ireland). The acetate buffer contained 1.5 g NaOAc and 6 g $MgSO_4$. The dispersive phase contained 150 mg PSA and 900 mg $MgSO_4$. A 2 g aliquot of homogenized sludge was weighed in a 50 mL polypropylene centrifuge tube containing NaOAc and $MgSO_4$. Then, 20 mL ACN + acetic acid 1% (v/v) were immediately added and manually shaken for 15 seconds and then swirled on a vortex mixer for 45 seconds to homogenize the sample. The extract was centrifuged at 10,000 rpm for 5 minutes. A 9.5 mL aliquot of the supernatant (ACN phase) was

transferred to a 15 mL centrifuge tube containing the PSA and $MgSO_4$ and was manually shaken for 10 seconds and swirled on a vortex mixer for 60 seconds. After this step, the extract was centrifuged again (10,000 rpm for 5 minutes) and 8 mL supernatant were transferred to a 12 mL glass tube. The extract was evaporated under a gentle stream of nitrogen till 0.5 mL. All samples were stored at -18 ºC until analysis.

3.5.6 Analysis by HPLC for organic contaminants determination

HPLC was performed on a Finnigan MAT HPLC system (Thermo Scientific, USA) equipped with a SP P4000 Pump, a AS 3000 Auto- sampler, the diode array detector (DAD) was a TSP SpectraSYSTEM UV6000LP with the wavelength set between 200 and 800 nm and a TSP SN 4000 Interface. The contaminants separation was carried out using Chromolith High Resolution RP-18e column with 100 mm x 4.6 mm from VWR (Darmstadt, Germany) and Onyx Security Guard C18 cartridges (5 - 4.6 mm) from Phenomenex (Torrance, USA).

All HPLC runs were performed at a constant flow rate of 1 mL/min, in gradient mode, with the oven set to 38 ºC. The eluents used were a mixture of ACN/MiliQ water/Formic acid (solution A: 5/94.5/0.5%; solution B: 94.5/5/0.5%). Solution A pH was 3.2 and Solution B was 3.6. Formic acid solution (50% in water) was from Fluka. The gradient run was set to: 5 min; 97% A from 0 to 15 min, then to 95% B until 50 min, where it was held until 53 min, then to 97% A until 55 min. The system re-equilibration was performed for 5 minutes with 97% A. All operations and data analysis were processed by the Xcalibur software v.1.3. (Thermo Scientific, USA).

Calibration curve and limits of detection and quantification

A calibration curve for Caf, BPA, EE2 and MBPh was prepared with six standards (0.5; 2.0; 3.5; 5.0; 6.5 and 8.0 ppm) of each compound in MeOH. A linear regression with the six standards was done and curve equations were obtained. **Table 3.2** shows calibration curves for each compound.

Table 3.2: Calibration curves obtained for Caf, BPA, EE2 and MBPh

Compound	Calibration curve*	R^2
Caf	y = 35092x - 7955.7	0.9973
BPA	y = 14181x - 2264.9	0.9987
EE2	y = 5311.6x - 1913.9	0.9872
MBPh	y = 143600x – 20666	0.9818

*where *y* is the area (count) and *x* the concentration (ppm) of the compound in the sample

The LD were calculated through the residual standard deviation of the calibration curve of each compound. On the other hand, LQ was obtained using the LD. **Table 3.3** shows the LD and LQ for BPA, EE2, Caf and MBPh in HPLC system.

Table 3.3: Limits of detection and quatification of BPA, EE2, Caf and MBPh (adaptated from Guedes et al., 2015)

Compound	LD* (mg/L)	LQ** (mg/L)
BPA	0.14	0.42
EE2	0.60	1.8
Caf	0.11	0.34
MBPh	0.16	0.47

*LD=3s_x, where s_x is the residual standard deviation **LQ=3LD

Method recoveries

Recovery assays were done to validate analytical processes, since they reflect the quantity of each analyte recovered during the process comparing to the real quantity present in the sample (Mesquita et al., 2003). Liquid and solid samples recoveries were calculated for each organic contaminant (Recovery %= [(obtained value - real value)/ (real value)] x100). Therefore, effluent and sludge samples were prepared adding a known concentration of a solution with BPA, EE2, Caf and MBPh (400 mg/L). **Table 3.4** shows the method recoveries percentages for each contaminant and matrix under study.

Table 3.4: Recoveries for BPA, EE2, Caf and MBPh

Compound	Recovery (%)		
	Effluent	Electrolyte	Sludge
BPA	85±13	96±10	87±5
EE2	87±9	87±13	84±7
Caf	86±12	97±9	99±4
MBPh	84±10	84±5	94±7

3.6Microbiological analysis

Sludge samples (2 g) were collected from each ED cell and beaker. A dilution was done, adding 6 ml of deionized water to the sample. One drop of the solution was applied in a lamina, covered with a lamella and observed in a *Leica* ATC 2,000 microscope to identify the organisms. The software LAS (Leica Application Suit) version 2.1.0 (2012) was used to record microbiological diversity observed in the microscope, through a camera (Leica EC3). The quantification of the organisms was performed using the inverted microscope method by Utermöhl (adapted from Lund et al, 1957).

In addition, 200 µl of the previous dilution were collected and a new dilution was prepared in a 50 ml volumetric flask. One drop of Lugol solution (Boney, 1975) was added to the flask in order to stop microbiological activity and preserve the samples. Then, 2.5 ml of the mixture were collected to a sedimentation chamber, with a diameter of 2.5 cm, and, after 4 hours (the time to

let the organisms to sediment), quantification was done. An inverted microscope from *Leica* (DMIL) was used to quantify the density of the microorganisms.

3.7Statistical analysis

In order to validate the results obtained during the experiments, significant differences between the samples were evaluated through ANOVA tests. The level of significance considered was 5% and it was applied Tukey Test using GraphPad software (version prism 6) to evaluate statistically significant differences among phosphorus and organic contaminants distribution within the cell compartments and organic contaminants degradation percentages.

4 Results and discussion

4.1 Initial sludge sample characterization

The several assays were conducted with fresh sewage sludge in order to minimize changes related to chemical, physical and microbiological properties. Therefore, the sludge samples from *Quinta do Conde* WWTP were collected from the secondary settler tank at 10 a.m, refrigerated and transported to the laboratory, where the experiments started at 11 a.m. During 72 hours the experiment ran continuously.

It is important to take into account that the experiments were carried out during spring and summer, and some variations in the weather affected sludge composition, such as thickness and microbiological diversity. Consequently, the sludge samples used presented differences between all assays (heterogeneous along the weeks).

Table 4.1 shows the initial characterization of the several sludge samples collected for each experiment. In all collected samples, high dry matter content is expected as, during biological treatment, many of the dissolved solids are incorporated into the cellular structures of microorganisms (Ebbers et al., 2015). Therefore, all samples presented an elevated TSS content, being the maximum 33,600 mg/L in control experiment (relative standard deviation, RSD ± 14%).

Total phosphorous content is higher in the samples used in the 50 mA experiments, 51.1±8.5 g/kg, and 100 mA experiment 56.9±1.3 g/kg (RSD ± 9%). The ability to conduct an electric field was somewhat constant along the several sludge samples, varying between 872 and 1,054 µS/cm (RSD, of ± 6%). Additionally, pH was also similar in all the initial samples, varying between 6.4 and 6.8 (RSD ± 2.5%).

Table 4.1: Inicial sewage sludge sample characterization

Assay	TSS (mg/L)	Total phosphorus (g/kg)	pH	Conductivity (µS/cm)	Day temperature T_{max}/T_{min} (ºC)
Control	33,600	45.5±3.1	6.4	872	26/17
50 mA	32,600	51.1±8.5	6.5	1,054	19/13
75 mA	28,250	45.5±3.0	6.7	983	26/13
100 mA	32,550	56.9±1.3	6.7	977	25/15
50-75-100 mA	27,400	41.0±5.5	6.8	989	27/17
100-75-50 mA	22,700	40.9±6.4	6.8	974	28/17

4.2 pH and conductivity variations along the experiments

During the ED assays, pH variations were recorded along the experiments (**Figure 4.1**). Since the sewage sludge was placed in the cathode compartment, it is visible a pH increase over the time in all experiments. This is mainly due to the continuous generation of OH^- that are produced by water electrolysis. The use of an AN prevents the migration of H^+ ions, from the anolyte compartment.

This results in a continuous pH increase till the 72 hours. The pH variation obtained in the experiments with an applied current was between 9.8±0.7 (100-75-50 mA) and 10.6±0.5 (50 mA), without statistically differences ($p<0.05$). It was observed the mean pH between 50 mA and 100 mA assays was the pH values recorded during the experiment conducted with 75 mA.

In addition, pH variations were also recorded in the electrolyte (**Figure 0.1**, appendix). When current was applied, generation of H^+ increased pH in this compartment, varying between 2.3 and 2.8 at the end of the assay (RSD ± 7%).

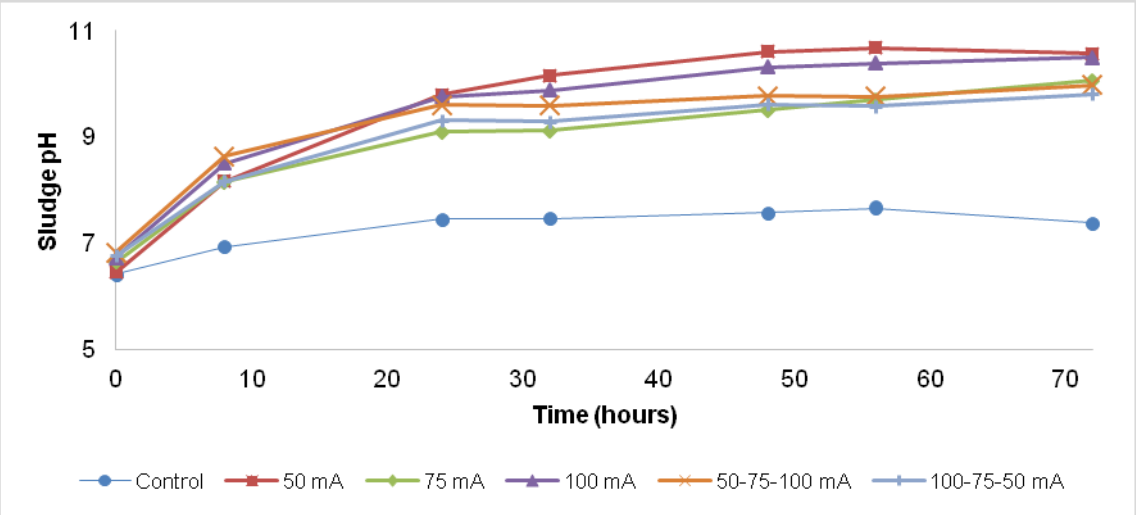


Figure 4.1: Development of pH in the sewage sludge compartment during ED

In the control experiment conductivity slightly increased at the end of the three days, 1,703 µS/cm. Since there is no electric field applied in this experiment, these differences may be explained by the composition of the initial sample. Sewage sludge may contain other ions, which may increase conductivity in the medium.

Sewage sludge conductivity tends to increase during the firsts 24 hours after which it is kept somewhat constant (**Figure 4.2**). This increase may be explained by the ions generation due to water electrolysis (H^+ and OH^-) and ions formation due to pH changes. The highest conductivity was achieved when 75 mA were applied (3,228 µS/cm) with the lowest being obtained in the experiment with 50 mA (1,502 µS/cm). As the current was kept constant, an increase in

conductivity means a decrease in the voltage drop between the working electrodes and, according to Ohm's law, a decrease in suspension resistivity. This was probably due to an increase in the amount of ions present in the sludge suspension.

In the electrolyte compartment (**Figure 0.2**, appendix), when current was applied, conductivity also tends to increase along the 72 hours, varying between 3,303 and 5,222 µS/cm (RSD ± 18%).

These differences observed between experiments may be related to the initial sewage sludge composition and consequent amount of compounds (ions) solubilized (due to the pH changes). The amount of mobilization of ions out of the sludge compartment to the electrolyte may be reflected by a decrease in conductivity.

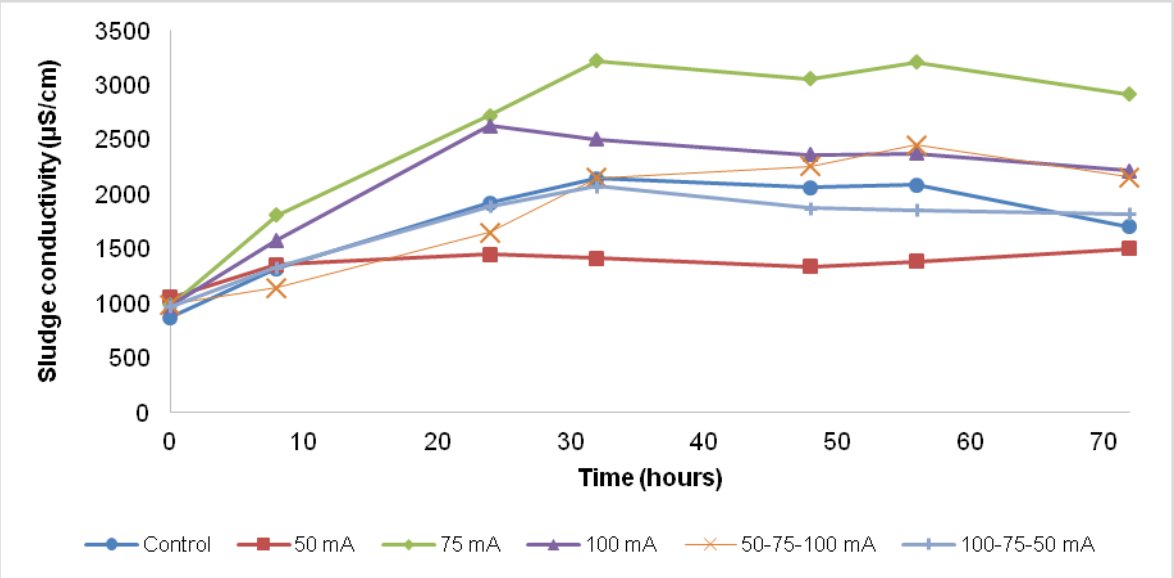


Figure 4.2: Development of conductivity in the sludge compartment during ED

4.3Identification and quantification of microbial communities in the ED cell

Since biological processes are extensively used in WWTP, samples were analyzed under the light microscope to observe how ED would affect the microbial community. In fact, these changes may affect the bioremediation potential of the contaminants, which is an important factor that needs to be considered when further experiments are being designed.

Samples were immediately observed after being collected in the WWTP. After the six experiments, it was possible to identify 35 taxa, belonging to four different groups: Algae, Protozoa, Bacteria and Metazoa, as shown in **Table 4.2**. In total, between 81,600-273,000 individuals *per* gram of initial sludge were observed and counted. **Tables 0.1, 0.2, 0.3, 0.4, 0.5** and **0.6** (appendix) shows the taxa and its quantification for experiment, over the time.

Table 4.2: Identified taxa *per* groups*

Algae	Metazoa
Filamentous algae n.i.; Filamentous Chloroficea n.i.; Centric Diatom n.i.; *Navicula* spp.; *Nitzschia* spp.; *Pirobotrys* sp.; *Euglena* sp.	Nematode: Free-living nematode n.i.; *Toxocara canis* (egg) Rotifers: *Philodenia* sp.; *Rotatoria* sp.; Rotífer (*mastax*); Rotífer (egg); Rotífer n.i. Tardigrade n.i
Bacteria	**Protozoa**
Cyanobacteria n.i.; *Oscillatoria* spp.; *Spirilum* sp.; *Spirulina* sp.; *Zoogloea* spp. (dendritic and globular growth)	Amoeba: Naked amoeba n.i.; *Arcella gibbosa; Difflugia* sp. Crawling ciliates: *Aspidisca cicada* Free-swimming ciliates: *Litonotus* sp.; *Didinium* sp. Stalked ciliates: Pendunclate cilliate n.i.; *Epistylis* spp.; *Opercularia* sp.; *Vorticella* spp.; *Zoothamnium* spp. Suctoreans: *Podoprhya* spp. Flagellates: *Bodo* sp.

*Pollen grains (*Pinus* spp.) were also identified

Samples are not homogenous, since environmental conditions were different for each sample collecting date and microbial community is sensible to these changes. **Figure 4.3** shows the initial microbiological characterization (*per* groups) for the six experiments. In the beginning (T0), it is possible to identify individuals from protozoa group in each studied sample. A special attention goes to control, 50 mA and 75 mA, where the percentage of protozoa individuals is between 98% and 99%.

Bacteria also assume a relevant position in microbial characterization in 100 mA, 50-75-100 mA and 100-75-50 mA assays. However, Bacteria group was not entirely analyzed in the experiments, e.g. filamentous bacteria. Bacteria are the most abundant microorganisms in the activated sludge process, representing around 95% of total biomass (Nsabimana et al.,1996) and should be considered in future developments.

Algae were found in the initial sample of the experience 50-75-100 mA, being this assay doted of a larger biodiversity in the beginning. In contrast, 50 mA and 75 mA samples have the smallest diversity. Metazoa have no significant expression in the initial sludge samples.

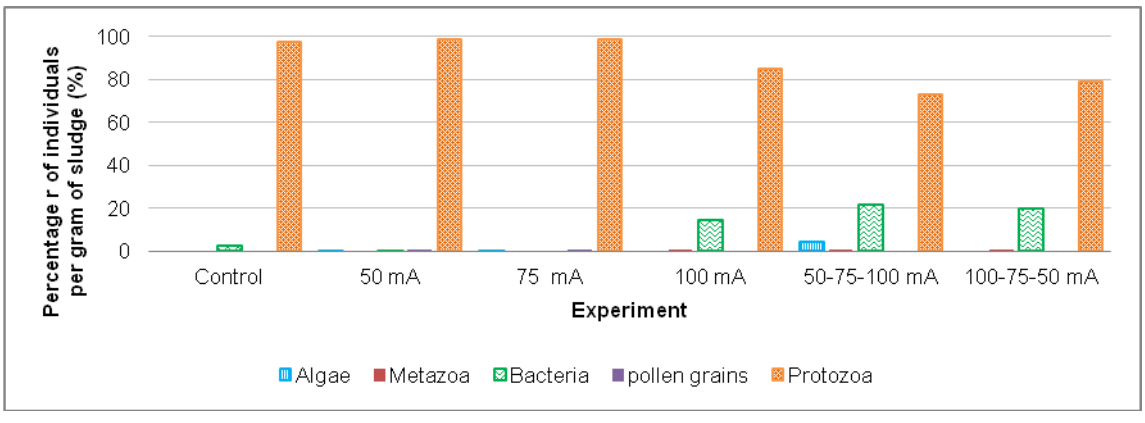


Figure 4.3: Percentage of individuals per gram of sludge in the begining of the experiments (T0)

Figure 4.4 shows the overall microorganisms declining along 72 hours of experiment. It is visible that after three days individuals tend to disappear. An exception is seen in the

experiment conducted with 75 mA, where an increase of individual is seen after half a day. This may have to do with the fact that after six hours microorganisms are more visible, since flocs are less dense and more dispersed. On the other hand, some studies have shown that the application of a low level of electrical field may enhance microorganisms' activity in wastewater (Zeyoudi and Hansan, 2014). Nevertheless, after 24 hours, individuals present in the 75 mA assay started to decrease until the end of the experiment.

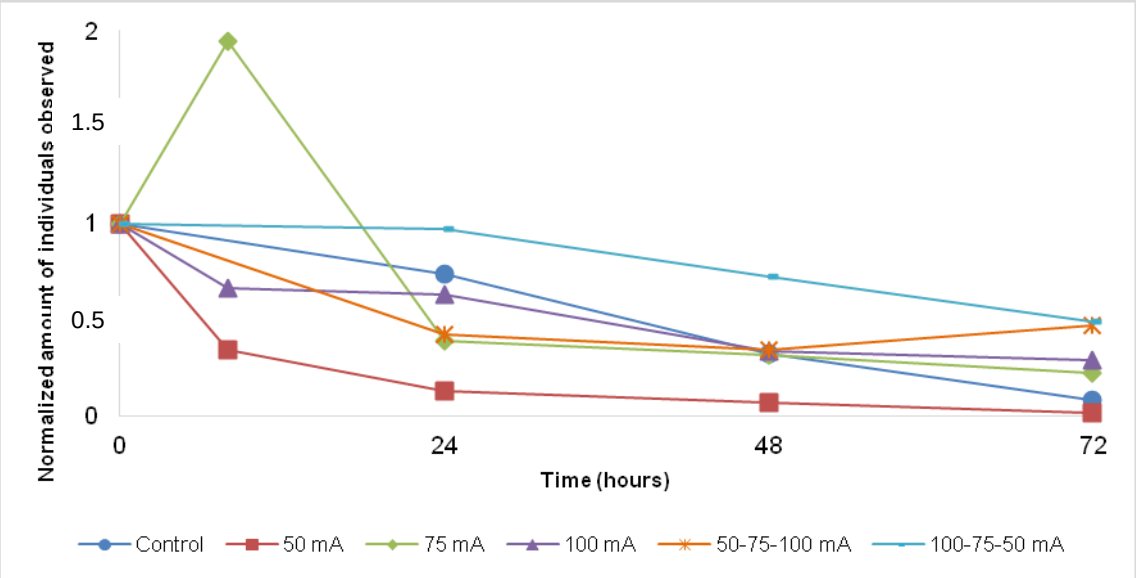


Figure 4.4: Amount of individuals normalized to the initial amount counted in the sewage sludge samples

Control experiment totalized 829,500 microorganisms *per* gram of sewage sludge (**Table 0.1** in appendix). In the begging of the assay (T0), Bacteria (*Zoogloea* spp.) and Protozoa (amoebae, free-swimming ciliates, stalked ciliates and flagellates) were identified. Stalked ciliates were the most abundant group and *Vorticella* spp. (**Figure 4.5**) was the most common specie, representing 33% of the total individuals *per* gram observed in this experiment.

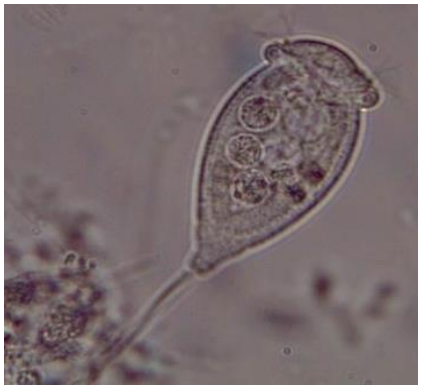


Figure 4.5: *Vorticella* sp. (ampliation of 110x)

After 24 hours, it was possible to identify other microorganisms, namely belonging to Algae group, *Navicula* sp., *Nitzschia* spp. and *Euglena* sp. (**Figure 4.6**). *Euglena, Nitzschia* and *Navicula* are microalgae that can be used as indicators of water pollution (Abdel-Raouf et al., 2012). Therefore, the quality of the sludge after 24 hours may have been deteriorated. The most abundant organisms were again stalked ciliates, but this time *Epistylis* spp. (**Figure 4.7**).

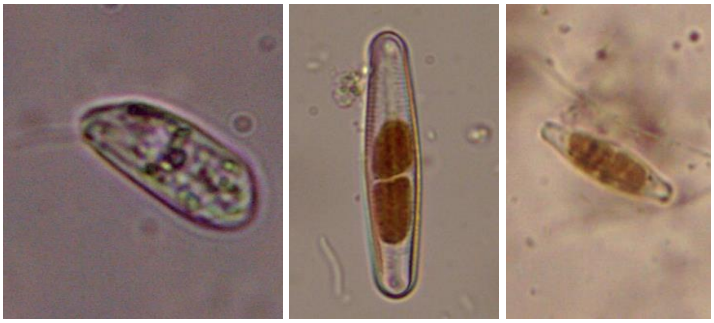


Figure 4.6: *Euglena* sp. on the left (ampliation of 290x), *Navicula* sp. in the middle (ampliation of 290x) and *Nitzschia* spp. on the right (ampliation of 110x)

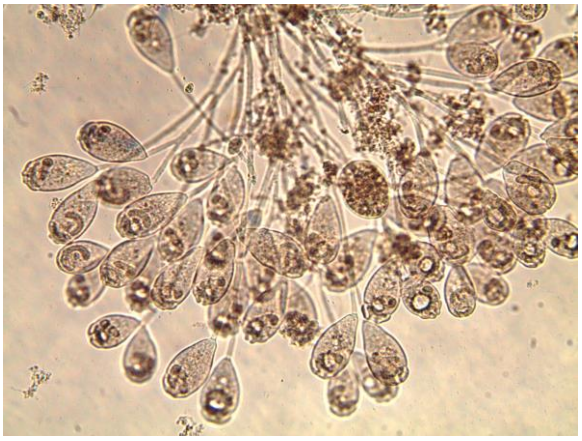


Figure 4.7: *Epistylis* spp. colony (ampliation of 290x)

The second day was characterized by microbial community reduction of 68%, in relation to T0. This may occur because ED medium conditions are different from the ones that these organisms find in a WWTP. The agitation in the ED cell may be not efficient to the community's needs, since the agitator is placed in the only hole existent in the cell. Therefore, oxygen quantities are very limited inside the ED cell. In the other hand, temperatures are higher than in secondary settling tank and since the experiment was performed in a fume hood protected from the sunlight, some organisms may not survive with these conditions. After 48 hours *Vorticella* spp. was the most common genera, representing 32% of the total individuals *per* gram observed after two days.

At the end of the control experiment, pollen grains and rotifers were also observed. Since the experiment took place during spring and the area where the WWTP is located is full of pines,

pollen grains may have been dragged by the wind and fell into the tanks. In addition, nematodes and rotifers (**Figure 4.8**) were observed in this experiment. These microorganisms are a bioindicator that sludge age is old (Kerry et al., 1989; Environmental Leverage, 2003). Control beakers (doped and non-doped) showed a similar microbiological characterization. After three days, control beaker doped had more 38% microorganisms than the non-doped One possible explanation is that microbiological community may have used doped solution to their own growth, besides flocs. Therefore, doped control has more available substrate than non-doped control, which may have enabled to feed more microorganisms. In addition, bioremediation may have been present during the ED process.

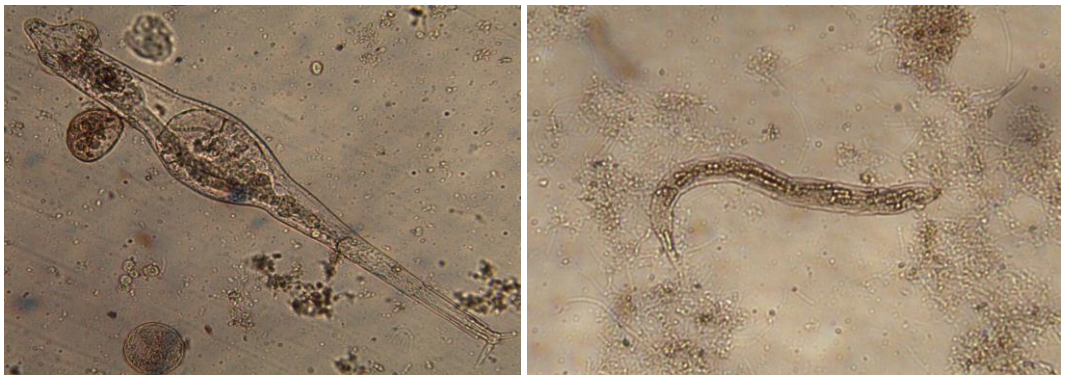


Figure 4.8: Rotifer on the left (ampliation of 110x) and nematode on the right (ampliation of 110x)

The experiment conducted with a current of 50 mA totalized a number of microorganisms of 557,550, according to **Table 0.2** (appendix). This experiment shows the lowest total of individuals *per* gram. In the begging of the assay (T0), Algae (*Navicula* spp.), Bacteria (*Zoogloea* spp.), Protozoa (amoebae, crawling ciliates, free-swimming ciliates, stalked ciliates, suctoreans and flagellates) and pollen grains were identified. Stalked ciliates were again the most abundant group and *Epistylis* spp. were the most common species identified, with a representativeness of 42% of the total individuals *per* gram identified.

Nematodes and rotifers were just observed after half a day and nematode eggs after 24 and 48 hours of experiment. On the other hand, rotifers mastax (mouthparts) is shown on **Figure 4.9** and appeared after 24 hours, which means that rotifers did not survive to the adverse conditions.

Figure 4.9: Rotifer mastax (ampliation of 290x)

Crawling and free swimming ciliates were just observed in the initial sample. It was possible to identify *Aspidisca cicada* and *Litonotus* sp. (**Figure 4.10**). These species are very common in activated sludge systems (Kerry et al., 1989). But there metabolism and physiognomy was not resistant enough to endure medium changes. Only 4% of the total individuals present in the final sample are *Aspidisca cicada* and *Litonotus* sp.

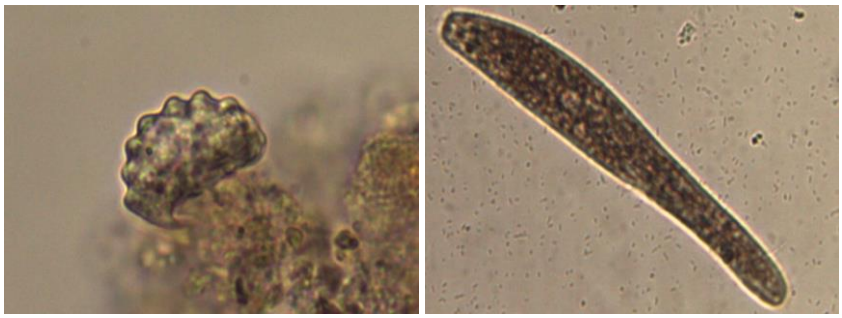


Figure 4.10: *Aspidisca Cicada* on the left (ampliation of 290x) and *Litonotus* sp. on the right (ampliation of 290x)

Cyanobacteria were identified after 24 hours of experiment. *Oscillatoria* spp. and *Spirulina* sp. were observed, but they were not seen in the next days. Control assays shown mainly *Zoogloea* spp. from Bacteria group, with a representativeness of 75% (**Figure 4.11**).

Figure 4.11:*Oscillatoria* spp. on the left (ampliation of 290x), *Spirulina* sp. on the middle (ampliation of 290x) and *Zoogloea* spp. on the right (ampliation of 290x)

After 48 hours it was possible to identify filamentous chloroficeae. On the other hand, *Navicula* spp. initially identified had a decrease of their individuals until the end of the experiment by 50%. *Navicula* was also observed in control beakers and was not found in the last day of the experiment.

Protozoa was identified in the overall of the experiment. *Arcella gibbosa*, an amoeba, was observed during the three days. However, *Arcella gibbosa* individuals had a decrease of 94% until the end of the assay. Their structure may have protected this microorganism against agitation, electric current and pH changes. In contrast with naked amoeba, *Arcella gibbosa* shows a kind of rough shell (**Figure 4.12**) which may have been favorable to the conditions generated inside the ED cell.

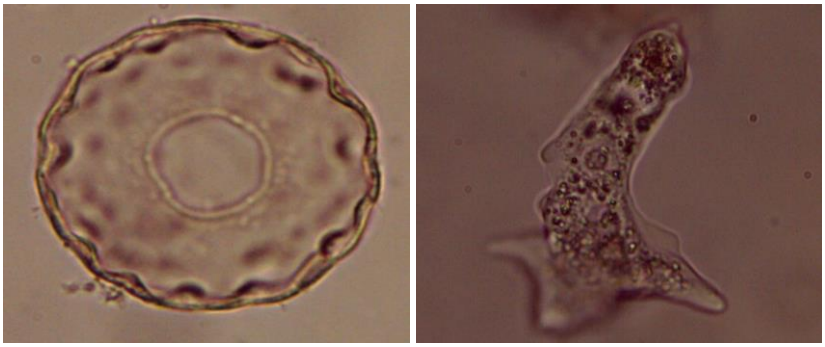


Figure 4.12: *Arcella gibbosa* on the left (ampliation of 290x) and naked amoeba on the right (ampliation of 290x)

Additionally, the genera *Epistylis* and *Vorticella* were also observed during the three days of the assay. Nevertheless, an abrupt declining over the time characterized these genera. *Epistylis* spp. individuals *per* gram declining 99% and *Vorticella* spp. 97%. *Arcella gibbosa, Epistylis* spp. and *Vorticella* spp. have shown a higher resistance to the medium conditions in the ED cell than the others, since it was possible to observe alive individuals in the end of this experiment.

When a higher current was applied, 75 mA, a number of microorganisms of 627,900 were found, according to **Table 0.3** (appendix). In the beginning of the assay (T0), Algae (*Pirobotrys* sp.), Protozoa (amoebae, free-swimming ciliates, stalked ciliates and flagellates) and pollen grains were identified. Amoeba was the most abundant group and *Arcella gibbosa* was the most common specie identified, representing 52% of the total individuals *per* gram observed in the initial sample.

Once more, *Arcella gibbosa* was present during the three days of the assay, with a decreasing of 61% of its individuals. Also, *Vorticella* spp. was in the ED cell along the experiment, with a population declining of 81%. Comparing with *Arcella gibbosa* and *Vorticella* spp. decrease in the experiment ran with 50 mA (94% and 97%, respectively), this decrease is less pronounced. A possible explanation for this divergence may be because when a current of 75 mA was applied, sewage sludge flocs stayed less aggregate and thick, which may have facilitate organisms visualization and consequently the individuals counting process. Therefore, more individuals were observed at the end of the experiment.

However, in contrast with 50 mA experiment, *Epistylis* spp. was not found in the end of the experiment. Their populations decreased 98% after two days and disappeared after 72 hours. An electric current of 75 mA has intensity not favorable to *Epistylis* spp. survival.

Algae were seen along the experiment, which means that the cellular structure can resist to high pH values (10.1±1.2), limited oxygen concentrations and current until 75 mA. Rotifers and nematodes were just found in the beginning of the experiment. Control beakers were mainly characterized by *Arcella gibbosa*, *Vorticella* spp., *Epistylis* spp. and Rotifers and its individuals

decreased along the time, although these species were observed during the three days. Therefore, Rotifers show an extremely sensibility to high pH and electric fields.

When a higher current of 100 mA was applied, a number of microorganisms of 1,559,100 was found (**Table 0.4** in appendix). This experiment shows the highest total of individuals *per* gram. The initial sample (T0) is characterized by Protozoa (amoebae, crawling ciliates, free-swimming ciliates, stalked ciliates, suctoreans and flagellates) and Rotifers. One more time Amoeba was the most abundant group and *Arcella gibbosa* was the most common specie identified, with a representativeness of 82% of the total individuals *per* gram observed in the assay.

Even with a current of 100 mA and a pH of 10.5±1.5, *Arcella gibbosa* was present during the three days of the assay, decreasing its individuals until the end of the experiment in 48%. However, in contrast to what happened with a current of 50 and 75 mA, *Epistylis* spp. and *Vorticella* spp. were just observed during the firsts 24 hours. After 24 hours, pH values achieved 9.8±0.5 when a current of 50 mA was applied 10.6±0.5. Therefore, it is possible to refer that *Epistilys* spp. and *Vorticella* spp. are more sensible to the electric field than to the pH changes. When pH was 10.6±0.5 and a current of 50 mA was applied, these two genera were found at the end of the experiment, and when the assay was carried out with a current of 100 mA, these taxa were not found at the end of the experiment.

In addition, Bacteria (*Zooglea* spp.) were seen along the 100 mA experiment. Nematodes eggs were also found between T1 and T3, which means this sludge may have nematodes initially despite they were not identified. Control beakers were mainly characterized by *Arcella gibbosa*, *Epistylis* spp. and *Vorticella* spp. Although the number of individuals decreased along the time, these species were observed during the three days. The exception was *Vorticella* spp. which was only observed until the second day. These results show again the influence of high values of current for individual's survival.

A gradual current of 50-75-100 mA was applied and 889,875 individuals were counted, according to **Table 0.5** (appendix). Initially, Algae (*Nitzschia* spp. and *Pirobotrys* sp.), Bacteria (*Oscillatoria* spp. and *Zoogloea* spp.) Protozoa (amoebae, free-swimming ciliates, stalked ciliates, suctoreans and flagellates), Nematode eggs and Rotifers were identified. Again, Amoeba was the most abundant group of protozoa and *Arcella gibbosa* was the most common specie identified, achieving its maximum representativeness in this assay (83% of the total individuals *per* gram observed).

During the 50-75-100 mA experiment, disappearance of the several species was not as abrupt as the other experiments. After 24 hours, it was possible to identify taxa from all the groups found in T0. Main differences were in Protozoa, where after one day of experiment, just Amoeba and stalked ciliates were observed. Two days later, the same diversity was found in the ED cell except rotifers. It was only observed rotifer mastax, which means rotifers did not survive to a current of 75 mA and a pH of 9.8±1.1.

At the end of the experiment, just Algae, Bacteria and Amoeba were present in the ED cell. In fact, *Arcella gibbosa* was present during the three days of the assay, decreasing its individuals only in 25%. Generally, this current sequence may have been favorable for the microbial community since they resisted longer than when a direct current of 100 mA was applied. They may have had time to adapt to the adverse medium conditions.

When a step current of 100-75-50 mA was applied, 698,250 microorganisms were found, according to **Table 0.6** (appendix). The initial sample (T0) contains Bacteria (*Zoogloea* spp.) Protozoa (amoebae, free-swimming ciliates, stalked ciliates and flagellates) and Rotifers. *Arcella gibbosa* was the most common specie identified once more, representing 83% of the total individuals *per* gram observed in the assay.

During this experiment, disappearance of the several species was again not as abrupt as the experiments conducted with a constant current. After 24 hours, it was possible to identify taxa from all the groups found in T0 and also from other groups, such as Algae and Metazoa (tardigrade). Main differences were in Protozoa, where after one day of experiment, just Amoeba and stalked ciliates were observed (similar to the assay carried out with a gradation of 50-75-100 mA). Two days later, the same groups were found in the ED cell except tardigrades. Rotifer eggs were also observed.

At the end of the experiment, just Algae, Bacteria and Protozoa (Amoeba and stalked ciliates) were present in the ED cell. *Arcella gibbosa* was present during the three days of the assay, decreasing 35% of its individuals. In contrast with the other sequence test, is seen that stalked ciliates (*Epistylis* spp.) can also be found in the end of this experiment. Once more, a current sequence is shown to be favorable for a microbial community since they resisted longer than when a direct current of 100 mA was applied, for example.

4.4Phosphorous

4.4.1 Phosphorous desorption

In order to understand phosphorus desorption from the sludge, some tests were made. In the **Figure 4.13** are visible the several flasks used for this test and is possible to observe different color tones in the sludge sampling according to the solution pH. When an acid pH was generated, sludge presents a light brown tone due to the presence of H^+ in the solution, which promotes the oxidation of the organic matter. In contrast, as the pH increases, sludge color starts to present a dark tone, since OH^- is significantly present and promotes the reduction of the organic matter.

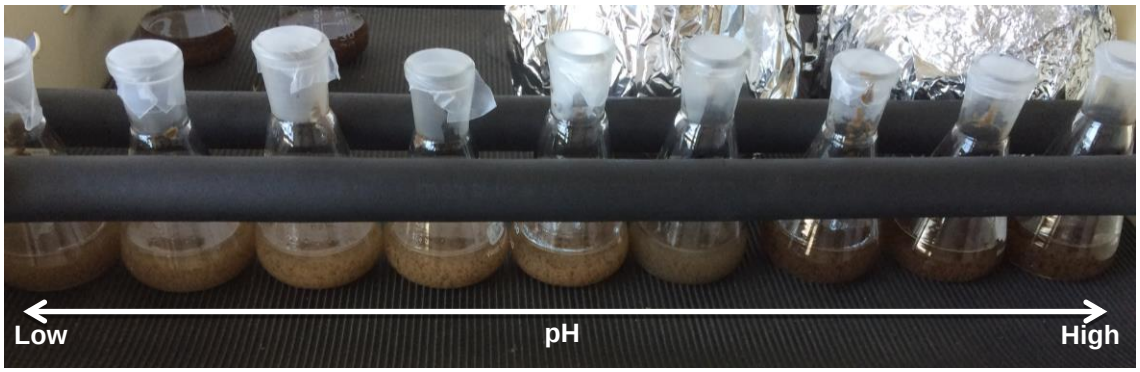


Figure 4.13: Desorption experiment

Figure 4.14 shows the phosphorus concentration in the aqueous phase as a function of pH. It is visible there is pH ranges where phosphorus concentration desorption is higher. Desorption tests showed that phosphorus desorption from the sludge was highest when pH was around 13, where 123.1 mg/L were solubilized from the sludge. On the other hand, phosphorus desorption from the sludge was lowest when pH was around 6.2, where just 7.4 mg/L were solubilized from the sample. According to phosphorus speciation (see section 2.2.1), a possible explanation is because phosphorus species present in solution are different when pH changes occur and some species can be more soluble and able to be desorbed by sludge.

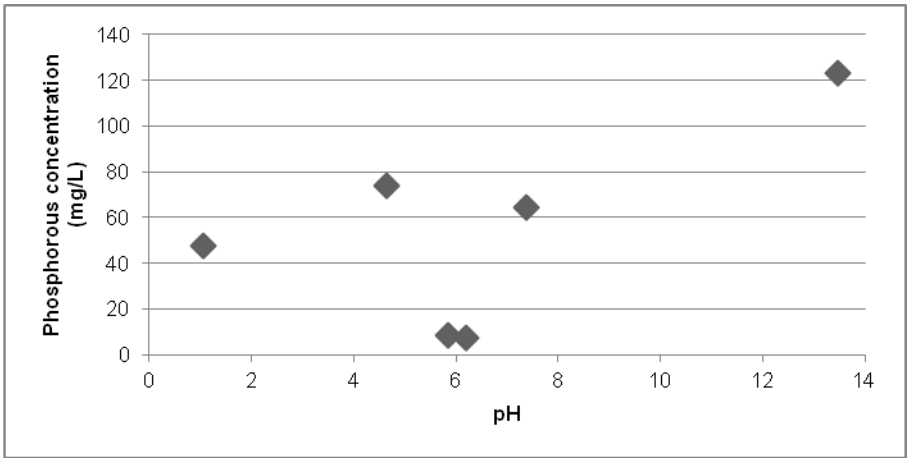


Figure 4.14: Phosphrous desorption

4.4.2 Phosphorous distribution and recovery in the ED cell

The distribution of total phosphorus in the different parts of the cell at the end of the ED experiments is shown in **Table 4.3**, sludge (solid collected after filtration of the sewage sludge), effluent (aqueous phase of the sewage sludge) and electrolyte. In the experiment control, after three days, 61.9±3.2% of the phosphorous was present in the sludge, 8.9±2.1% in the effluent and 29.2±1.1% in the electrolyte solution (due to diffusion). Although the liquid phase of sludge resulting from biological treatment should contain virtually no phosphorous, it is possible to observe small percentages of this compounds in the effluent. This may have happened due to

the anaerobic conditions in the bottom sludge after settling and before sampling (Ebbers et al., 2015).

In the experiment where a current of 50 mA was applied, 53.3±9.7% of phosphorus was recovered in the electrolyte with only 40.7±11.1% remaining in the sludge. This means that the alkaline pH achieved in this experiment helped to solubilize the phosphorus from the sludge to the effluent. This result corroborates with desorption tests (see section 4.4.1), where the highest amount of phosphorus solubilized observed from the sludge was at pH around 13. As an AN was used, once in the effluent, phosphorus was transported to the anode compartment through electromigration, as it should be mainly present as HPO_4^{2-}/PO_4^{3-}, since pH was 9.8±0.7.

Using the same cell set up, Guedes et al.(2015), after five days of experience at 50 mA with frozen sewage sludge obtained better results: 78±2% of the total phosphorous was recovered in the electrolyte and 19%±3% was found in the remaining sludge. The final sludge pH was approximately 12.5±2.9, so it is also expected to found phosphorous as HPO_4^{2-}/PO_4^{3-}. Since Guedes et al. (2015) run the assay longer than the present work, pH achieved higher values which may have promoted a better solubilization of phosphorous compounds and consequently its recovery. On the other hand, the present work (done with fresh sewage sludge) was richer in microbiological diversity than the study conducted by Guedes et al. (2015). In this case, microbial community may have used phosphorous for their own growth, which limited its recovery.

The best results were achieved when a current of 100 mA was applied, where 70.3±2.0% of the total phosphorus was recovered in the electrolyte, after three days. The higher current used may have promoted a faster solubilization of phosphorus from the sludge to the effluent. Once in the effluent, phosphorus was transported to the anode compartment as it should be mainly present as HPO_4^{2-}/PO_4^{3-}. The experiment done with 75 mA showed an intermediate recovery in the electrolyte (62.9±0.7%). It is possible to verify a relation between the applied electric current and phosphorus concentration, since when current increased also phosphorus concentration in the electrolyte increased (R^2=99%).

The experiments conducted with a gradation sequence of current showed similar results. When a gradation of 50-75-100 mA was applied, 59.9±3.7% of the total phosphorus was recovered in the electrolyte while 56.7±2.1% was recovered when a current of 100-75-50 mA was applied. Therefore, the sequence current did not improve phosphorus solubilization and recovery.

Table 4.3: Total phosphorus distribution in the ED cell (ICP analysis)

Compartment	Time (days)	Assay					
		Control	*50 mA*	*75 mA*	*100 mA*	*50-75-100 mA*	*100-75-50 mA*
Sludge (%)	3	61.9±3.2^{C,b}	40.7±11.1^{P,c}	35.0±0.1^{Q,c,d}	26.0±0.5^{B}	37.5±4.2^{O,c,h}	40.8±0.3^{a,c,}
Effluent (%)	3	8.9±2.1^{c,j}	5.9±1.4^{f,k,p,r}	2.1±0.8^{d,g,q,s}	3.7±2.5^{b,e,I,n}	2.5±0.5^{H}	2.5±1.9^{A}
Electrolyte (%)	1	11.3±0.6^{M,c,e,f,g}	47.0±5.4^{F}	51.7±3.8^{G}	55.4±3.5^{E,b}	48.1±4.1^{h,m}	45.4±10.1^{a,m}
	2	16.7±1.3^{I,c,d}	51.3±7.5^{K,i}	64.8±5.2^{D}	59.6±2.4^{N,b,i}	55.5±3.4^{h,i,o}	54.9±2.7^{a,i}
	3	29.2±1.1^{J,c}	53.3±9.7^{R,j}	62.9±0.7^{S,j,i,q}	70.3±2.0^{L,b}	59.9±3.7^{h,o}	56.7±2.1^{a,j}

Statistics tests: values statistically significantly different at $p < 0.05$ (95% confidence interval) comparing to: a Effluent T3 100-75-50 mA (A); b Sludge T3 100 mA (B); c Sludge T3 Control (C); d Electrolyte T2 75 mA (D); e Electrolyte T1 100 mA (E); f Electrolyte T1 50 mA (F); g Electrolyte T1 75 mA (G); h Effluent T3 50-75-100 mA (H); i Electrolyte T2 Control mA (I); j Electrolyte T3 Control mA (J); k Electrolyte T2 50 mA (K); l Electrolyte T3 100 mA (L); m Electrolyte T1 Control (M); n Electrolyte T2 100 mA (N); o Sludge T3 50-75100 mA (O); p Sludge T3 50 mA (P); q Sludge T3 75 mA (Q); r Electrolyte T3 50 mA (R); s Electrolyte T3 75 mA (S)

Figure 4.15 shows the variation of the total phosphorus in the electrolyte compartment along the three days of experiment. It is visible a high increase during the first 24 hours of all experiments. After just one day, 88.0% of the total phosphorus recovered (53.3±9.7%) applying an electric field of 50 mA can be found in the electrolyte compartment. Lower percentages were achieve, in relation to the total phosphorus recovered, after one day, when an electric field of 75 mA, 100 mA, 50-75-100 mA and 100-75-50 mA was applied, where 82.2%, 78.7%, 80.2% and 80.0% of the total phosphorus recovered was found in the electrolyte, respectively.

However, the highest amount of phosphorus recovered in the electrolyte after 24 hours, related to the initial phosphorus concentration, was achieved in the experiment conducted with 100 mA (55.4±3.5%). In contrast, since there was no electric field, control experiment showed the smallest variations along the three days. Only 11.3±0.6% was recovered in the control electrolyte after 72 hours.

In addition, it was possible to verify a relation between the time (24-72 hours) and the phosphorus concentration. Strong relations were recorded to control (R^2=95%), 50 mA (R^2=96%), 100 mA (R^2=94%) and 50-75-100 mA (R^2=98%). Lower relations were found to 75 mA (R^2=73%) and 50-75-100 mA (R^2=87%). In order to corroborate these results, more tests should be carried out.

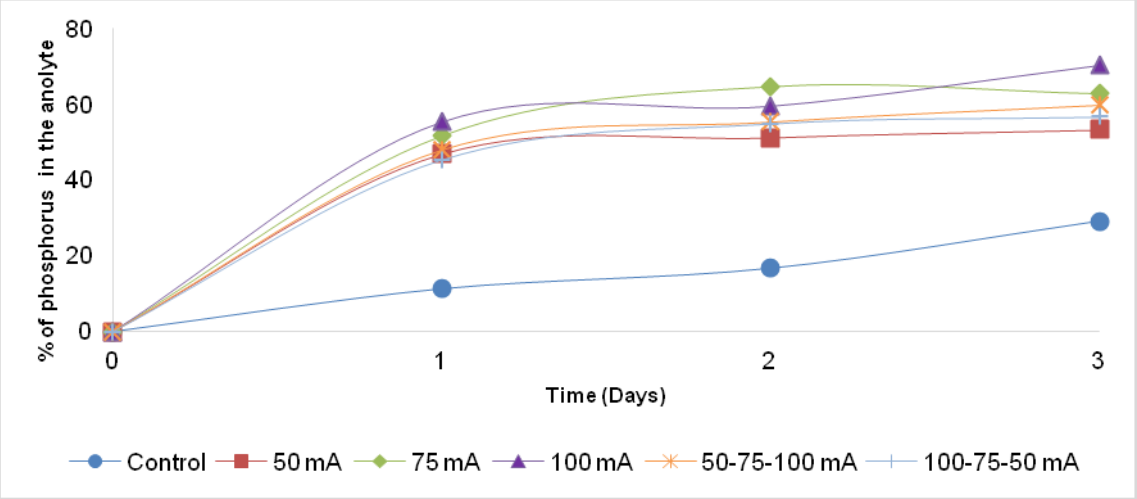


Figure 4.15: Percentage of phosphorus reaching the anolyte along the experiments

About 95% of the phosphorous in municipal WWTP is as phosphate species, including organic phosphate, polyphosphate and orthophosphate (Snoeyink and Jenkins, 1980). The colorimetric method was used aiming at understanding phosphorus speciation within the different matrices in the ED cell. **Figure 4.16** shows the distribution of the organic phosphorus and inorganic phosphorus (composed by polyphosphates and orthophosphates) in each experiment.

Independently of the electric field applied, the effluent and sludge compartment are mainly characterized by inorganic phosphorus, predominantly orthophosphates. On the other hand, organic phosphorus is mainly found in the electrolyte compartment and its content increased with the electric field increase (R^2=75%).

Organophosphorus compounds are chemical compounds containing carbon-phosphorus bonds The reaction of phosphorus ions with other compounds containing carbon that were present in the system may explain the increase in the organophosphorus content. Phosphorus may have reacted in the sludge compartment and then migrated to electrolyte as organophosphorus or the reaction may have occurred directly in the electrolyte compartment. Organophosphorus active compounds are normally esters, amides, or thiol derivatives of phosphoric or phosphonic acid (Mazzacurati, 2007).

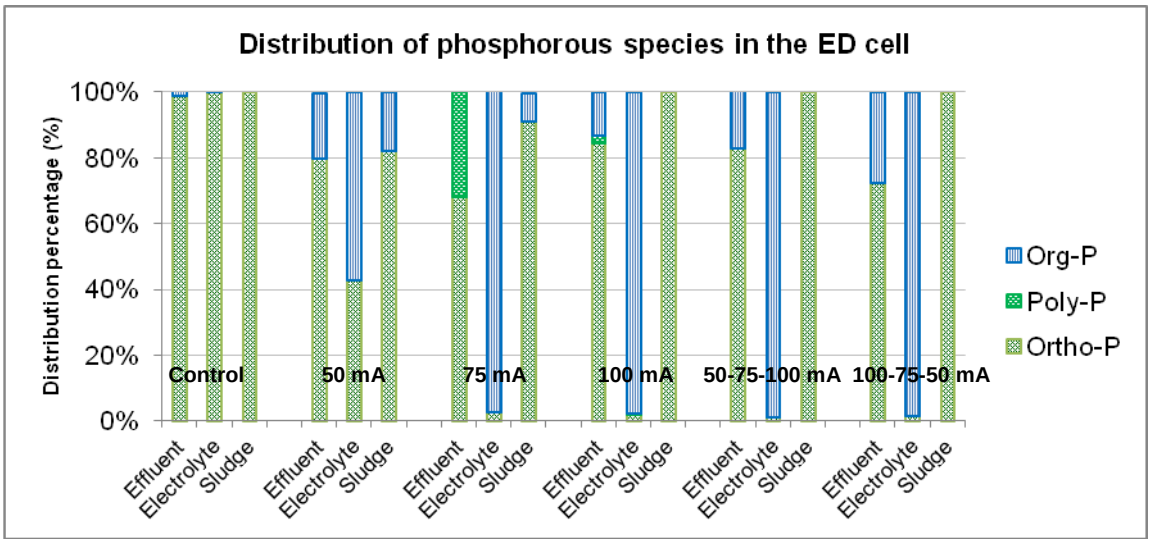


Figure 4.16:Organic and inorganic phosphorous distribution in the ED cell (colorimetric method)

4.5Organic contaminants distribution and degradation in the ED cell

In order to achieve the total amount of organic contaminants after ED process, chromatograms from HPLC, of each sample (sludge, effluent and electrolyte), were analyzed. Each studied compound has different maximum absorbency wavelengths: EE2 at 281 nm, BPA at 280 nm, Caf at 273 nm and MBPh at 288 nm. Additionally, the retention time is different for each compound, since the affinity in the HPLC column depends of the chemical structure of the substances (polarity). Caf peak is found after 6-8 min of sequence running, EE2 between 33-34 min, BPA 30-31 min and MBPh at 36-37 min.

First, standard solutions were introduced in the HPLC and the obtained chromatograms are shown in **Figure 4.17**. The four peaks correspondent to the compounds under study are represented in the figure. Only these four peaks were integrated in order to find the total area and the final concentration of each organic compound in each cell matrice (sludge, effluent and electrolyte), according to the calibration curves shown in **Table 3.2** of section 3.5.6.

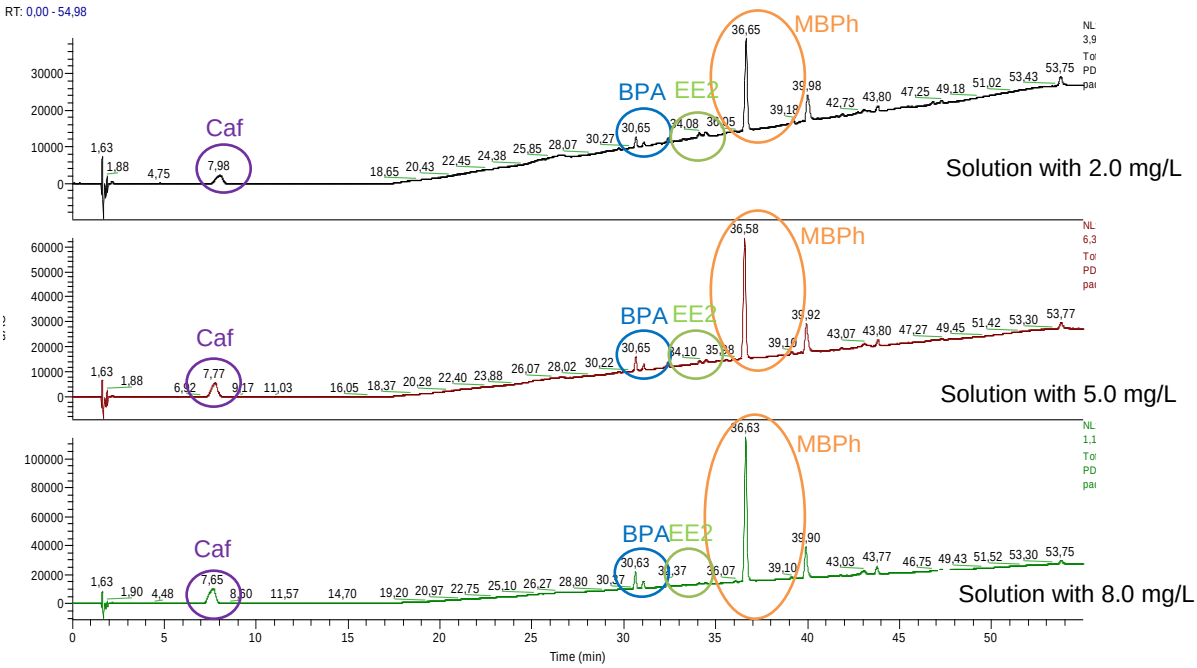


Figure 4.17: Standard solutions (2.0; 5.0 and 8.0 mg/L) HPLC chromatograms

Since the matrix under study is more complex than the calibration standards, more peaks were observed in the chromatograms (**Figure 4.18**). For this, some of the compounds peaks under study can be co-eluting with other compounds and/or be hindered due to the matrix effect. Still, the amount of organic contaminants not detected, was assumed to have suffered electro and/or biodegradation.

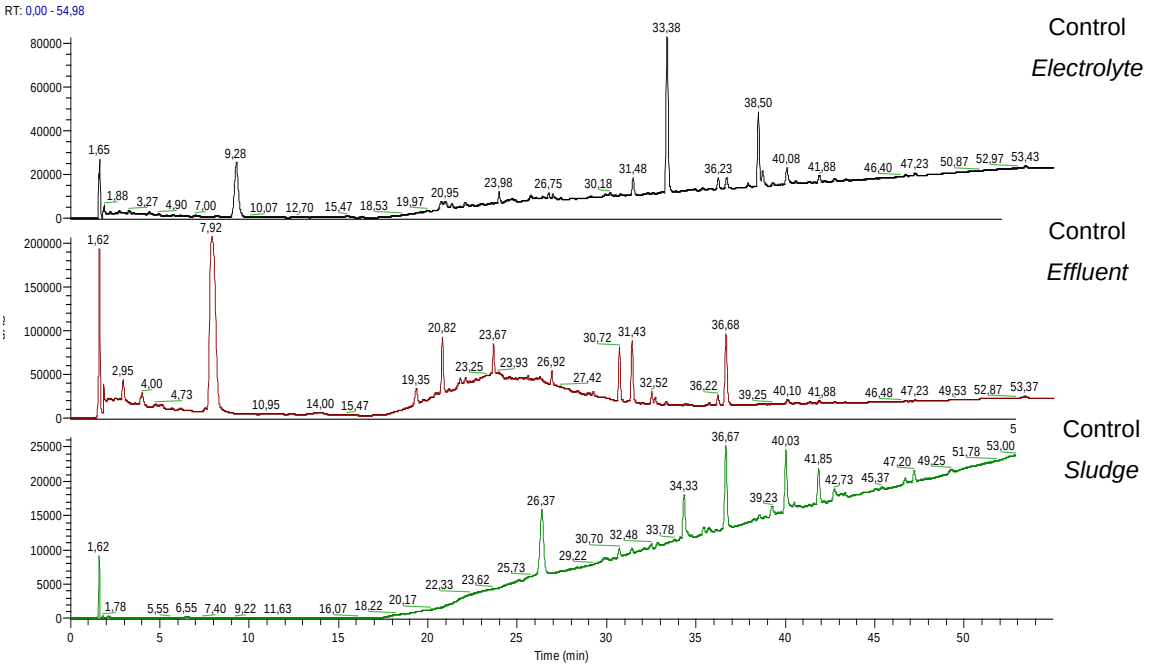


Figure 4.18: Control experiment HPLC chromatograms

The percentage of contaminants detected at the end of the ED experiments and the compounds degradation percentage in the sludge, effluent and electrolyte are presented in **Table 4.4.**

The majority of the organic contaminants have a log Kow higher than 3.32 (**Table 2.2**) and, consequently, at the end of the control experiment, they were mainly detected in the solid phase, sludge (**Table 4.4**), together with phosphorus. Caf was the exception, due to the low log Kow (-0.07) it was mainly detected in the effluent together with a lower amount of the other contaminants. Caf was also able to pass through the AN to the electrolyte compartment, 2.7±0.3% of Caf for control.

Table 4.4: Organic contaminants distribution and degradation in the ED cell

Assay	Compartment	Compound			
		EE2	*BPA*	*Caf*	*MBPh*
Control	Sludge (%)	33.9±0.8^{A}	12.9±5.7^{a}	<LD^{a,c}	27.9±7.2^{C}
	Effluent (%)	0.9±0.5^{a}	1.2±1.3	10.7±11.9	0.3±0.2^{c}
	Electrolyte (%)	<LD^{a}	<LD	2.7±0.3	<LD^{c}
	Degradation (%)*	65.2±1.3	85.9±7.0	86.7±11.6	71.8±7.3
50 mA	Sludge (%)	13.1±2.7^{a}	<LD	8.4±0.4	15.9±1.4
	Effluent (%)	1.1±0.6	2.2±1.2	3.1±4.4**^{b}	1.3±0.2
	Electrolyte (%)	2.7±0.4	6.0±3.4	α	1.2±0.5
	Degradation (%)*	83.1±1.7	91.8±4.6	88.3±4.0	81.6±0.6
75 mA	Sludge (%)	25.8±7.6^{D}	12.7±10.9	4.5±0.2^{d}	18.1±1.6
	Effluent (%)	2.2±0.5^{d}	5.1±0.4	6.0±2.7	0.8±0.3
	Electrolyte (%)	2.4±2.1^{d}	7.9±2.9	α	0.2±0.2
	Degradation (%)*	69.5±5.0	74.3±7.6	89.4±2.5^{f}	80.9±1.0
100 mA	Sludge (%)	18.1±3.1	4.1±5.9**	<LD	13.6±1.7
	Effluent (%)	2.7±0.8	5.1±2.8	3.8±0.2	0.9±0.5
	Electrolyte (%)	0.1±0.2**	10.0±13.1	<LD	0.7±0.8
	Degradation (%)*	79.1±2.0	80.8±4.5	96.2±0.2^{f}	84.8±1.3
50-75-100 mA	Sludge (%)	17.4±0.0	6.3±8.9**	14.2±8.8	19.2±14.3
	Effluent (%)	2.2±1.0^{b}	3.8±4.4	22.7±4.2^{B}	0.8±0.3^{b}
	Electrolyte (%)	<LD	6.7±8.8	<LD^{b}	0.8±0.7
	Degradation (%)*	80.4±1.0	83.2±4.5	63.1±13.0^{F}	79.2±13.9
100-75-50 mA	Sludge (%)	24.7±8.4^{E}	6.9±0.2	6.9±9.8**	16.0±3.9
	Effluent (%)	<LD^{e}	2.0±1.0	0.6±0.2^{b}	0.6±0.7
	Electrolyte (%)	<LD^{e}	0.6±0.9	<LD	0.2±0.1
	Degradation (%)*	75.3±8.4	90.5±1.7	92.5±10.0^{f}	83.2±4.7

*% of contaminant degraded = [1 - ∑(mass of contaminant detected in all cell compartments)/(mass of contaminant added to the sewage sludge)] x100

**One of the duplicates is below LD or LQ

$^{\alpha}$ Contaminant was not possible to quantify as it was masked by another compound that was co-eluting.

Statistics tests: values statistically significantly different at $p < 0.05$ (95% confidence interval) comparing to: a EE2 Sludge Control (A); b Caf Effluent 50-75-100 mA(B); c MBPh Sludge Control (C); d EE2 Sludge 75 mA (D); e EE2 Sludge 100-75-50 mA (E); f Caf 50-75-100 mA (F).

(LD values were considered 0 for statistic tests)

When a current of 50 mA was applied, lower peaks (**Figure 4.19**) compared to the control experiment (**Figure 4.18**) were obtained. This was expected, since the electric current should help to degrade the organic compounds. Also, the current transported other matrix components to the electrolyte, which changed the chromatogram base line (**Figure 4.19**) compared to the control experiment (**Figure 4.18**).

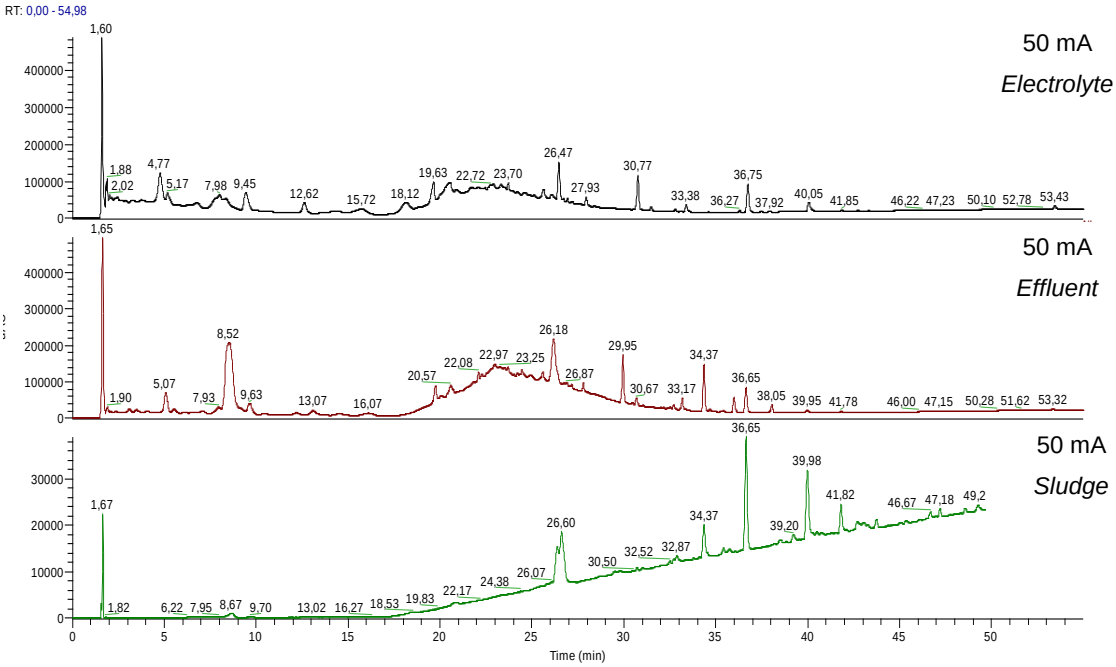


Figure 4.19: 50 mA experiment HPLC chromatograms

After the application of an electric current, the pH in the sewage sludge compartment achieved values around 10. Consequently, all compounds, except Caf, were mainly present in their ionized form, which made them more soluble and able to migrate to the anode compartment through the AN.

Compounds were mainly detected in sludge. EE2 presented the smaller degradation percentage when a current of 75 mA was applied, where 25.8±7.6% of this compound was detected in the sludge. The same happened to BPA, with 12.7±10.9% detected in the sludge. MBPh achieved its worst degradation when a current of 100-75-50 mA was applied, with a representativeness of 19.2±14.3% in the sludge. Caf was mostly found in the effluent (22.7± 4.2%) when a current of 50-75-100 mA was applied, showing the lowest percentage removal for this compound. In contrast, better removals from the sludge to EE2 (only 13.1±2.7% detected in sludge) and BPA (<LD detected in sludge) were obtained when a current of 50 mA was applied. Caf (<LD detected in sludge) and MBPh (only13.6±1.7% detected in sludge) showed better degradations when 100 mA were applied.

Comparing with Guedes et al.(2015) results, it is possible to observe some differences. When a current of 50 mA was applied during five days using an AN, all the compounds in the sludge

were under LQ, except MBPh with approx. 16.7% being detected. In **Table 4.4**, when the experiment was done applying 50 mA, just BPA was under the limit of detection. EE2, Caf and MBPh concentration in sludge were higher than in the study done by Guedes et al. (2015), 13.1±2.7%, 8.4±0.4% and 15.9±1.4%, respectively.

In terms of degradation, the compound that presented higher degradation in the control experiment was Caf (86.7±11.6), followed by BPA (85.9±7.0), MBPh (71.8±7.3) and EE2 (65.2±1.3). This degradation was mainly attributed to bioremediation mechanisms, as after the three days experiment, microbial activity was observed in the collected samples (see section 4.3). Volatilization is not expected to be an important fate process based upon the estimated Henry's Law constant of the studied compounds. Also, photodegradation is not a relevant fate process since the assay was conducted in dark conditions.

It is expected that the degradation/removal of the compounds in the cell increases after the application of electric current as the compounds may suffer anodic oxidation and/or cathodic reduction (Camões, 2005). Also, chlorides may occur in the electrolyte solution (Ribeiro and Rodríguez-Maroto, 2006). Chloro is commonly used to disinfect and oxidize organic matter, since chloride is a strong oxidizing agent. Generally, chloro reactivity increases with pH reduction (Meyer, 1994). Therefore, in the electrolyte compartment, where pH achieved values under 2.0, the presence of chloro could have improved compounds degradation.

However, the electric field applied during the experiments promoted the death or modifications of the microorganisms present in the sewage sludge, causing a decrease in the bioremediation potential of the medium which influenced the results.

Compounds degradation when compared to the control experiment is shown in **Figure 4.20** (Compounds degrade % [electric current]-Compounds degrade % [control]). Different behaviors were observed for the several compounds when different electric fields were applied. For Caf, when a progression of 50-75-100 mA was applied, less 23.5% of this compound was degraded comparing with the control experiment. A current of 100 mA enabled to degrade more 9.6% of Caf than in the control assay. Also, MBPh shows better results with 100 mA, with more 13.0% of degradation than the control.

BPA and EE2 achieved better results when 50 mA were applied, where more 5.9% and 17.9% was degraded in relation to the control experiment, respectively. Just when a current of 50 mA and a progression of 100-75-50 mA were applied all compounds degradations were increased comparing to the control experiment.

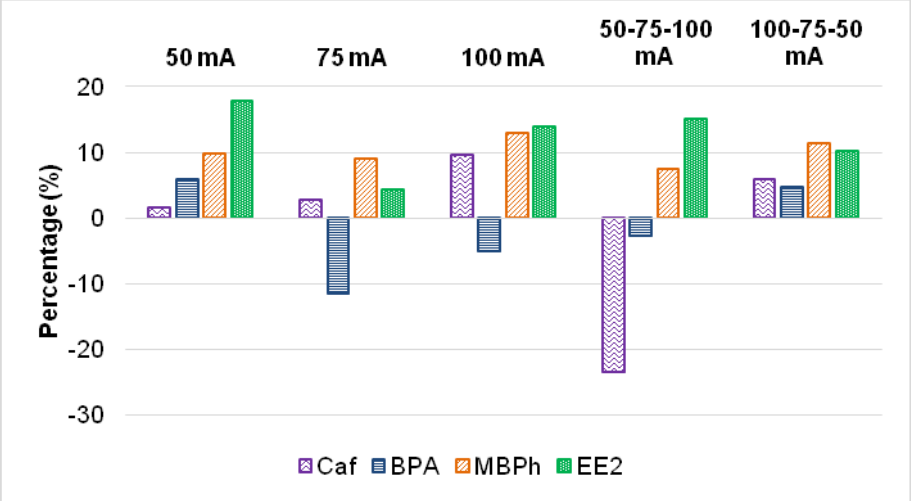


Figure 4.20: Percentage of compounds degradation in relation to control experiment

In general, ED showed potential to enhance the degradation of the contaminants being the process mainly dependent on the intensity of the applied DC field. Comparing to the control experiment (0 mA) the experiment that presented the best results for phosphorus recovery (100 mA) was also one of the most promising for increasing MBPh and Caf removal.

Along the ED experiments, the differences found in the degradation percentages are mainly attributed to their chemical structures, reactivity and mechanisms previously studied by different authors (Yuan et al., 2013; Xu et al., 2013).

Degradation percentages show improvements comparing to Guedes et al.(2015) study. After five days, EE2, BPA, Caf and MBPh degradations percentages in the ED cell were 83±12%, 87±15%, 71±4.6% and 83±12%, respectively. The present study, just during three days, got higher percentages, improving 4.8% BPA degradation and 17.3% Caf removal. About EE2 and MBPh degradation, percentages were somewhat similar to the previous study.

A possible explanation for this fact is related with the reactivity of the sludge. Guedes et al.(2015) collected the sludge in the same WWTP and froze it till use. This action stopped the microbiological activity, and many organisms did not resist. The present study was done with fresh sewage sludge, so initial samples were richer in diversity than the study conducted with frozen sludge. In three days, it is possible to observe similar and, in some cases, higher degradations of organic compounds than in the assay carried out for five days, which may be due to the bioremediation potential observed in the present study.

Also, the present study improves energetic consumption, being economical and environmentally more attractive. Less two days of operation may reduce around 40% of the total energy consumed in five days.

In the conducted experiments, a decrease in the microbial community was observed, which may have led to a low bioremediation potential within the ED cell. Therefore, if the conditions are optimized to combine the effect of both bio and electrodegradation, higher remediation efficiencies may be achieved. Also, in this study, the initial contaminants concentration was approximately 8 mg/L and at lower concentrations, more similar to the ones entering the WWTP, higher electrodegradation percentages may be achieved e.g. 52% for BPA at 10 mA (Guedes et al., 2014).

5 Conclusions

Phosphorous solubilization from the sludge to the liquid phase was mainly affected by pH. Once in the liquid phase, phosphorous electromigrated to the anode end from where it can be recovered by, e.g, precipitation. In total, a maximum of 70.3 ±2.0% of phosphorous were recovered in the anolyte, when a current of 100 mA was applied. Independently of the electric field applied, the effluent and sludge compartment are mainly characterized by inorganic phosphorus (orthophosphates). On the other hand, organic phosphorus is mainly found in the electrolyte compartment and its content increased with the electric field.

The application of a low level electric field improved the degradation of the several compounds studied. A current of 100 mA allowed degrading more 9.6% of Caf (88.3±4.0%) than in the control assay. Also, MBPh (84.8±1.3%) shows better results with 100 mA, with more 13% of degradation than the control. BPA (91.8±9.6%) and EE2 (83.1±1.7%) achieved better results when 50 mA were applied, where more 5.9% and 17.9% was degraded in relation to the control experiment, respectively. Just when a current of 50 mA and a progression of 100-75-50 mA were applied all compounds degradations increased comparing to the control experiment.

After the six experiments, it was possible to identify 35 taxa, belonging to four different groups: Algae, Protozoa, Bacteria and Metazoa. Bacteria, Algae and some Protozoa were the groups that shew more resistance to current and pH changes. The Ameobae *Arcella gibbosa* (protozoa) was the most common organism found in the total of the experiments, with a representativeness of 61%. This organism was able to support an electric field of 100 mA and a pH of 10.5±1.5.

6 Future Developments

At the end of this study, it was possible to see that phosphorous recovery is good when ED is applied to sewage sludge. In this way, future studies can focus on a real scale and find out the best way to introduce this step in a WWTP considering operations costs, energetic balance and emissions. Also, it is important to see the efficiency of the process in a real scale, considering environmental, social and economic aspects.

A study of the several phosphorous species which are formed during the assay, such as organophosphorus compounds should be carried out. This would allow a better understanding of the reactions that may affect P recovery, as well as P bioavailability for agriculture.

Organic compounds are substances hard to work, since its behavior is difficult to predict over the time. In the present dissertation, three days of degradation were tested. It would be interesting from an environmental and economic point of view to repeat the experiments just for 24 hours and assess organic compounds degradations. In addition, in order to increase compounds degradation, different electrodes could be tested, namely others than inert electrodes as reaction with the electrode could increase electrodegradation.

In addition, more attention should be given to microbial community present in sewage sludge. Commonly species characteristics and behaviors should be studied more exhaustively in order to find its “optimal conditions” and boost microbiological community performance at the level of bioremediation. Also, a study related to species richness could be done in order to improve the knowledge about community’s evolution along the time. Parameters such as biotic index, dissolved oxygen (DO), TSS, CBO, COD, SVI and Nitrites may be monitored during the assay for a better understanding of medium conditions.

Combine physical and chemical processes with bioremediation brings benefits to the process. Not only would the process be more effective, but also more efficient, since it would allow reducing current application. A way to test this matter consists in the application of an alternating current during the experiments. An example as it can be done is applying during one hour a constant current and after one hour no current, in order to let the microorganisms recover.

Finally, it would be interesting to developed statistical studies and mathematical models for the optimization of methods.

References

Abdel-Raouf, N., Al-Homaindan, A.A. and Ibraheem, I.B.M. (2012). Microalgae and wastewater treatment. King Saud University. Saudi Journal of Biological Sciences19(3), 257–275. doi:10.1016/j.sjbs.2012.04.005

Acar,Y.B. and Alshawabkeh, A.N. (1993). Principles of electrokinetic remediation. Environmental Science and Technology 27(13), 2638–2647. doi:10.1021/es00049a002

Alcock, R. E. and Jones, K. C. (1993). Polychlorinated biphenyls in digested UK sewage sludges.Chemosphere 26(12), 2199–2207. doi:10.1016/0045-6535(93)90346-7

Andersen, H., Siegrist, H., Bent, H.S. and Ternes, T.A. (2003). Fate of estrogens in a municipal sewage treatment plant. Environmental Science and Technology 37(18), 4021–4026. doi:10.1021/es026192a

Asada, T., Oikawa, K., Kawata, K., Ishihara, S., Iyobe, T. and Yamada, A. (2004). Study of removal effect of Bisphenol A and β-estradiol by porous carbon. Journal of Health Science 50(6), 588–593. doi:10.1248/jhs.50.588

Bila, D. M. and Dezotti, M. (2007). Desreguladores endócrinos no meio ambiente: efeitos e consequências. Quimíca Nova 30(3), 651–666. doi:10.1590/S0100-40422007000300027

Bixio, D., Thoeye, C., De Koning, J., Joksimovic, D., Savic, D., Wintgens, T. and Melin, T.(2006). Wastewater Reuse in Europe. Elsevier. Desalination 187(1-3): 89–101. doi:10.1016/j.desal.2005.04.070

BisphenolA (2015). Bisphenol A general toxicity. USA. Accessed in April 2015, http://www.bisphenol-a.org/human/herGeneralToxicity.html

Blöcher, C., Niewersch, C. and Melin, T. (2012). Phosphorus Recovery from Sewage Sludge with a Hybrid Process of Low Pressure Wet Oxidation and Nanofiltration. Water Research 46(6): 2009–19. doi:10.1016/j.watres.2012.01.022

Boney, A.D (1975). Phytoplankton. Studies in Biology, 52, Edward Arnold, London, 116 pp.

Boney, A. D. (1976). Phytoplankton. Limnology and Oceanography, 21(2), 347. doi:10.4319/lo.1976.21.2.0347b

Horne, A. J. (1976). Boney, A. D. 1975. Phytoplankton. Studies in Biology Ser., 52. Limnology and Oceanography, 21(2), 347. doi:10.4319/lo.1976.21.2.0347b

Boström, B., Persson, G. and Broberg, B. (1988). Bioavailability of Different Phosphorus Forms in Freshwater Systems. Kluwer Academic Publishers. Hydrobiology 170(1): 133–55. doi:10.1007/BF00024902

Braga, O., Smythe, G.A., Schafer Feitz, A.J. (2005). Fate of steroid estrogens in Australian inland and coastal wastewater treatment plants. Environmental Science and Technology 39(9), 3351–3358. doi:10.1021/es0501767

Buerge, I.J, Poiger, P., Buser,H. and Muller, M.D. (2003). Caffeine , an Anthropogenic Marker for Wastewater Contamination of Surface Waters. Environmental Science and Technology 37(4), 691–700. doi:10.1021/es020125z

Câmara Municipal de Sesimbra (2015). Quinta do Conde. Portugal. Acessed in May 2015, http://www.cm-sesimbra.pt/pt/conteudos/juntas+de+freguesia/quinta+do+conde/.

Camões, M. F. (2005). Ânodos e cátodos - positivos ou negativos? Química - Boletim da SPQ: Folhas de Química.:Série II, 99(Out.- Dez), 70–72. Accessed in September 2015, http://www.spq.pt/magazines/BSPQ/624/article/30001286/swf

Chaney, R.L., Ryan, J.A. and O'Connor, G.A.(1996). Organic contaminants in municipal biosolids: risk assessment, quantitative pathways analysis, and current research priorities. Science of the Total Environment 185(1-3), 187–216. doi:10.1016/0048-9697(96)05051-6

Chang, H. S., Choo, K.H., Lee, B., and Choi, S.J. (2009). The Methods of Identification, Analysis, and Removal of Endocrine Disrupting Compounds (EDCs) in Water. Journal of Hazardous Materials 172(1), 1–12. doi:10.1016/j.jhazmat.2009.06.135

Chemspider (2015). Substances. Royal Society of Chemistry. Accessed in April 2015, http://www.chemspider.com/.

Cherif, M., Mgaidi, A., Ammar, M.N., Abderrabba, M. and Fürst, W. (2000). Modelling of the Equilibrium Properties of the System H3PO4-H2O: Representation of VLE and Liquid Phase Composition. Fluid Phase Equilibria 175(1-2), 197–212. doi:10.1016/S0378-3812(00)00458-1.

Choi, K.J., Kim, S.G., Kim, C.W. and Kim, S.H. (2005). Effects of activated carbon types and service life on removal of endocrine disrupting chemicals: amitrol, nonyphenol and bisphenol A. Chemosphere 58(11), 1535–1545. doi:10.1016/j.chemosphere.2004.11.080

Clarke, B.O., Porter, N.A., Marriott, P.J. and Blackbeard, J.R. (2010). Investigating the levels and trends of organochlorine pesticides and polychlorinated biphenyl in sewage sludge. Environmental International 36(4), 323–329. doi:10.1016/j.envint.2010.01.004.

Clarke, B. and Smith, S. (2011). Review of 'emerging' organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids, Environmental International 37(1), 226–247. doi:10.1016/j.envint.2010.06.004

Cornel, P., and Schaum, C. (2009). Phosphorus Recovery from Wastewater: Needs, Technologies and Costs. Water Science and Technology 59(6), 1069. doi:10.2166/wst.2009.045

Coronado, M., De Haro, H., Deng, X., Rempel, M. A., Lavado, R. and Schlenk, D. (2008). Estrogenic activity and reproductive effects of the UV-filter oxybenzone (2-hydroxy-4-methoxyphenyl-methanone) in fish. Aquatic Toxicology, 90(3), 182–187. doi:10.1016/j.aquatox.2008.08.018

Couto, N., Guedes, P., Mateus, E.P., Santos, C., Ribau Teixeira, M., Nunes, L.M., Hansen, K.K., Gutierrez, C., Ottosen, L.M. and Ribeiro, A.B.(2013). Phosphorus Recovery from a Water Reservoir-Potential of Nanofiltration Coupled to Electrodialytic Process. Springer. Waste and Biomass Valorization 4(3), 675–681. doi:10.1007/s12649-012-9194-7

Daniel, M. and Lima, E. (2014). Determinação Simultânea de Estriol, B-Estradiol, 17a-Etinilestradiol e Estrona Empregando-se Extracção Em Fase Sólida (SPE) e Cromatografia Líquida de Alta Eficiência (HPLC). Revista Ambiente & Agua-An Interdisciplinary Journal of Applied Science, 4(2), 57–65. doi:10.4136/1980-993X

Decreto-Lei nº. 276/2009 de 2 de Outubro do Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional. Diário da República: 1ª série nº.192 (2009).

Decreto-Lei nº. 348/98 de 9 de Novembro do Ministério do Ambiente. Diário da República: I série A nº.259 (1998).

Denisov, G., Hicks, R., and Probstein, R. F. (1996). On the Kinetics of Charged Contaminant Removal from Soils Using Electric Fields. Journal of Colloid and Interface Science 178, 309–323. doi:10.1006/jcis.1996.0119

Desbrow, C., Routledge, E.J., Brigthy, G.C., Sumpter, J.P. and Waldock, M. (1998). Identification of estrogenic chemicals in STW effluents. 1. Chemical fractionation and in vitro biological screening. Environmental Science and Technology 32(11), 1549–1558. doi:10.1021/es9707973.

Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. . Official Journal of the European Comunities. L 181/6

Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013. Official Journal of the European Union. L 226/1.

Dong, M. (2013). The Essence of Modern HPLC: Advantages, Limitations, Fundamentals, and Opportunities. LCGC solutions for separation scientists. Accessed in May 2015, http://www.chromatographyonline.com/essence-modern-hplc-advantages-limitations-fundamentals-and-opportunities?id=&sk=&date=&%0A%09%09%09&pageID=4.

Ebbers, B., Ottosen, L.M. and Jenssen, P.E. (2015). Electrodialytic Treatment of Municipal Wastewater and Sludge for the Removal of Heavy Metals and Recovery of Phosphorus. Electrochimica Acta. doi:10.1016/j.electacta.2015.04.097.

Environmental Leverage (2003). Worms. California. Accessed in June 2015, http://www.environmentalleverage.com/Worms.htm.

EPA:Environmental Protection Agency (2002). Use or Disposal of Sewage Sludge Biosolids.Chapter 1. United States Environmental Protection Agency.

EPA:Environmental Protection Agency (2012). Application Note HPR-EN-23. US EPA 3051.Milestone productivity tools.

European Commission (2010). Environmental, economic and social impacts of the use of sewage sludge on land. Final Report. Part III: Project Interim Reports.

European Comission (2013). Regional and Urban Policy: Green Public Procurement Criteria for Waste Water Infrastructure.

European Comission (2015). Urban Waste Water Directive Overview. Accessed in March 2015, http://ec.europa.eu/environment/water/water-urbanwaste/index_en.html.

Feng, Y., Wang, C., Liu, J. and Zhang, Z., (2010). Electrochemical degradation of 17-alpha-ethinylestradiol (EE2) and estrogenic activity changes. Journal of Environmental Monitoring 12(2), 404. doi:10.1039/b923495k

Fukuhara, T. I., Kawashima, S., Shinohara, M. and Abe I.O. (2006). Adsorbability of estrone and 17-βestradiol in water onto activated carbon. Water Research. 40(2), 241–248. doi:10.1016/j.watres.2005.10.042

Gardner, K. (2005). Electrochemical Remediation and Stabilization of Contaminated Sediments: 34. Cooperative Institute for Coastal and Estuarine Environmental Technology

Gomes, R.L., Scrimshaw, M.D. and Lester, J.N. (2009). Fate of conjugated natural and synthetic steroid estrogens in crude sewage and activated sludge batch studies. Environmental Science and Technology 43(10), 3612–8. doi:doi: 10.1021/es801952h

Guedes, P., Mateus, E.P., Couto, N., Rodriguez, Y. and Ribeiro, A.B. (2014). Electrokinetic Remediation of Six Emerging Organic Contaminants from Soil. Chemosphere 117, 124–131. doi:10.1016/j.chemosphere.2014.06.017.

Guedes, P., Couto,N., Ottosen, L., Ribeiro, A.B (2014b). Incinerated sewage sludge ashes – Potential and challenges. in Fly Ash: Chemical Composition, Sources and Potential Environmental Impacts, Sarker, P.K. (Ed.) Nova Science Publishers. 432-437.

Guedes, P., Magro, C., Couto, N., Mosca, A., Mateus, E.P. and Ribeiro, A. B. (2015). Potential of the Electrodialytic Process for Emerging Organic Contaminants Remediation and Phosphorus Separation from Sewage Sludge. Electrochimica Acta. doi:10.1016/j.electacta.2015.03.167

Han, J., Qiu,W., Meng,S. and Gao,W. (2012). Removal of Ethinylestradiol (EE2) from Water via Adsorption on Aliphatic Polyamides. Water Research 46(17), 5715–5724. doi:10.1016/j.watres.2012.08.001

Holzer, K. and Horak, O. (1994). Catalytic wet oxidation without flame burn as a method for wastewater and sewage sludge treatment. Chemical Industry,7-8.

Indermuhle, C., Martín de Vidales, M.J., Sáez,C., Robles,J., Cañizares,P., García-Reyes,J.F., Molina-Diaz,A., Comninellis, C. and Rodrigo, M. (2013). Degradation of Caffeine by Conductive Diamond Electrochemical Oxidation. Chemosphere, 93(9), 1720–1725. doi:10.1016/j.chemosphere.2013.05.047

Iwasaki, S., Fukuhara, T., Abe, I., Yanagi, J., Mouri, M. and Iwashima, Y. (2001). Adsorption of alkylphenols onto microporous carbons prepared from coconut shell. 125(2), 207–211. doi:10.1016/S0379-6779(01)00530-6.

Jaffer, Y., Clark, T.A., Pearce, P. and Parsons, S.A. (2002). Potential phosphorus recovery by struvite formation. Water Research. 36(7), 1834–1842. doi:10.1016/S0043-1354(01)00391-8

Jakobsen, M.R., Fritt-Rasmussen, J., Nielsen, S. and Ottosen, L.M. (2004). Electrodialytic removal of cadmium from wastewater sludge. Journal of Hazardous Materials 106(2-3), 127–132. doi:10.1016/j.jhazmat.2003.10.005.

Jansen, R., Osterwalder, U., Wang, S. Q., Burnett, M., and Lim, H. W. (2013). Photoprotection: Part II. Sunscreen: Development, efficacy, and controversies. Journal of the American Academy of Dermatology 69(6), 867.e1–867.e14. doi:10.1016/j.jaad.2013.08.022

Jensen, P.E. (2005). Application of microbial products to promote electrodialytic remediation of heavy metal contaminated soil. Ph.D Thesis. Technical University of Denmark.

Kah, M. and Brown, C.D. (2007). Changes in pesticide adsorption with time at high soil to solution ratios. Chemosphere, 68(7), 1335–1343. doi:10.1016/j.chemosphere.2007.01.024

Kerry, K. D., Dendy, B.B., Crooks, W. and Brady, J. (1989). Operation of Wastewater Treatment Plants: A field study training program. Sacramento State college, Department of Civil Engineering. California water pollution control association.

Kocerba-Soroka, W., Fiatkowska, E., Pajdak-Stós, A., Klimek, B., Kowalska,E., Drzewicki, A., Salvado, H. and Fyda, J. (2013). The Use of Rotifers for Limiting Filamentous Bacteria Type 021n, a Bacteria Causing Activated Sludge Bulking. Water Science and Technology 67(7), 1557-63. doi:10.2166/wst.2013.028

Lima, A.T. (2008). Fly ashes contaminants removal:The electrodialytic process potentialities. Ph.D. Thesis. Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Portugal.

Lima, D.L.D., Schneider, R.J., Scherer, H.W., Duarte, A.C., Santos, E.B.H. and Esteves, V.I. (2010). Sorption– desorption behavior of atrazine on soils subjected to different organic long-term amendments. Journal of Agricultural and Food Chemistry, 58(5), 3101–6. doi:10.1021/jf903937d

Lima, D., Calisto, V. and Esteves, V.I. (2011). Adsorption Behavior of 17α-Ethynylestradiol onto Soils Followed by Fluorescence Spectral Deconvolution. Chemosphere, 84(8), 1072–1078. doi:10.1016/j.chemosphere.2011.04.060.

Lima, D., Schneider, R.J. and Esteves, V.I. (2012). Sorption Behavior of EE2 on Soils Subjected to Different Long-Term Organic Amendments.Science of the Total Environment 423, 120–124. doi:10.1016/j.scitotenv.2012.02.014.

Liu, Z., Kanjo,Y. and Mizutani,S. (2009). Removal Mechanisms for Endocrine Disrupting Compounds (EDCs) in Wastewater Treatment - Physical Means, Biodegradation, and Chemical Advanced Oxidation: A Review. Science of the Total Environment 407(2), 731–748. doi:10.1016/j.scitotenv.2008.08.039.

Lovett, R. (2005). Coffee: The demon drink? | New Scientist. 187, 38–41.

Lund, J.W.G, Kipling, C. and Le Cren, E.D. (1957). The Inverted Microscope Method of Estimating Algal Numbers and Statistical Basis of Estimations by Counting. Hydrobiology, 11, 143-170.

Magi, E., Di Carro, M., Scapolla, C. and Nguyen, K.T.N. (2012). Stir Bar Sorptive Extraction and LC–MS/MS for Trace Analysis of UV Filters in Different Water Matrices. Chromatographia 75(17), 973–982. doi:10.1007/s10337-012-2202-z

Malvern Instruments (2005). Zeta potential: an Introduction in 30 Minutes. Technical note. UK. Accessed in June 2015, http://www3.nd.edu/~rroeder/ame60647/slides/zeta.pdf

Matamoros, V., Gutiérrez, R., Ferrer, I., García, J. and Bayona, J.M. (2015). Capability of Microalgae-Based Wastewater Treatment Systems to Remove Emerging Organic Contaminants: A Pilot-Scale Study. Journal of Hazardous Materials 288, 34–42. doi:10.1016/j.jhazmat.2015.02.002

Matsui, Y., Knappe, D.R. and Takagi, R. (2002). Pesticide adsorption by granular activated carbon adsorbers.1. Effect of natural organic matter preloading on removal rates and model simplification. Environmental Science and Technology 36(15):3426–31.

Mazzacurati, M. (2007). Advanced studies on the synthesis of organophosphorus compounds. Phd thesis. University of Bologna. Accessed in May 2015, http://amsdottorato.cib.unibo.it/470/1/Tesi_Dottorato.pdf

McIntyre, A. and Lester, J. (1984). Occurrence and distribution of persistent organochlorine compounds in U.K. sewage sludges. Water Air Soil Pollution 23:397–415.

Meina, E.G., Lister, A., Bosker, T., Servos, M., Munkittrick,K. and MacLatchy, D. (2013). Effects of 17α-Ethinylestradiol (EE2) on Reproductive Endocrine Status in Mummichog (Fundulus Heteroclitus) under Differing Salinity and Temperature Conditions. Aquatic Toxicology, 134-135, 92–103. doi:10.1016/j.aquatox.2013.03.014

Mesquita Brito, N., Possidônio Amarante Junior, O. De, Polese, L., & Lúcia Ribeiro, M. (2003). Validação De Métodos Analíticos: Estratégia E Discussão. Pesticidas: Revista de Ecotoxicologia e Meio Ambiente 13, 129–146.

Metcalf and Eddy (2003). Wastewater Engineering: Treatment and Reuse. 4th ed. McGraw-Hill, New York.

Meyer, S.T. (1994). Chlorine Use in Water Disinfection, Trihalomethane Formation, and Potential Risks to Public Health. Caderno de Saúde Pública, Rio de Janeiro, 10 (1): 99-110.

Miège, C., Peretti, A., Labadie, P., Budzinski,H., Le Bizec,B., Vorkamp,K., Tronczyński, J., Persat, H., Coquery, M. and Babut, M. (2012). Occurrence of Priority and Emerging Organic Compounds in Fishes from the Rhone River (France). Analytical and Bioanalytical Chemistry 404(9), 2721–2735. doi:10.1007/s00216-012-6187-0.

Muller, J., Schlupmann, O., Gunther, L., Dockhorn, T., Dichtl, N.,Phan, L.C., Weichgrebe, D., Rosenwinkel, K.H., Bayerle, N. and Vesterager, N.O. (2008). Results of the modified seaborne process in Gifhorn 41. Essen conference for water and waste management. Essen 49, 1-13.

Nakanishi, A., Tamai, M., Kawasaki, N., Nakamura, T. and Tanada, S. (2002). Adsorption characteristics of bisphenol A onto carbonaceous materials produced from wood chips as organic waste. Journal of Colloid Interface Science 252(2), 393–396. doi:10.1006/jcis.2002.8387.

Niewersch, C., Meier, K., Wintgens, T. and Melin, T. (2010). Selectivity of polyamide nanofiltration membranes for cations and phosphoric acid. Desalination 250(3), 1021–1024. doi:10.1016/j.desal.2009.09.097.

Nsabimana, E., Bohatier, J., Belan, A., Pepin, D. and Charles, L. (1996). Effects of the Herbicide Atrazine on the Activated Sludge Process: Microbiology and Functional Views. 33(3), 479–494. doi:10.1016/0045-6535(96)00182-8

Nystrøm, G. M. (2001). Investigations of soil solution during enhanced electrodialytic soil remediation. Master Thesis. Technical University of Denmark.

Nystrom, G.M. (2004). Electrodialytic removal of heavy metals from contaminated harbor sediments. Phd thesis. Technical University of Denmark.

Ottosen, L. M., Hansen, H.K. and Hansen, C.B. (2000). Water Splitting at Ion-Exchange Membranes and Potential Diferences in Soil during Electrodialytic Soil Remediation. Journal of Applied Electrochemistry 30, 1199–1207.

Ottosen, L.M., Lima, A.T., Pedersen, A.J. and Ribeiro, A.B. (2006). Electrodialytic extraction of Cu, Pb and Cl from MSWI fly ash suspended in water. Journal of Chemical Technology and Biotechnology 81(4), 553–559. doi:10.1002/jctb.1424.

Ottosen, L.M., Jensen, P.E., Kirkelund, G.M., and Ebbers, B. (2014). Electrodialytic recovery and purification of phosphorous from sewage sludge ash, sewage sludge and wastewater. Filed in 2013, Denmark.

Peccia, J., and Westerhoff, P. (2015). We Should Expect More from Our Sewage Sludge. Environmental Science and Technology 49(14), 8271–8276. doi:10.1021/acs.est.5b01931

Pedersen, A.J. (2002). Electrodialytic removal of heavy metals from fly ashes. Ph.D. Thesis. Technical University of Denmark.

Pedersen, A. J., Ottosen, L. M., & Villumsen, A. (2003). Electrodialytic removal of heavy metals from different fly ashes. Journal of Hazardous Materials 100(1-3), 65–78. doi:10.1016/S0304-3894(03)00064-5

Peysson, W. and Vulliet, E. (2013). Determination of 136 Pharmaceuticals and Hormones in Sewage Sludge Using Quick, Easy, Cheap, Effective, Rugged and Safe Extraction Followed by Analysis with Liquid Chromatography-Time-of-Flight-Mass Spectrometry. Journal of Chromatography A 1290, 46–61. doi:10.1016/j.chroma.2013.03.057.

Pilon-Smits, E. (2005). Phytoremediation. Annual Review of Plant Biology, 56(1), 15–39. doi:10.1146/annurev.arplant.56.032604.144214

Plotan, M., Elliott, C.T., Frizzell, C. and Connolly,L. (2014). Estrogenic Endocrine Disruptors Present in Sports Supplements. A Risk Assessment for Human Health. Food Chemistry 159, 157–165. doi:10.1016/j.foodchem.2014.02.153

Philips Innovation Services. (2013). Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). Phillips inovation services. Accessed in June 2015, http://www.innovationservices.philips.com/sites/default/files/materials-analysis-icp-aes.pdf.

Pubchem (2015). Substances - NCBI. USA. Accessed in April 2015, https://pubchem.ncbi.nlm.nih.gov/.

Reinhold, D., Vishwanathan, S., Park, J.J., Oh, D. and Michael Saunders, F. (2010). Assessment of plant- driven removal of emerging organic pollutants by duckweed. Chemosphere 80(7), 687–692. doi:10.1016/j.chemosphere.2010.05.045.

Ribeiro, A.B. (1998). Use of electrodialytic remediation technique for removal of selected heavy metals and metalloids from soils. Ph.D Thesis. Technical University of Denmark.

Ribeiro, A. B., Réffega, A. J. M., Villumsen, A. and Bech-Nielsen, G. (1999). O processo electrodialítico na remediação de solos contaminados por metais pesados. Paper presented at the 6th National Conference on Environmental Quality performed, Lisbon, 20-22 Oct.

Ribeiro, A. B., Mateus, E. P., Ottosen, L. M. and Bech-Nielsen, G. (2000). Electrodialytic removal of Cu. Cr and As from chromated copper arsenate-treated timber waste. Environmental Science and Technology 34(5), 784–788. doi:10.1021/es990442e.

Ribeiro, A., & Rodríguez-Maroto, J. (2005). Electroremediation of Heavy Metal-Contaminated Soils -Processes and Applications. In Trace Elements in the Environment (pp. 325–340). CRC Press. doi:doi:10.1201/9781420032048.ch18

Rodil, R., Quintana, J.B., Concha-Graña, E., López-Mahía, P., Muniategui-Lorenzo, S. and Prada-Rodríguez, D. (2012). Emerging Pollutants in Sewage, Surface and Drinking Water in Galicia (NW Spain). Chemosphere 86(10), 1040–1049. doi:10.1016/j.chemosphere.2011.11.053

Safe, S.H. (2000). Endocrine Disruptors and Human Health: Is There a Problem? An Update. Environmental health perspectives 108(6), 487–93. Retrieved from http://europepmc.org/articles/PMC1638151/?report=abstract.

Sawyer, C.N. and McCarty, P.L., (1978), Chemistry for Environmental Engineering (3rd ed.). New York: McGraw-Hill Book Company.

Sewart, A., Harrad, S.J., McLachlan, M.S., Mcgrath, S.P. and Jones, K.C. (1995). PCDD/Fs and non-o-PCBs in digested U.K. sewage sludges. Chemosphere 30:51

Sigma-Aldrich Co. (1998). Guide to Solid Phase Extraction. Accessed in April 2015, http://www.sigmaaldrich.com/Graphics/Supelco/objects/4600/4538.pdf.

Sigma-Aldrich (2015). Substances. USA. Accessed in April 2015, http://www.sigmaaldrich.com/portugal.html.

Simarsul (2012). Estação de Tratamento de Águas Residuais (ETAR) da Quinta do Conde. Sistema Integrado Multimunicipal de Águas Residuais da Península de Setúbal.

Sjöström, E., Collins, C.D., Smith, S.R. and Shaw, G. (2008). Degradation and plant uptake of nonylphenol (NP) and nonylphenol-12-ethoxylate (NP12EO) in four contrasting agricultural soils. Environmental Pollution 156(3), 1284–1289. doi:10.1016/j.envpol.2008.03.005.

Snoeyink, V.L. and Jenkins, D.(1980). Water Chemistry. Wiley. University of Michingan.

Snyder, S.A., Westerhoff, P., Yoon, Y. and Sedlak, D.L. (2003). Pharmaceuticals, personal care products, and endocrine disruptors in water: implications for the water industry. Environmental Engineering Science 20(5), 449–469. doi:10.1089/109287503768335931.

Sørensen, B. L., Dall, O. L. and Habib, K. (2015). Environmental and Resource Implications of Phosphorus Recovery from Waste Activated Sludge. Waste Management. doi:10.1016/j.wasman.2015.02.012

Stevens, J., Green, N.J.L. and Jones, K.C. (2001). Survey of PCDD/Fs and non-ortho PCBs in UK sewage sludges. Chemosphere 44(6), 1455–1462. doi:10.1016/S0045-6535(00)00474-4.

Stevens, J.L., Northcott, G.L., Stern, G.A., Tomy, G.T. and Jones, K.C. (2003). PAHs, PCBs, PCNs, organochlorine pesticides, synthetic musks, and polychlorinated n-alkanes in U.K. sewage sludge: Survey results and implications. Environmental Science and Technology 37(3), 462–467. doi:10.1021/es020161y.

Stina, C. (1998). Sweden Creates Sludge Products. World Wastes, 10648429, Oct98, Vol. 41, Issue 10. Sweeden. Accessed in June 2015, http://web.b.ebscohost.com/ehost/detail/detail?vid=1&sid=bedc5811-3450-4757-8dbd61c0ed0de02e%40sessionmgr113&hid=110&bdata=JnNpdGU9ZWhvc3QtbGl2ZQ%3d%3d#db=bth&AN=1207208.

Stumpe, B. and Marschner, B. (2009). Factors controlling the biodegradation of 17β-estradiol, estrone and 17α-ethinylestradiol in different natural soils. Chemosphere 74(4), 556–562. doi:10.1016/j.chemosphere.2008.09.072.

Svenson, A., Allard, A.S. and Ek, M. (2003). Removal of estrogenicity in Swedish municipal sewage treatment plants. Water Research 37(18), 4433–4443. doi:10.1016/S0043-1354(03)00395-6

Ternes, T.A., Bonerz, M. and Schmidt, T. (2001).Determination of neutral pharmaceuticals in wastewater and rivers by liquid chromatographyelectrospray tandem mass spectrometry. Journal of Chromatography A, 938(1-2), 175–185. doi:10.1016/S0021-9673(01)01205-5.

Tsai, W.T., Lai, C.W. and Su, T.Y. (2006). Adsorption of bisphenol-A from aqueous solution onto minerals and carbon adsorbents. Journal of Hazardous Materials 134(1-3), 169–75. doi:10.1016/j.jhazmat.2005.10.055.

Vaccari, D. (2011). Chemosphere Phosphorus Cycle Issue - Introduction. Chemosphere 84(6), 735–736. doi:10.1016/j.chemosphere.2011.06.050

Van Der Bruggen, B., Schaep, J., Maes, W., Wilms, D., & Vandecasteele, C. (1998). Nanofiltration as a treatment method for the removal of pesticides from ground waters. Desalination, 117(1-3), 139–147. doi:10.1016/S0011-9164(98)00081-2

Vermeulen, F., Covaci, A., D'Havé, H., Van den Brink, N. W., Blust, R., De Coen, W. and Bervoets, L. (2010). Accumulation of background levels of persistent organochlorine and organobromine pollutants through the soil-earthworm-hedgehog food chain. Environment International 36(7), 721–727. doi:10.1016/j.envint.2010.05.006.

Voulvoulis, N. and Lester, J.N. (2006). Fate of organotins in sewage sludge during anaerobic digestion. Science of the Total Environmental 371(1-3), 373–382. doi:10.1016/j.scitotenv.2006.08.024.

Voulvoulis, N., Scrimshaw, M.D. and Lester, J.N.(2004). Removal of organotins during sewage treatment: a case study. Environmental Technology 25(6), 733–740. doi:10.1080/09593330.2004.9619363.

Westerhoff, P., Yoon, Y., Snyder, S. and Wert, E.(2005). Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes. Environmental Science and Technology 39(17), 6649–6663. doi:10.1021/es0484799.

Wild, S.R., Waterhouse, K.S., McGrath, S.P. and Jones, K.C. (1990). Organic contaminants in an agricultural soil with a known history of sewage sludge amendments: polynuclear aromatic hydrocarbons. Environmental Science and Technology 24, 1706–1711. doi:10.1021/es00081a013.

Wilkowska, A. and Biziuk, M. (2011). Determination of Pesticide Residues in Food Matrices Using the QuEChERS Methodology. Food Chemistry 125(3): 803–12. doi:10.1016/j.foodchem.2010.09.094

Wilson, S. C., Alcock, R. E., Sewart, A. P., & Jones, K. C. (1997). Persistence of Organic Contaminants in Sewage Sludge-Amended Soil: A Field Experiment. Journal of Environment Quality, 26(6), 1467. doi:10.2134/jeq1997.00472425002600060004x

World Bank Group (2015). Introduction to wastewater treatment processes. New Hampshire, EUA. Accessed in May 2015, http://water.worldbank.org/shw-resource-guide/infrastructure/menu-technical-options/wastewater-treatment.

Wzorek, Z., and Gorazda,K. (2007). Phosphorus Recovery from Waste - Methods Review. Polish Journal of Chemical Technology 9(2): 57–60. doi:10.2478/v10026-007-0027-6

Yaroshchuk, A., Martınez-Llado, X., Llenas, L., Rovira, M. and de Pablo, J. (2011). Solution-diffusion model for the description of pressure-driven trans-membrane transfer of electrolyte mixtures: one dominant salt and trace ions. Journal of Membrane Science 368(1-2), 192–201. doi:10.1016/j.memsci.2010.11.037.

Ye, X., Zhou,X., Needham, L.L. and Calafat, A.M. (2011). In-Vitro Oxidation of Bisphenol A: Is Bisphenol A Catechol a Suitable Biomarker for Human Exposure to Bisphenol A? Analytical and Bioanalytical Chemistry 399(3), 1071–1079. doi:10.1007/s00216-010-4344-x.

Yuan, S., Gou, N., Alshawabkeh, A.N. and Gu, A.Z. (2013). Efficient degradation of contaminants of emerging concerns by a new electro-Fenton process with Ti/MMO cathode, Chemosphere 93(11), 2796–804. doi:10.1016/j.chemosphere.2013.09.051.

Xu, N., Zhang, Y., Tao, H., Zhou, S. and Zeng, Y. (2013). Bio-electro-Fenton system for enhanced estrogens degradation, Bioresource Technology 138. 136–140. doi:10.1016/j.biortech.2013.03.157

Zeyoudi, M. and Hasan, S.W. (2014). Evaluation of microbial communities, growth rates and susbtrate consumption under electrical field, in: J.M.R. Maroto, R. García-Delgado, F. García-Herruzo, C. Gómez-Lahoz, C. Vereda-Alonso, M. Villén-Guzmán (Eds.), 13th Symposium on Electrokinetic Remediation, EREM14, Málaga, Spain, pp. 194.

Zha, J.M. and Wang, Z.J. (2005). Assessing technological feasibility for wastewater reclamation based on early life stage toxicity of Japanese medaka (Oryzias latipes). Agriculture, Ecosystems and Environmental 107(2-3), 187–198. doi:10.1016/j.agee.2004.11.009.

Zhou, J.L., Liu, R., Wilding, A. and Hibberd, A. (2007). Sorption of selected endocrine disrupting chemicals to different aquatic colloids. Environmental Science and Technology 41(1), 206–13. Accessed in june 2015, http://www.ncbi.nlm.nih.gov/pubmed/17265949.

Appendix

Table 0.1: Microorganisms counting results for Control experiment

Taxa	**Control (**number of individuals *per* gram of sludge)													
	ED-T0	ED-T1	RSU	ED-T2	RSU	ED-T3	RSU	CBd*-T1	CBnd**-T1	CBd-T2	CBnd-T2	CBd-T3	CBnd-T3	**Total**
Arcella gibbosa	16800	23400	15698	22200	14425	6000	1273	3300	12000	0	300	300	0	84300
Aspidisca cicada	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bodo sp.	2700	19350	10819	3300	4667	0	0	4200	4800	0	0	0	0	34350
Centric Diatom n.i.	0	0	0	0	0	0	0	0	0	1200	0	900	0	2100
Cyanobacteria n.i.	0	0	0	0	0	0	0	0	0	0	0	23400	7500	30900
Didinium sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Difflugia sp.	2400	0	0	0	0	0	0	0	0	0	0	0	0	2400
Epistylis spp.	58500	69750	58336	11550	16334	2100	2970	23400	31800	1200	2700	5100	8100	214200
Euglena sp.	0	600	849	0	0	0	0	0	0	0	0	0	0	600
Filamentous algae n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Filamentous Chloroficea n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Free-living nematode n.i.	0	0	0	1350	1909	150	212	0	0	0	0	0	0	1500
Litonotus sp.	13500	0	0	0	0	0	0	0	0	0	0	0	0	13500
Naked amoeba n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula spp.	0	1200	1697	150	212	300	424	1500	3600	300	0	300	0	7350
Nitzschia spp.	0	450	636	0	0	0	0	0	0	0	0	0	0	450
Opercularia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oscillatoria spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pendunclate cilliate n.i.	0	22650	18880	16950	2758	3900	5515	42300	43200	8400	3300	0	0	140700
Philodenia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pirobotrys sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Podoprhya spp.	0	1050	1485	0	0	1350	636	4500	0	0	0	300	600	7800
Pollen grain (Pinus spp.)	0	0	0	0	0	300	424	0	0	0	0	600	600	1500
Rotatoria sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rotífer (egg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rotífer (*mastax*)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rotífer n.i.	0	0	0	0	0	150	212	0	0	0	0	0	0	150
Spirilum sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spirulina sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tardigrade n.i	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Toxocara canis (egg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vorticella spp.	151500	45450	21850	25950	16334	6150	2758	20100	12300	2100	3000	3000	4200	273750
Zoogloea spp. (dendritic growth)	3000	1800	1697	0	0	0	0	2700	0	0	0	0	0	7500
Zoogloea spp. (globular growth)	3300	150	212	0	0	0	0	2400	0	300	300	0	0	6450
Zoothamnium spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	251700	185850	132158	81450	56639	20400	14425	104400	107700	13500	9600	33900	21000	829500

*Control Beaker doped **Control Beaker non doped

Table 0.2:Microorganisms counting results for 50 mA experiment

Taxa	**50 mA (**number of individuals *per* gram of sludge)																	
	ED-T0	ED-T1/2	RSU	ED-T1	RSU	ED-T2	RSU	ED -T3	RSU	CBd*-T1/2	CBnd**-T1/2	CBd-T1	CBnd-T1	CBd-T2	CBnd-T2	CBd-T3	CBnd-T3	**Total**
Arcella gibbosa	10200	10800	424	3600	424	1350	636	600	849	14400	16500	8700	3300	3900	1500	0	1200	76050
Aspidisca cicada	1500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1500
Bodo sp.	600	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	600
Centric Diatom n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyanobacteria n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Didinium sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Difflugia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	300	0	300	600
Epistylis spp.	60900	11250	1061	600	849	300	0	150	212	27900	28800	17700	27600	2700	37500	0	16500	231900
Euglena sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Filamentous algae n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Filamentous Chloroficea n.i.	0	0	0	0	0	4500	6364	0	0	0	0	0	0	0	0	0	0	4500
Free-living nematode n.i.	0	150	212	0	0	0	0	0	0	0	300	0	0	0	0	0	0	450
Litonotus sp.	4200	0	0	0	0	0	0	0	0	0	300	0	0	0	0	0	0	4500
Naked amoeba n.i.	0	0	0	300	0	0	0	150	212	0	600	0	0	0	0	0	300	1350
Navicula spp.	300	0	0	300	0	300	0	150	212	300	600	600	300	0	300	0	0	3150
Nitzschia spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Opercularia sp.	0	0	0	0	0	0	0	0	0	0	300	0	0	0	0	0	0	300
Oscillatoria spp.	0	0	0	1050	1485	0	0	0	0	0	0	0	0	0	0	0	0	1050
Pendunclate cilliate n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Philodenia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pirobotrys sp.	0	0	0	0	0	450	636	0	0	0	0	0	0	0	0	0	0	450
Podoprhya spp.	1500	150	212	0	0	0	0	0	0	0	300	600	900	0	0	0	0	3450
Pollen grain (Pinus spp.)	600	300	0	600	424	300	0	0	0	300	600	300	0	0	0	0	0	3000
Rotatoria sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rotífer (egg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rotífer (*mastax*)	0	0	0	150	212	300	0	150	212	0	0	0	300	300	300	0	300	1800
Rotífer n.i.	0	300	0	150	212	0	0	0	0	300	0	0	0	0	0	0	0	750
Spirilum sp.	0	0	0	0	0	0	0	0	0	300	0	0	0	0	0	0	0	300
Spirulina sp.	0	0	0	8250	11667	0	0	0	0	0	0	0	0	0	0	0	0	8250
Tardigrade n.i	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Toxocara canis (egg)	0	0	0	750	636	150	212	0	0	600	300	300	0	0	0	0	0	2100
Vorticella spp.	34800	10950	636	1500	849	1200	424	1050	636	12300	11100	11100	7800	11100	9300	2700	6000	120900
Zoogloea spp. (dendritic growth)	600	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	600
Zoogloea spp. (globular growth)	0	600	849	0	0	0	0	0	0	300	300	300	0	0	0	0	0	1500
Zoothamnium spp.	0	600	849	0	0	0	0	0	0	300	300	300	0	0	0	0	0	1500
Total	131700	45600	3394	17250	16758	8850	8273	2250	2333	76200	70800	46500	47400	20700	62400	3300	24600	557550

*Control Beaker doped **Control Beaker non doped

Table 0.3: Microorganisms counting results for 75 mA experiment

Taxa	**75 mA (**number of individuals *per* gram of sludge)																	
	ED-T0	ED-T1/2	RSU	ED-T1	RSU	ED-T2	RSU	ED - T3	RSU	CBd*-T1/2	CBnd**-T1/2	CBd-T1	CBnd-T1	CBd-T2	CBnd-T2	CBd-T3	CBnd-T3	**Total**
Arcella gibbosa	42000	97500	26729	26100	9758	19500	10607	16350	1909	78300	54900	4800	22800	9000	12000	1200	24300	408750
Aspidisca cicada	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bodo sp.	9900	20550	5303	0	0	0	0	0	0	12600	0	0	0	0	0	0	0	43050
Centric Diatom n.i.	0	900	1273	0	0	0	0	0	0	0	0	0	0	0	0	0	9000	9900
Cyanobacteria n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Didinium sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Difflugia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Epistylis spp.	18600	15150	7000	300	0	450	212	0	0	33000	7800	3600	3300	600	0	0	900	83700
Euglena sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Filamentous algae n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Filamentous Chloroficea n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Free-living nematode n.i.	0	150	212	0	0	0	0	0	0	0	0	0	0	0	0	0	0	150
Litonotus sp.	1500	300	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1800
Naked amoeba n.i.	0	0	0	0	0	0	0	300	424	0	0	0	0	0	0	0	0	300
Navicula spp.	0	0	0	0	0	300	424	300	424	0	0	0	0	0	0	0	600	1200
Nitzschia spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Opercularia sp.	1200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1200
Oscillatoria spp.	0	2250	3182	0	0	3900	5515	0	0	0	0	0	0	0	0	0	0	6150
Pendunclate cilliate n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Philodenia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pirobotrys sp.	900	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	900
Podoprhya spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pollen grain (Pinus spp.)	300	600	424	150	212	150	212	0	0	900	1500	0	300	300	0	0	300	4500
Rotatoria sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rotífer (egg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rotífer (*mastax*)	0	0	0	0	0	150	212	0	0	0	0	0	0	0	0	0	0	150
Rotífer n.i.	0	600	424	450	212	0	0	0	0	900	300	0	0	0	0	0	0	2250
Spirilum sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spirulina sp.	0	0	0	3600	5091	0	0	0	0	0	0	0	0	0	0	0	0	3600
Tardigrade n.i	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Toxocara canis (egg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vorticella spp.	7200	20700	1697	1050	636	1200	0	1350	212	14400	9900	1800	900	900	300	0	600	60300
Zoogloea spp. (dendritic growth)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Zoogloea spp. (globular growth)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Zoothamnium spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	81600	158700	46245	31650	15910	25650	17183	18300	2970	140100	74400	10200	27300	10800	12300	1200	35700	627900

*Control Beaker doped **Control Beaker non doped

Table 0.4: Microorganisms counting results for 100 mA experiment

Taxa	**100 mA (**number of individuals *per* gram of sludge)																	
	ED-T0	ED-T1/2	RSU	ED-T1	RSU	ED-T2	RSU	ED - T3	RSU	CBd*-T1/2	CBnd**-T1/2	CBd-T1	CBnd-T1	CBd-T2	CBnd-T2	CBd-T3	CBnd-T3	**Total**
Arcella gibbosa	135375	147938	25721	153000	39457	81900	50063	70950	20153	180750	127875	135375	79200	106200	18300	31200	2700	1270763
Aspidisca cicada	1500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1500
Bodo sp.	1875	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1875
Centric Diatom n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1200	0	1200
Cyanobacteria n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Didinium sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Difflugia sp.	0	0	0	0	0	0	0	150	212	0	0	0	0	0	0	0	0	150
Epistylis spp.	58875	6188	1326	450	212	0	0	0	0	28500	13125	7500	10200	6000	300	900	300	132338
Euglena sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Filamentous algae n.i.	0	2813	3977	0	0	0	0	1650	2333	0	4125	0	0	0	0	0	0	8588
Filamentous Chloroficea n.i.	0	0	0	2250	3182	2400	3394	0	0	8250	0	6000	2100	0	0	0	0	21000
Free-living nematode n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Litonotus sp.	4875	0	0	150	212	0	0	0	0	0	0	0	0	0	0	0	0	5025
Naked amoeba n.i.	0	0	0	0	0	0	0	150	212	0	0	0	300	0	0	0	0	450
Navicula spp.	0	0	0	150	212	150	212	0	0	375	0	0	0	0	0	0	300	975
Nitzschia spp.	0	0	0	0	0	0	0	0	0	0	0	375	0	0	0	0	0	375
Opercularia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oscillatoria spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pendunclate cilliate n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Philodenia sp.	750	0	0	150	212	150	212	0	0	0	0	0	0	300	0	0	0	1350
Pirobotrys sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Podoprhya spp.	3000	0	0	0	0	0	0	0	0	0	0	375	0	0	0	0	0	3375
Pollen grain (Pinus spp.)	0	188	265	450	636	300	0	0	0	0	375	375	0	900	0	300	0	2888
Rotatoria sp.	375	938	265	0	0	0	0	0	0	1125	375	0	0	0	0	0	0	2813
Rotífer (egg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rotífer (*mastax*)	0	0	0	150	212	0	0	0	0	0	375	0	0	0	0	0	0	525
Rotífer n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spirilum sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spirulina sp.	0	0	0	0	0	0	0	0	0	0	0	8625	0	0	0	0	0	8625
Tardigrade n.i	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Toxocara canis (egg)	0	0	0	150	212	150	212	0	0	0	0	0	0	0	0	0	0	300
Vorticella spp.	6375	3563	1856	300	424	0	0	0	0	8625	6000	1875	2700	600	0	0	0	30038
Zoogloea spp. (dendritic growth)	21750	1500	530	0	0	0	0	0	0	0	1125	0	0	0	0	0	0	24375
Zoogloea spp. (globular growth)	14625	1875	530	1350	636	0	0	600	424	3000	3000	4500	900	0	0	0	0	29850
Zoothamnium spp.	2625	1500	0	0	0	0	0	0	0	3750	1500	750	600	0	0	0	0	10725
Total	252000	166500	34471	158550	45608	85050	54094	73500	23335	234375	157875	165750	96000	114000	18600	33600	3300	1559100

*Control Beaker doped **Control Beaker non doped

Table 0.5: Microorganisms counting results for 50-75-100 mA experiment

Taxa	**50-75-100 mA (**number of individuals *per* gram of sludge)													
	ED-T0	ED-T1	RSU	ED-T2	RSU	ED-T3	RSU	CBd*-T1	CBnd*-T1	CBd-T2	CBnd-T2	CBd-T3	CBnd-T3	**Total**
Arcella gibbosa	160500	101550	18880	90900	19092	120900	70428	47625	115875	22500	55125	4500	21300	740775
Aspidisca cicada	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bodo sp.	375	0	0	0	0	0	0	0	0	0	0	0	0	375
Centric Diatom n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyanobacteria n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Didinium sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Difflugia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Epistylis spp.	7875	750	636	150	212	0	0	375	375	300	0	0	0	9825
Euglena sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Filamentous algae n.i.	0	8250	11667	0	0	0	0	0	0	0	0	0	0	8250
Filamentous Chloroficea n.i.	0	0	0	0	0	4950	7000	0	0	0	0	0	0	4950
Free-living nematode n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Litonotus sp.	1500	0	0	0	0	0	0	0	0	0	0	0	0	1500
Naked amoeba n.i.	375	0	0	0	0	150	212	750	0	0	0	0	0	1275
Navicula spp.	0	0	0	0	0	150	212	0	375	300	0	0	0	825
Nitzschia spp.	375	0	0	150	212	0	0	0	0	0	0	0	0	525
Opercularia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oscillatoria spp.	5625	0	0	0	0	0	0	0	0	0	0	3600	0	9225
Pendunclate cilliate n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Philodenia sp.	0	150	212	0	0	0	0	0	0	0	0	0	0	150
Pirobotrys sp.	12000	0	0	0	0	0	0	0	0	0	0	0	0	12000
Podoprhya spp.	750	0	0	0	0	0	0	0	0	0	0	0	0	750
Pollen grain (Pinus spp.)	0	600	0	300	424	450	212	0	0	0	0	0	0	1350
Rotatoria sp.	0	0	0	0	0	0	0	0	1500	0	0	0	0	1500
Rotífer (egg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rotífer (*mastax*)	0	0	0	150	212	0	0	0	0	0	0	0	0	150
Rotífer n.i.	375	300	424	0	0	0	0	0	0	300	0	0	0	975
Spirilum sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spirulina sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tardigrade n.i	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Toxocara canis (egg)	750	0	0	0	0	0	0	0	0	0	0	0	0	750
Vorticella spp.	24750	150	212	450	212	0	0	0	375	300	0	0	0	26025
Zoogloea spp. (dendritic growth)	42000	900	849	0	0	0	0	0	375	0	0	300	0	43575
Zoogloea spp. (globular growth)	12000	2100	0	1500	849	900	1273	2250	2625	0	0	0	0	21375
Zoothamnium spp.	3750	0	0	0	0	0	0	0	0	0	0	0	0	3750
Total	273000	114750	32880	93600	21213	127500	79337	51000	121500	23700	55125	8400	21300	889875

*Control Beaker doped **Control Beaker non doped

Table 0.6: Microorganisms counting results for 100-75-50 mA experiment

Taxa	**100-75-50 mA (**number of individuals *per* gram of sludge)													
	ED-T0	ED-T1	RSU	ED-T2	RSU	ED-T3	RSU	CBd*-T1	CBnd*-T1	CBd-T2	CBnd-T2	CBd-T3	CBnd-T3	**Total**
Arcella gibbosa	160500	101550	18880	90900	19092	120900	70428	47625	115875	22500	55125	4500	21300	740775
Aspidisca cicada	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bodo sp.	375	0	0	0	0	0	0	0	0	0	0	0	0	375
Centric Diatom n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyanobacteria n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Didinium sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Difflugia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Epistylis spp.	7875	750	636	150	212	0	0	375	375	300	0	0	0	9825
Euglena sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Filamentous algae n.i.	0	8250	11667	0	0	0	0	0	0	0	0	0	0	8250
Filamentous Chloroficea n.i.	0	0	0	0	0	4950	7000	0	0	0	0	0	0	4950
Free-living nematode n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Litonotus sp.	1500	0	0	0	0	0	0	0	0	0	0	0	0	1500
Naked amoeba n.i.	375	0	0	0	0	150	212	750	0	0	0	0	0	1275
Navicula spp.	0	0	0	0	0	150	212	0	375	300	0	0	0	825
Nitzschia spp.	375	0	0	150	212	0	0	0	0	0	0	0	0	525
Opercularia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oscillatoria spp.	5625	0	0	0	0	0	0	0	0	0	0	3600	0	9225
Pendunclate cilliate n.i.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Philodenia sp.	0	150	212	0	0	0	0	0	0	0	0	0	0	150
Pirobotrys sp.	12000	0	0	0	0	0	0	0	0	0	0	0	0	12000
Podoprhya spp.	750	0	0	0	0	0	0	0	0	0	0	0	0	750
Pollen grain (Pinus spp.)	0	600	0	300	424	450	212	0	0	0	0	0	0	1350
Rotatoria sp.	0	0	0	0	0	0	0	0	1500	0	0	0	0	1500
Rotífer (egg)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rotífer (*mastax*)	0	0	0	150	212	0	0	0	0	0	0	0	0	150
Rotífer n.i.	375	300	424	0	0	0	0	0	0	300	0	0	0	975
Spirilum sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spirulina sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tardigrade n.i	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Toxocara canis (egg)	750	0	0	0	0	0	0	0	0	0	0	0	0	750
Vorticella spp.	24750	150	212	450	212	0	0	0	375	300	0	0	0	26025
Zoogloea spp. (dendritic growth)	42000	900	849	0	0	0	0	0	375	0	0	300	0	43575
Zoogloea spp. (globular growth)	12000	2100	0	1500	849	900	1273	2250	2625	0	0	0	0	21375
Zoothamnium spp.	3750	0	0	0	0	0	0	0	0	0	0	0	0	3750
Total	181875	1768122	37388	131625	41896	89063	31555	34125	41625	19500	15000	4125	4500	698250

*Control Beaker doped **Control Beaker non doped

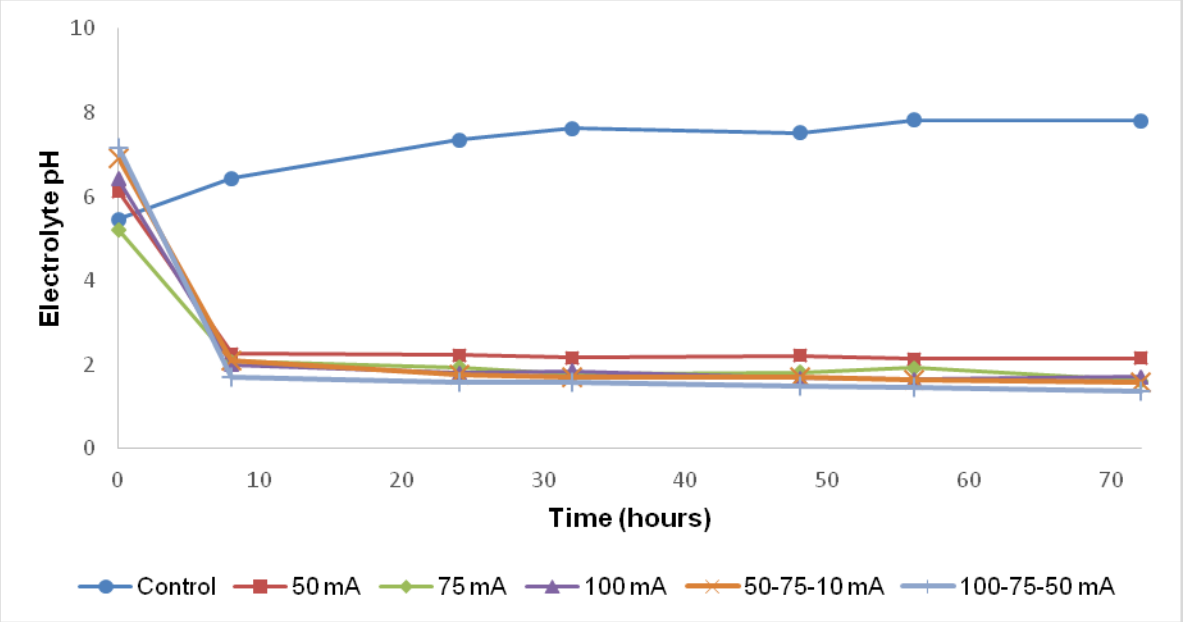


Figure 0.1: Development of pH in the electrolyte compartment during ED

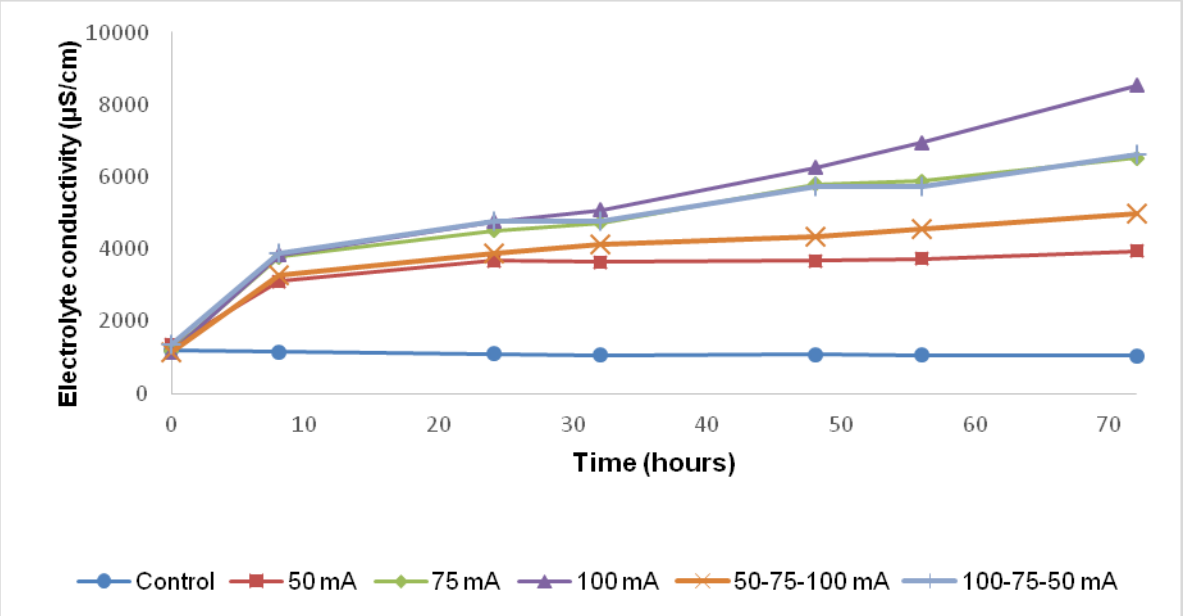


Figure 0.2: Development of conductivity in the electrolyte compartment during ED5