EXPLORATION OF BIOLOGICAL TREATMENT SYSTEMS FOR THE REMOVAL OF PERSISTENT LANDFILL LEACHATE CONTAMINANTS AND NANOPARTICLES

by

Francisco Gomez-Rivera

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF CHEMICAL AND ENVIRONMENTAL ENGINEERING

In Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

WITH A MAJOR IN ENVIRONMENTAL ENGINEERING

In the Graduate College

THE UNIVERSITY OF ARIZONA

2011

THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Francisco Gomez-Rivera
entitled Exploration of Biological Treatment Systems for the Removal of Persistent Landfill Leachate Contaminants and Nanoparticles
and recommended that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy

_____Date: 11/24/10
James A. Field

_____Date: 11/24/10
Maria Reyes Sierra Alvarez

_____Date: 11/24/10
Raina M. Maier

_____Date: 11/24/10
Robert G. Arnold

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copies of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommended that it be accepted as fulfilling the dissertation requirement.

_____Date: 11/24/10
Dissertation Director: James A. Field

STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Francisco Gomez-Rivera

ACKNOWLEDGEMENTS

I would like to take this opportunity to express my deepest gratitude and appreciation to my advisors, Dr. James A. Field and Dr. Maria Reyes Sierra Alvarez, for their excellent guidance, assistance, and invaluable support. My advisors provided me with the tools and knowledge required to successfully complete my degree. I would like to thank as well to my Committee members, Dr. Raina M. Maier and Dr. Robert G. Arnold, for their time and expertise to improve my work.

I am heartily thankful to all the people that in any way assisted me and supported me while pursuing my degree; especially to those who directly helped me in effectively achieving my goals, including professors, technicians, administrative staff, and graduate and undergraduate students.

I would like to thank to the Consejo Nacional de Ciencia y Tecnologia (CONACyT) for providing the financial support. The labor that CONACyT does is remarkable, giving the opportunity to numerous students to study an advanced degree in renown Universities.

I am especially grateful to Aida Tapia-Rodriguez. Her company and understanding is the motor that keeps me going. Finally I would like to deeply thank my family. I would not have gotten this far without their unconditional trust and encouragement.

TABLE OF CONTENTS

TABLE OF CONTENTS – *Continued*

TABLE OF CONTENTS – *Continued*

TABLE OF CONTENTS – *Continued*

TABLE OF CONTENTS – *Continued*

TABLE OF CONTENTS – *Continued*

LIST OF FIGURES

LIST OF FIGURES - *Continued*

LIST OF FIGURES - *Continued*

LIST OF FIGURES - *Continued*

LIST OF FIGURES - *Continued*

LIST OF TABLES

ABSTRACT

The integrity of groundwater sources is constantly threatened by contaminant plumes generated by accidental gasoline leakages and leachates escaping landfills. These plumes are of concern due to their proven toxicity to living organisms. Aromatic and chlorinated hydrocarbons, volatile fatty acids, phenols, and ammonia have been found in these leachates. In addition, benzene, toluene, and xylenes (BTX) are major components of gasoline. The lack of oxygen in groundwater makes anaerobic bioremediation desired for the treatment of groundwater contaminated with BTX and chlorinated solvents. With the objective of finding microorganisms capable of BTX and *cis*-dichloroethylene (*cis*-DCE) degradation under anaerobic conditions for their use in permeable reactive barriers, different inocula were tested in batch experiments. Toluene was rapidly degraded by several inocula in the presence of alternative electron acceptors. Benzene and *m*-xylene were eliminated by few of the inocula tested after incubation periods ranging from 244 to 716 days. *cis*-DCE was highly recalcitrant as no degradation was observed over 440 days. Biological processes have been successfully applied for the treatment of landfill leachates as well. In an effort to provide an effective and economical alternative, an anaerobic-aerobic system was evaluated using a synthetic media simulating the organic and ammonia content of real leachates. The removal of the organic content reached 98% in an upflow anaerobic sludge blanket reactor, and resulted in the formation of methane. During the aerobic process, in an innovative down-flow sponge

reactor, ammonia was highly transformed to nitrite and nitrate. Complete nitrification was eventually achieved.

The capacity of current wastewater treatment plants for removing nanoparticles has been questioned during the last years. Nanoparticles have been incorporated into numerous applications and their presence in wastewater seems to be inevitable. A laboratory-scale secondary treatment system was set-in to study the behavior of cerium and aluminum oxide nanoparticles during wastewater treatment. The nanoparticles were highly removed, suggesting that secondary treatment is suitable for their elimination. The removal of these nanoparticles was influenced by the pH and organic content of the wastewater. Aluminum nanoparticles proved to be toxic; however the performance of the system for eliminating the organic content was recovered over time.

OBJECTIVES

The aim of this research was to assess the capability of different biological technologies for the removal of organic and inorganic contaminants commonly found in gasoline plumes and landfill leachates and oxide nanoparticles present in municipal wastewater. The particular objectives of this PhD dissertation are as follows:

I. Evaluate the presence of microorganisms, in different inocula, capable of degrading BTX and *cis*-DCE under anaerobic conditions using nitrate and chlorate as alternative electron acceptors.

II. Study the feasibility of obtaining pure cultures of BTX and *cis*-DCE degrading organisms by serial transfers.

III. Evaluate the performance of an integrated anaerobic-aerobic system for the treatment of a synthetic media simulating a real landfill leachate.

IV. Evaluate the partial nitrification of ammonia in the synthetic leachate to nitrite to promote the elimination of nitrogen under aerobic conditions, eliminating the implementation of a post-treatment process.

V. Study the behavior of cerium and aluminum oxide nanoparticles during activated sludge secondary treatment.

VI. Elucidate the mechanisms that promote the removal, if any, of the nanoparticles during treatment.

VII. Investigate the effect of the nanoparticles over the performance of the treatment system for removing the organic content of the wastewater.

CHAPTER 1

INTRODUCTION

1.1 Hydrocarbons

Hydrocarbons are organic compounds composed entirely of carbon and hydrogen and can be found as gases, liquids, or solids. They can have different shapes, including straight and branched chains and cyclic arrangements. According to the carbon-carbon (C-C) bonding features in the molecule, hydrocarbons can be classified into single-bonded (alkanes), double-bonded (alkenes), triple-bonded (alkynes), and mono- or polycyclic aromatics. Hydrocarbons are widely abundant in nature and are formed by geochemical reactions or during the degradation of organic matter mediated by living organisms [1]. Hydrocarbons can be synthetically produced as well, for example, by the catalytic mediated reaction between hydrogen and carbon monoxide (CO) [2]. Hydrocarbons are the most important source of energy around the globe [3] and find numerous industrial and commercial applications. It is believed that the use of hydrocarbons as fuels is of major importance for the human development in main areas such as the economic and social sectors [4]. The economic importance of hydrocarbons is

not only related to their use as fuels, but also to their utilization in the manufacturing of plastics, dyes, paints, pesticides, among others. However, the dependence of the society on hydrocarbons may pose a risk to the environment, as different studies have demonstrated that anthropogenic activities are the main source of hydrocarbon contamination in different ecosystems [5-8].

1.1.1 Aromatic hydrocarbons

Aromatic hydrocarbons comprise the second most abundant group of organic compounds in nature [9]. They alternate single and double bonds between carbons in the molecule and contain one or more benzene rings. Benzene, toluene, and (*o-*, *m-*, *p-*) xylene (BTX) are volatile monoaromatic hydrocarbons of great importance to society since they are used in diverse processes and commercial products. Figure 1.1 provides the structural formula of each of the BTX compounds. They are found in petroleum and petroleum derivatives and are extensively utilized for the manufacturing of plastics, fibers, pesticides, paints, inks, dyes, and nylon. In 1997, the industrial production of these compounds was estimated in the range of megatons per year (1×10^{12} grams) [10]. It is not surprising that BTX compounds are recognized as one of the major causes of environmental pollution [11], resulted from landfill leachates reaching groundwater, leakage of underground storage tanks (USTs) and pipelines, spills at production wells, refineries, and distribution terminals, and improper disposal and accidents during transport [12]. BTX are so prevalent as environmental contaminants, that these chemicals

have been listed in the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) priority list. Concerns about exposure to BTX are due to their known toxic effects [13] and their relatively high mobility, which allows them to travel long distances. According to the Agency for Toxic Substances and Disease Registry (ATSDR), long term exposure to toluene [14] and the three xylene isomers [15] can result in damage of the central nervous system, the liver and/or the kidneys; whereas benzene is a suspected carcinogen [16]. For this reason, the Environmental Protection Agency (EPA) has set a maximum contaminant level (MCL) in potable water of 0.005, 1, and 10 mg L^{-1}, for benzene, toluene, and (*o-, m-, p-*) xylene, respectively (Table 1.1). Although BTX are so widespread as pollutants in nature, other organic compounds of concern as well.

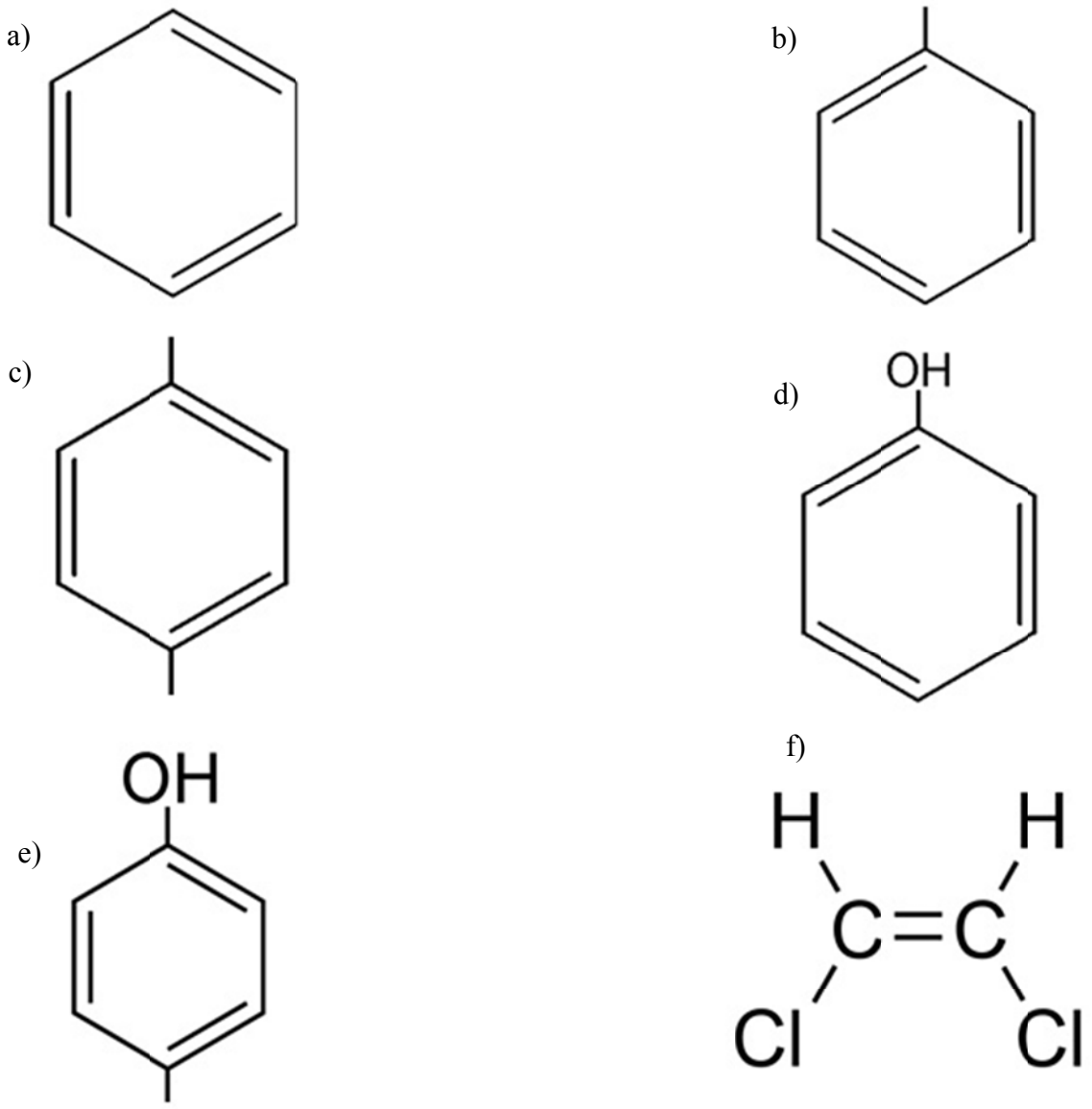

Figure 1.1. Structural formula of a) benzene, b) toluene, c) p-xylene, d) phenol, c) p-cresol, and f) *cis*-dichloroethylene.

1.1.2 Phenols

Phenolic compounds, also called phenols, consist of one or more aromatic molecules bonded to one or more hydroxyl groups (-OH). Although phenols are similar to alcohols, they are not classified as such, since the –OH group is bonded to an aromatic ring, what makes phenols weak acids [17]. Phenolic compounds are naturally produced organic molecules essential for the growth, development, and defense of plants; phenols are commercially employed as dyes and food additives [17], among other applications. Phenol is the simplest of the phenolic compounds and consists of a single benzene ring bonded to a single –OH group (Figure 1.1). It is commonly used as disinfectant and it is employed in numerous synthetic chemical processes since phenol is the basic structural unit for different organic compounds [18, 19]. Phenol production was estimated in 1.25 billion Kg in 1980 [19]. Other important phenolic compounds are cresols, which are methylated phenols. Cresols can be obtained from coal tar and petroleum or from the oxidation of toluene; and like phenol, cresols are employed in the synthesis of other organic compounds, and are extensively used in the manufacture of pesticides and resins [20]. The structural formula of p-cresol, or 4-methylphenol, is shown in Figure 1.1. Releases of phenolic compounds are of concern since they are known to be toxic at high concentrations and they are genotoxic at low concentrations [21]. Dissemination of phenolic compounds into the environment is common due to their presence in countless products and processes; they have been frequently identified in landfill leachates [22, 23] posing a threat to groundwater.

1.1.3 Chlorinated hydrocarbons

Chlorinated hydrocarbons are organic molecules bonded to at least one chlorine atom. These compounds are produced in large quantities since they are extensively used as solvents for degreasing fats, oils, waxes, and resins [24]. *cis*-1,2- Dichloroethylene (*cis*-DCE) is industrially used as solvent for polymers, waxes, and resins; extended exposure to this contaminant results in damage to the central nervous system, and it is a suspected carcinogen [25]. The structural formula of *cis*-DE is shown in Figure 1.1. In order to reduce the risk of exposure to *cis*-DCE, the EPA has set a MCL for *cis*-DCE equal to 0.07 mg L^{-1} (Table 1.1). *cis*-DCE can contaminate groundwater due to improper disposal of chlorinated solvents and/or from landfill leachates escaping the landfill. Although large amounts of *cis*-DCE are produced each year, its occurrence in groundwater is primarily due to the microbial degradation of higher chlorinated solvents, mainly tetrachloriethylene (PCE) and trichloroethylene (TCE) [26]. Due to the risk that all these contaminants pose to human health and the environment, their removal from groundwater and landfill leachates is a high priority.

Table 1.1. Maximum contaminant level (MCL) of benzene, *cis*-1,2- dichloroethylene, phenol, toluene, and xylene.

Contaminant	MCL ($mg\ L^{-1}$)	Potential health effects above the MCL	Common sources of contaminant in drinking water
Benzene	0.005	Anemia; increased risk of cancer	Discharge from factories; leaching from gas storage tanks and landfills
cis-1,2-Dichloroethylene	0.07	Liver problems; risk of cancer	Discharge from industrial chemical factories
*Phenol	10	Gastrointestinal and skin damage	Discharge from industrial chemical factories
Toluene	1	Nervous system, kidney, or liver problems	Discharge from petroleum factories
Xylene	10	Nervous system damage	Discharge from petroleum factories; discharge from chemical factories

* Phenol is listed only in the national recommended water quality criteria. p-Cresol is not classified by the EPA as a priority pollutant. Source: Environmental Protection Agency, 2011 [18, 27, 28].

1.2 Hydrocarbons in landfill leachates

Sanitary landfills are the preferred option for the disposal of household waste, since they are the most economical alternative for handling the waste and allow its decomposition under controlled conditions [29]. It is estimated that around 70% of the household refuse is disposed off in landfills worldwide [30] and normally consists of paper, food waste, vegetative matter, plastics, textiles and glass [31]. However, hazardous waste can be found as well, due to illegally dumped industrial material and the presence of toxic compounds in household products [32]. Landfills have been recognized to pose a threat to the environment since they can emit pollutants that have the potential to jeopardize the quality of the air [33, 34] and surface water and groundwater [35, 36] surrounding the landfill.

1.2.1 Characterization of landfill leachates

Landfill leachates are formed as percolating water mixes with chemicals leached from the buried waste due to the intrinsic physicochemical and microbial conditions in landfills. Landfill leachates pose a threat to groundwater as numerous organic contaminants have been identified in the leachates, including BTX, phenol, p-cresol, and *cis*-DCE [37-41]. Baun et al [22] found 55 xenobiotic organic compounds and 10 degradation byproducts in leachates from 10 Danish landfills. In Japan, Yasuhara et al [42] identified more than 100 organic compounds, including toxic chemicals such as

1,4-dioxane, phthalates, and bisphenol, in leachates from 11 different landfills. Paxeus [23] detected more than 200 organic compounds in leachates from 3 landfills in Sweden. The composition of the leachates can vary with the age of the landfill. Leachates produced during the initial aerobic phase have a neutral pH and a high concentration of complex compounds. Once oxygen is depleted, anaerobic conditions develop. Further degradation of the high molecular weight organics results in a leachate of acidic pH and high biological oxygen demand (BOD) content due to the presence of soluble organic compounds, such as volatile fatty acids (VFA). Methanogenic conditions gradually dominate over time as VFA and other compounds are degraded, which causes a decrease of the BOD content of the leachate and an increase of the pH to circumneutral values [43-45].

1.2.2 Physicochemical technologies for treating landfill leachates

Different technologies exist for the treatment of landfill leachates and can be divided in three major groups, including (a) leachate recirculation, (b) physicochemical methods, and (c) membranes [40, 45, 46]. Leachate recirculation was a preferred option for treating landfill leachates and consists in the recirculation of the leachate to the top of the landfill. Although this technique is efficient and relatively inexpensive, ammonia (NH_4^+), an inorganic contaminant occurring at high concentrations in landfill leachates, is not treated and it accumulates. Physicochemical processes, which include coagulation-flocculation, adsorption, and advance oxidation, are used to remove suspended solids,

colloidal particles, and biological refractory compounds. Due to their high operation costs they are normally used as a pre- or post-treatment. Finally, techniques employing membranes, such as reverse osmosis (RO) and ultra-, micro-, and nanofiltration, are mainly utilize for the polishing of treated effluents to avoid a rapid saturation of the membranes.

1.2.3 Physicochemical processes for groundwater treatment

Landfill leachates can contaminate groundwater if landfills lack of contention systems or if such systems fail. Hydrocarbons have shown to be a constant threat to groundwater streams. Only in the US it is estimated that groundwater from 300,000 to 400,000 sites is contaminated with organic chemicals [47]. Different techniques have been utilized for the removal of aromatic and chlorinated compounds in groundwater; perhaps the most commonly used are air striping, carbon adsorption, and chemical oxidation. The used of these technologies relies on the physical and chemical properties of the contaminants, such as density, henry's law constant, and water solubility. Table 1.2 provides information about these physicochemical characteristics for BTX, phenol, p-cresol, and *cis*-DCE. Air stripping consists in exposing the contaminated groundwater to a clean air supply, resulting in the release of the organic compounds into the gas phase [48]. It can enhance the biodegradation of the pollutants by providing oxygen that may be lacking on the site. Depending on the type and concentration of the organic contaminants, the gas phase might require treatment before being discharge into the atmosphere [49].

Another important method for treating contaminated groundwater involves the use of activated carbon (AC). Processes based on AC technologies are so widely used that are normally employed as the primary treatment, preceding polishing processes [50]. AC can be obtained from different raw materials, including wood, coconut shells, peats, and coals [51], and it is characterized by a its high porosity and large surface area. When groundwater is treated using AC, it is generally pumped out and passed through filters containing granules of AC. The removal of the contaminant from the groundwater is achieved by adsorption between the contaminant and the AC; which occurs when the attractive forces between the organic molecules and the liquid are overcome by the attractive forces at the carbon surface. The main factors affecting the adsorption of the contaminant onto the AC are the pore size distribution and the surface chemistry of the AC [50]. After time, the AC must be replaced as it reaches its maximum adsorption capacity, increasing the operation costs. Finally, chemical oxidation is another viable option for treating contaminated groundwater. During chemical oxidation, the organic contaminants are degraded by the addition of oxidizing agents, including ozone and hydrogen peroxide [52]. Higher degradation rates are achieved when advance oxidation processes are used instead of chemical oxidation. During advance oxidation processes hydroxyl radicals (OH-) are generated most commonly by cavitation (ultrasonic irradiation), photocatalytic oxidation (ultraviolet radiation), or Fenton's chemistry, to oxidize the organic molecules [53]. Although oxidation processes have demonstrated to be a promising alternative for the degradation of organic contaminants, operation costs can be high due to the use of expensive reactants [54].

Table 1.2. Physical and chemical properties of benzene, *cis*-1,2-dichloroethylene, phenol, p-cresol, toluene, and p-xylene.

Contaminant	Density at 20°C (Kg L^{-1})	Henry's constant at 25°C (atm m^3 mol^{-1})	Solubility at 25°C (g L^{-1})	Octanol-water partitioning coefficient (Log K_{ow})
Benzene	0.8787 (15°C)	5.50×10^{-3}	0.188 (%)	2.13
cis-1,2-Dichloroethylene	1.284	4.08×10^{-3}	3.50	1.86
Phenol	1.0545	3.00×10^{7}	82.4	1.46
p-Cresol	1.0341	7.92×10^{1}	21.5	1.94
Toluene	0.8669	5.94×10^{-3}	0.535	2.72
p-Xylene	0.8611	6.90×10^{-3}	0.162	3.15

Data obtained from the Agency for Toxic Substances and Disease Registry (ATSDR) [14-16, 18, 20, 25].

1.2.4 Bioremediation. An alternative for groundwater and landfill leachate treatment

Physicochemical processes are not the only viable alternative for treating contaminated groundwater and landfill leachates. Processes relying in bioremediation technologies have gained importance during the last 30 years since it is suggested that these technologies are safer, less costly, and less disruptive than some current physicochemical processes [55]. Bioremediation is defined by the EPA as any process by which microorganisms, or their enzymes, transform the structure of harmful chemicals released into the environment. Many microorganisms, including bacteria and fungi, are known to be capable of degrading a diverse range of hydrocarbons. It is believed that, since hydrocarbons are ubiquitously present in the environment, microorganisms have developed pathways to use these compounds as growth substrates [1, 56]. Moreover, it has been suggested that one of the primary removal mechanisms of petroleum and other organic contaminants involves the biodegradation of such compounds by natural populations of microorganisms in the environment [57]. Many microorganisms are known to be capable of degrading numerous natural and synthetic organic compounds in the presence of oxygen. In this case, the initial attack requires the use of molecular oxygen as co-substrate [58]. Hydrocarbons were thought to resist biodegradation in the absence of oxygen since aromatic and aliphatic compounds are very stable, resulted from the resonance energy in the aromatic ring and the inertness of C-C and H-C bonds in the organic molecules [56]. The energy associated to these bonds is shown in Table 1.3. However, it is now widely known that different organic contaminants can be degraded in

the presence of alternative electron acceptors [1, 3, 12, 58]. Anaerobic biodegradation of hydrocarbons in the presence of alternative electron acceptors, including nitrate (NO_3^-), ferric iron (Fe_3^+), sulfate (SO_4^{2-}), manganese (Mn^{4+}) and carbon dioxide (CO_2), has been demonstrated [3]. In contrast to the aerobic degradation, the initiation reactions under anoxic conditions are widely diverse [58]. During aerobic degradation, the organic contaminants are transformed mainly to CO_2 and biomass sludge, whereas the main products of the anaerobic degradation are methane (CH_4) and CO_2 [29]. Bioremediation has been successfully applied in the cleanup of groundwater, soils, wastewater, among others; it has even proved to be effective for large scale applications as demonstrated by the remediation of the Exon oil spill in Alaska [59]. However, certain environmental, biological, and physicochemical factors can impede bioremediation to occur. Thus, more research is needed to make bioremediation processes a more feasible treatment alternative.

Table 1.3. Average bond energies of some covalent bonds relevant to hydrocarbons.

Bond	Bonds order	Bond energy (KJ mol^{-1})
H – C	1	411
C – C	1	346
C = C	2	602
C ≡ C	3	835

Data obtained from "Inorganic chemistry: principles of structure and reactivity" by J.E. Huheey et al, 1993 [60].

1.3 Removal of emerging contaminants during wastewater treatment

Wastewater commonly contains pollutants, pathogenic microorganisms, and nutrients that pose a risk to human health and environmental welfare if released untreated. With the objective of removing these contaminants and hazardous microorganisms, wastewater treatment plants (WWTPs) have implemented physicochemical and biological processes to produce effluents that can be safely discharged into the environment. Wastewater treatment has evolved over the years. The elimination of colloidal and suspended material, biodegradable organics, and pathogenic organisms based on aesthetic and environmental concerns was the primary goal of the treatment from the 1900s until the 1980s; during the last 30 years the goal of WWTPs has been the removal of constituents that may pose a threat to health and the environment in the long-term [61]. However, as new emerging pollutants previously unexpected or unrecognized to reach WWTPs are being found in wastewater, the capability of WWTPs for eliminating these contaminants is being questioned. Nanoparticles (NPs) are one important group of emerging pollutants. These particles are defined by the National Nanotechnology Initiative (NNI) as materials with at least one dimension of 100 nm or less and can be naturally formed (volcanic dust, colloids in fresh water, soil erosion) or synthetically engineered. NPs are much smaller in diameter than bacteria (500 – 5000 nm) and many viruses (10 – 300 nm).

1.3.1 Nanoparticles

Engineered NPs (ENPs) can be manufactured by mechanical and chemical processes and by gas-phase synthesis [62]. NPs characteristics such as size, shape, chemical composition, and surface area and charge influence their complex colloid and aggregation chemistry. The size of the NPs has a great impact over the charge distribution within the particle and their surface area. The reactivity of the nanomaterials is mainly affected by their surface structure which is defined by the shape and chemical composition of the NP. The molecular bonding pattern of the material greatly changes as its shape is modified. Finally, the chemical composition refers mainly to the crystallinity of the NP and the presence of functional organic and/or inorganic groups on its surface. All these properties make ENPs to have a dual behavior between the quantum effects of atoms and bulk properties of large materials.

The special characteristics of ENPs have caused an exponential increase in the utilization of these materials in countless processes and products. According to a 2001 report by the National Science Foundation (NSF), nanotechnology is estimated to reach a $1 trillion dollar market by 2015 [63] and visualizes this field as a relevant technology that could revolutionize the science and the industry. ENPs are now found in stain-resistant clothing, cosmetics, food packing, drugs and many other applications. Sunscreen production is one of the most important markets for ENPs, where titanium dioxide (TiO_2) and zinc oxide (ZnO) are regularly used. These nano-oxides are the most common inorganic agents utilize in sunscreens for scattering purposes [64]. It has been

estimated that sunscreen products sales reached near a half billion dollars in the year 2005 [65]. ENPs can also be used for interesting applications in the field of environmental engineering [66-68], resulting in cheaper and more effective remediation techniques. The most common nanomaterial used to date in environmental applications has been zero-valent iron (ZVI); however, many other nanomaterials are being considered such as nano-zeolites, nano-oxides such as TiO_2, and carbon nanotubes [69]. The most important characteristic that makes nanomaterials interesting for environmental applications is their high surface area to volume ratio that could enhance their reactivity with pollutants [70].

Semiconductor industrial processes have incorporated NPs in many operations. The most important nanomaterials utilize in this market are the inorganic nano-oxides including silica (SiO_2), alumina (Al_2O_3), and ceria (CeO_2), which are used in slurries used for the chemical mechanical polishing (CMP) of wafers. The objective of the CMP is to obtain wafers with smooth surfaces and free of defects by mechanical abrasion and chemical reaction when in contact with the slurries. Mechanical grinding can achieve planarization of the surface of the wafer; however it leaves a rough pattern. To eliminate the damage on the wafer the chemical components of the slurries react with the wafer to achieve the smooth surfaces. The slurry market for CMP applications was calculated to reach $ 400 million in 2003 and accounted for the 74.2% of the worldwide NP market in 2005, including magnetic and optoelectric applications [71]. According to the Semiconductor Industry Association sales jumped 11% from 2000 to 2005, due in large part to CMP. The extensive and increasing use of engineered NPs has led to increasing

concern about their potential toxicological effects and environmental impact. Table 1.4 shows current uses of different nanomaterials and their estimated production.

Table 1.4. Current applications of certain nanomaterials and their forecasted production.

Materials/Devices	**Application**	**Estimated production from 2011 – 2020 (tonnes per year)**
Ceramics, catalysts composites, coating, powders, metals	Structural	10^4 - 10^5
Metal oxides (TiO_2, ZnO, Fe_2O_3)	Skincare products	10^3 or less
Nanoelectronics, optoelectronic materials	Information and communication technology	10^3 or more
Nanoencapsulates, quantum dots	Biotechnology	10
Nanofiltration, membranes	Environmental	10^3 - 10^4
Nanoelectronic-mechanical systems	Instruments, sensors, characterization	10^2 - 10^3

Adapted from "Nanomaterials – the driving force" by M.J. Pitkethly, 2004 [62].

1.3.2 Health effects and environmental fate of nanoparticles

The use of nanotechnology has been associated to certain benefits, including improvements in energy production, storage and efficiency as well as pollution and waste minimization by implementing "green" technologies that will generate less undesirable by-products and reducing consumption of resources [72]. Nanotechnology, together with biotechnology, was thought to pose the potential to eliminate the concept of waste and pollution by creating products and services at the molecular scale [73]. However it has been argued that the same characteristics that provide NPs their unique behavior could influence their toxicological effects to humans and the environment. In some cases, cell membranes seem to be an ineffective barrier to certain NPs under 100 nm of diameter, which can readily pass through them [74]. The inherent small size of NPs results in large surface area, as previously discussed. This feature, which is concentration dependent, is of concern because some of these NPs are very reactive, posing a hazard to organisms. The capacity to generate reactive oxygen species (ROS) [75] is a clear example of the potential risks associated to the large surface area and reactivity of these materials. Awareness of the possible adverse effects of the production, manipulation, release, and exposure to NPs has increased in the last years. The US federal government will invest $480 million dollars between 2005 and 2011 to support research related to the potential environmental, health, and safety (EHS) impacts of nanoparticles, as stated by the National Nanotechnology Initiative (NNI). Despite the increasing concern about the

potential environmental impact and adverse health effects of NPs, nothing has been done towards the regulation of such materials [76].

Engineered nanomaterials have modified surfaces that could threaten the well-being of living organisms if exposed to these particles. The possible routes of exposure to NPs in humans include inhalation and ingestion of ultrafine particles, as well as direct contact. Particles of 50 nm, or smaller, behave more like gas molecules, thus they can deposit anywhere in the respiratory track; moreover they can reach the liver or the kidneys when ingested and can penetrate the skin rather easy [77]. Interactions of NPs with other molecules affect their fate in the organisms since they can bind to proteins forming complexes that are more mobile and that can penetrate tissue sites otherwise inaccessible [78]. The intrinsic characteristics of NPs play a major role in their excretion as well, normally via urine, feces or sweat; properties such as surface charge, size, and chemical composition greatly determine their toxicity and whether or not these materials are expulse out of the body and at what extent. In general, smaller particles are thought to be more toxic than their larger counterparts since the surface area increases as the diameter of the particles decreases. Documented adverse effects of NPs include oxidative stress by formation of ROS, briefly discussed above. Under normal coupling conditions in the mitochondrion ROS species are readily neutralized; however, as the concentration of ROS increases the capacity of the cells are surpassed, causing inflammation as observed in the lungs [79] during ambient and occupational exposure. Other effects are particular to each NP species. For example, to mention a few, silver nanomaterials have shown to cause lung cancer, nano TiO_2 and ZnO may damage nucleic acids, and carbon

nanotubes may cause dermal toxicity [72]. A comprehensive risk assessment about the use and exposure to NPs is not possible at this stage due to the lack of knowledge about their fate in the organisms and the environment. Despite data about the toxicity of these materials constantly emerge, most of his information is obtained from observational studies and limited to species used for regulatory toxicology [80]. Information about NPs in the environment is even more restricted. As recognized by the EPA [81], little is known about the environmental fate of nanomaterials released directly or indirectly from manufacturing and processing of nanomaterials, oil refining processes, chemical and materials manufacturing processes, among others.

1.3.3 Surface chemistry of nanoparticles

Contrary to bulk materials, a large fraction of the atoms in NPs are exposed on the surface [82]; therefore adsorption processes are thought to control their behavior in the environment. In general, in aqueous solutions, the fate of NPs is determined by their interactions with organic and inorganic chemicals, the naturally occurring biotic/abiotic processes, and the stability of the dispersions they form [81]. In aquatic environments NPs are rarely dissolved; instead they form colloidal dispersions [83] whose stability greatly depend on pH and ionic strength (IS), since colloids are generally charged. When particles are small and have negligible settling velocity, Brownian motion is considered to be the most important collision mechanism. Brownian motion refers to the random movement of particles suspended in a fluid and is characterized by (i) continuous motion

and (ii) independent gaussian increments [84]. Particles may collide or not into each other depending on their concentration and affinity; after collision they may adhere depending once again on their affinity and agitation of the media. The affinity between NPs is defined by the zeta potential (ζ-potential) of the colloidal particles and represents the magnitude of the electrostatic forces between the surface of the particle and the bulk media, which can be repulsive or attractive.

Solid oxide particles dispersed in aqueous media are often electrically charged due to an imbalance between the densities of adsorbed H^+ and OH^- and the presence of ionic groups and/or the adsorption of metal hydroxo complexes on the surface of the particle [85]. The ions adsorbed on the surface of the particle attract ions of opposite charge, present in the aqueous phase, forming two parallel charge layers that surround the particle called electrostatic diffuse double layer (EDL) and is shown in Figure 1.2. The EDL can be either positive or negative, depending on the surface ligands of the particle. The forces associated with the EDL are generally repulsive due to the electrostatic surface charge of the materials and dominate as the distance between particles increases. Attraction between neutral NPs is the result of London-var der Waals (LVW) forces that dominate in the short range, as the separation of such NPs decreases. LVW interactions are exhibited by nonpolar materials and are originated by the dipoles in the molecules. As the absolute charge of the particles increases (positive or negative), the absolute value of ζ-potential exhibited by the particles increases as well, resulting in stable dispersions. On the other hand, as the particles loose charge and become neutral, the absolute ζ-potential

decreases (positive or negative) and the dispersion becomes unstable, which can lead to the sedimentation of the particles.

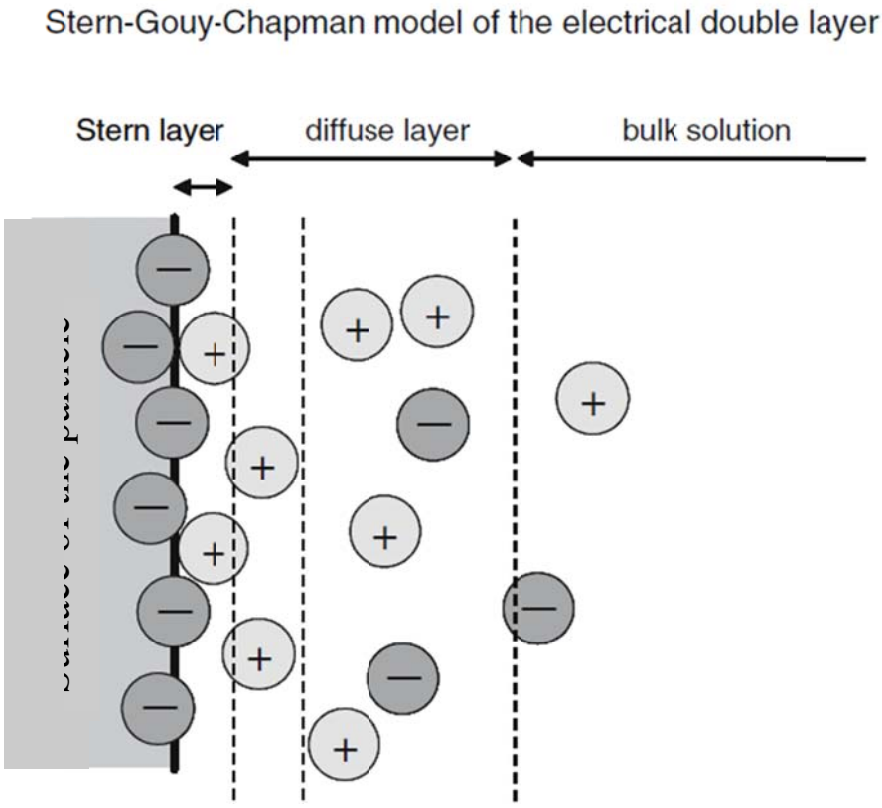

Figure 1.2 Schematic diagram showing the electrical double layer at the surface of a particle. Adapted from Handy et al, 2008 [86].

The pH of the media has an important influence over the ζ-potential of the particles, affecting their average particle size. Any change in the pH of the media is followed by change in the ζ-potential, resulting in an increase or decrease of the surface charge of the NPs. At low pH values, metallic NPs become positively charged (+),

whereas at high pH the charge becomes negative (-). The pH at which the surface of the NPs is neutral is called the isoelectric point (IP) (Figure 1.3) and particles are expected to agglomerate. At pH values distant from the IP particles are stabilized. Different studies have demonstrated the effect of pH over particle size and stability of NPs dispersions [87-90]. Since the stability of the particles is function of the charge of the NPs, the ionic strength (IS) of the dispersion plays an important role as well. The IS is an indicator of the concentration of ions in solution and increases in the presence of salts, since they normally dissociate into ions. Salts reduce the repulsion between the double layers of the particles, making it possible for NPs to get very close to each other. In the case of positively charged NPs, the surface charge layer is compressed, thus subsequent collisions of the NPs will cause aggregation, and probably further sedimentation [80]. Studies have demonstrated the influence of salt concentration over particle size in solution [91-93]. Amal et al [94] observed that the agglomeration velocity of haematite NPs was directly dependent of the salt concentration. Thus, higher agglomeration velocities were observed as the salt content in solution was increased.

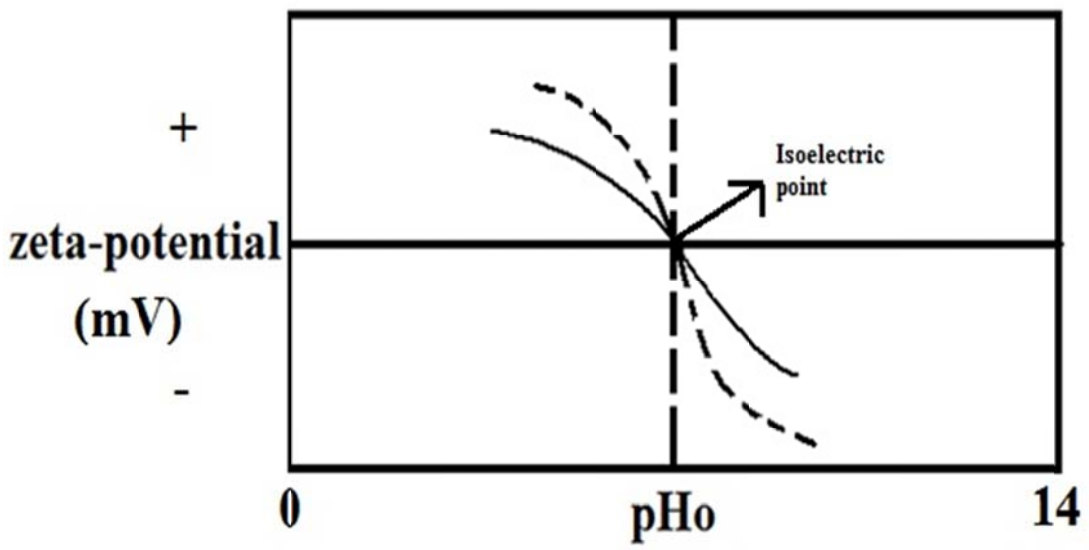

Figure 1.3 Schematic showing the dependence of the zetal-potential on the surface of the particle on the pH of the media.

The stability and particle size distribution (PSD) can be modified by encapsulating the NPs in organic or inorganic polyelectrolytes, or stabilizers [95, 96]. Polyelectrolytes dissociate when in solution and can impart surface charge to the NPs, increasing the stability of the dispersion. The most common stabilizers in the market are Carbowax-20 M, poly-N-vinyl-2-pyrrolidone, poly-N-isopropylacrylamide, poly-acrylicacid-b-polystyrene-6, 3-aminopropyl-trimethoxysilane (APS), sodium polyacrylate, dendrimers, and cubic silsesquioxane [97]. Stabilizers are pH dependent, thus different stabilizers can behave distinctively at the same pH, enhancing or not agglomeration of the NPs [98]. In general, NP dispersions utilized in the industry contain stabilizers and dispersants that stabilize the NPs at neutral pH values. This allows NPs to remain dispersed for extended periods of time and travel long distances, increasing the

likelihood to reach WWTPs. Most of the engineered nanomaterials are released into the environment via sewage and industrial wastewater discharges [99]. Different computer models have suggested the potential of many ENPs to reach municipal WWTPs [100, 101], which was confirmed by the finding of TiO_2 NPs in effluents of municipal WWTPs across the nation [102]. Not only industrial wastewater can contain significant concentrations of ENPs. These materials can be found in municipal sewage due to their increased use in numerous household products. The fate of ENPs during wastewater treatment is limited and more research is needed to determine if current technologies are appropriate for their removal.

1.4 References

1. Widdel, F. and R. Rabus, *Anaerobic biodegradation of saturated and aromatic hydrocarbons.* Current Opinion in Biotechnology, 2001. 12(3): p. 259-276.
2. Underwood, A.J.V., *Industrial synthesis of hydrocarbons from hydrogen and carbon monoxide.* Industrial and Engineering Chemistry, 1940. 32: p. 449-454.
3. Chakraborty, R., *Anaerobic degradation of monoaromatic hydrocarbons.* Appl Microbial Biotechnol, 2004. 64: p. 437-446.
4. Hall, C., et al., *Hydrocarbons and the evolution of human culture.* Nature, 2003. 426(6964): p. 318-322.

5. Bicego, M.C., et al., *Results from a 15-year study on hydrocarbon concentrations in water and sediment from Admiralty Bay, King George Island, Antarctica.* Antarctic Science, 2009. 21(3): p. 209-220.

6. Steinhauer, M.S. and P.D. Boehm, *The composition and distribution of saturated and aromatic-hydrocarbons in nearshore sediments, river sediments, and coastal peat of the alaskan beafort sea - implications for detecting anthropogenic hydrocarbon inputs.* Marine Environmental Research, 1992. 33(4): p. 223-253.

7. El Deeb, K.Z., et al., *Distribution and sources of aliphatic and polycyclic aromatic hydrocarbons in surface sediments, fish and bivalves of Abu Qir Bay (Egyptian Mediterranean Sea).* Bulletin of Environmental Contamination and Toxicology, 2007. 78(5): p. 373-379.

8. Bicego, M.C., et al., *Assessment of contamination by polychlorinated biphenyls and aliphatic and aromatic hydrocarbons in sediments of the Santos and Sao Vicente Estuary System, Sao Paulo, Brazil.* Marine Pollution Bulletin, 2006. 52(12): p. 1804-1816.

9. Brackmann, R. and G. Fuchs, *Enxymes of anaerobic metabolism of phenolic-compounds - 4-hydroxybenzoyl-CoA reductase (dehydroxylating) from a denitrifying Pseudomonas species.* European Journal of Biochemistry, 1993. 213(1): p. 563-571.

10. Jindrova, E., et al., *Bacterial aerobic degradation of benzene, toluene, ethylbenzene and xylene.* Folia Microbiologica, 2002. 47(2): p. 83-93.

11. Fries, M.R., et al., *Isolation, characterization, and distribution of denitrifying toluene degraders from a variety of habitats.* Appl Environ Microbiol, 1994. 60(8): p. 2802-10.

12. Holliger, C. and A.J.B. Zehnder, *Anaerobic biodegradation of hydrocarbons.* Current Opinion in Biotechnology, 1996. 7(3): p. 326-330.

13. Dean, B.J., *Recent findings on the genetic toxicology of benzene, toluene, xylenes and phenols.* Mutation Research, 1985. 154(3): p. 153-181.

14. ATSDR, *Toxicological profile for toluene*, U.S.D.O.H.A.H. SERVICES, Editor. 2000: Atlanta.

15. ATSDR, *Toxicological profile for xylene*, U.S.D.O.H.A.H. SERVICES, Editor. 2007: Atlanta.

16. ATSDR, *Toxicological profile for benzene*, U.S.D.O.H.A.H. SERVICES, Editor. 2007: Atlanta.

17. Vermerris, W. and R. Nicholson, *Phenolic Compound Biochemistry* 2006: Springer. 276.

18. ATSDR, *Toxicological profile for phenol*, U.S.D.O.H.A.H. SERVICES, Editor. 2010: Atlanta.

19. Boyd, S.A., et al., *Anaerobic biodegradation of phenolic-compounds in digested sludge.* Applied and Environmental Microbiology, 1983. 46(1): p. 50-54.

20. ATSDR, *Toxicological profile for cresols*, U.S.D.O.H.A.H. SERVICES, Editor. 2008: Atlanta.

21. Young, L.Y. and M.D. Rivera, *Methanogenic degradation of 4 phenolic compounds - reply.* Water Research, 1987. 21(7): p. 859-861.

22. Baun, A., et al., *Xenobiotic organic compounds in leachates from ten Danish MSW landfills - chemical analysis and toxicity tests.* Water Research, 2004. 38(18): p. 3845-3858.

23. Paxeus, N., *Organic compounds in municipal landfill leachates.* Water Science and Technology, 2000. 42(7-8): p. 323-333.

24. USGS. *Chlorinated Solvents Contamination*. 2008 [cited 2011; Available from: http://toxics.usgs.gov/investigations/chlorinated_solvents.html.

25. ATSDR, *Toxicological profile for 1,2-dichloroethene*, U.S.D.O.H.A.H. SERVICES, Editor. 1996: Atlanta.

26. Bradley, P.M., *Microbial degradation of chloroethenes in groundwater systems.* Hydrogeology Journal, 2000: p. 104-111.

27. EPA. *Drinking Water Contaminants*. 2009 [cited 2011; Available from: http://water.epa.gov/drink/contaminants/upload/mcl-2.pdf.

28. EPA. *National Recommended Water Quality Criteria* 2009; Available from: http://water.epa.gov/scitech/swguidance/waterquality/standards/current/upload/nrwqc-2009.pdf.

29. Lema, J.M., R. Mendez, and R. Blazquez, *Characteristics of landfill leachates and alternatives for their treatment - a review.* Water Air and Soil Pollution, 1988. 40(3-4): p. 223-250.

30. Slack, R.J., J.R. Gronow, and N. Voulvoulis, *Household hazardous waste in municipal landfills: contaminants in leachate.* Sci Total Environ, 2005. 337(1-3): p. 119-37.

31. Reinhart, D.R. and F.G. Pohland, *The assimilation of organic hazardous wastes by municipal solid-waste landfills.* Journal of Industrial Microbiology, 1991. 8(3): p. 193-200.

32. Murray, H.E. and J.N. Beck, *Concentrations of Synthetic organic chemicals in leachate from a municipal landfill.* Environ Pollut, 1990. 67(3): p. 195-203.

33. James, K.J. and M.A. Stack, *The impact of leachate collection on air quality in landfills.* Chemosphere, 1997. 34(8): p. 1713-1721.

34. Wilkins, K., *Volatile organic compounds from household waste.* Chemosphere, 1994. 29(1): p. 47-53.

35. Christensen, T.H., et al., *Biogeochemistry of landfill leachate plumes.* Applied Geochemistry, 2001. 16(7-8): p. 659-718.

36. Kjeldsen, P., et al., *Characterization of an old municipal landfill (Grindsted, Denmark) as a groundwater pollution source: landfill history and leachate composition.* Waste Management & Research, 1998. 16(1): p. 3-13.

37. Jimenez, L., R. Alzaga, and J.M. Bayona, *Determination of organic contaminants in landfill leachates: A review.* International Journal of Environmental Analytical Chemistry, 2002. 82(7): p. 415-430.

38. Kettunen, R.H. and J.A. Rintala, *Performance of an on-site UASB-reactor treating leachate at low temperature.* Water Research, 1998. 32(3): p. 537-546.

39. Kjeldsen, P., et al., *Present and long-term composition of MSW landfill leachate: A review.* Critical Reviews in Environmental Science and Technology, 2002. 32(4): p. 297-336.

40. Renou, S., et al., *Landfill leachate treatment: Review and opportunity.* Journal of Hazardous Materials, 2008. 150(3): p. 468-493.

41. Sikkema, S.D., E.; Ahlert, R. and Kosson, D., *Identification and Quantification of Volatile Organic Species During Microbial Treatment of Leachate.* Environmental Progress, 1988. 7: p. 77-83.

42. Yasuhara, A., et al., *Organic components in leachates from hazardous waste disposal sites.* Waste Management & Research, 1999. 17(3): p. 186-197.

43. Lo, I.M.C., *Characteristics and treatment of leachates from domestic landfills.* Environment International, 1996. 22(4): p. 433-442.

44. Christensen, T.H., et al., *Attenuation of landfill leachate pollutants in aquifers.* Critical Reviews in Environmental Science and Technology, 1994. 24(2): p. 119-202.

45. Salem, Z., et al., *Evaluation of landfill leachate pollution and treatment.* Desalination, 2008. 220(1-3): p. 108-114.

46. Wiszniowski, J., et al., *Landfill leachate treatment methods: A review.* Environmental Chemistry Letters, 2006. 4(1): p. 51-61.

47. Kunukcu, Y.K., *In situ bioremediation of groundwater contaminated with petroleum constituents using oxygen release compounds (ORCs).* Journal of

Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering, 2007. 42(7): p. 839-845.

48. Nyer, E.K., *Groundwater Treatment Technology*. 2nd Edition ed. 1992, New York: Van Nostrand Reinhold. 320.

49. Pankow, J.F., R.L. Johnson, and J.A. Cherry, *Air sparging in gate wells in cutoff walls and trenches for control of plumes of volatile organic-compounds (VOCs).* Ground Water, 1993. 31(4): p. 654-663.

50. Dias, J.M., et al., *Waste materials for activated carbon preparation and its use in aqueous-phase treatment: A review.* Journal of Environmental Management, 2007. 85(4): p. 833-846.

51. Pis, J.J., et al., *Preparation of active carbons from coal .1. Oxidation of coal.* Fuel Processing Technology, 1996. 47(2): p. 119-138.

52. Freese, S.D., et al., *Comparison of ozone and hydrogen peroxide/ozone for the treatment of eutrophic water.* Water Science and Technology, 1999. 39(10-11): p. 325-328.

53. Gogate, P.R. and A.B. Pandit, *A review of imperative technologies for wastewater treatment I: oxidation technologies at ambient conditions.* Advances in Environmental Research, 2004. 8(3-4): p. 501-551.

54. Andreozzi, R., et al., *Advanced oxidation processes (AOP) for water purification and recovery.* Catalysis Today, 1999. 53(1): p. 51-59.

55. Van Stempvoort, D. and K. Biggar, *Potential for bioremediation of petroleum hydrocarbons in groundwater under cold climate conditions: A review.* Cold Regions Science and Technology, 2008. 53(1): p. 16-41.

56. Boll, M., G. Fuchs, and J. Heider, *Anaerobic oxidation of aromatic compounds and hydrocarbons.* Curr Opin Chem Biol, 2002. 6(5): p. 604-11.

57. Leahy, J.G. and R.R. Colwell, *Microbial-degradation of hydrocarbons in the environment.* Microbiological Reviews, 1990. 54(3): p. 305-315.

58. Heider, J., et al., *Anaerobic bacterial metabolism of hydrocarbons.* Fems Microbiology Reviews, 1998. 22(5): p. 459-473.

59. Boopathy, R., *Factors limiting bioremediation technologies.* Bioresource Technology, 2000. 74(1): p. 63-67.

60. Huheey, J.E., E.A. Keiter, and R.L. Keiter, *Inorganic Chemistry:Principles of Structure and Reactivity*. 4th ed. 1993, New York: Prentice Hall. 964.

61. Tchobanoglous, G., F.L. Burton, and H.D. Stensel, *Wastewater Engineering: Treatment and Reuse*. 4th ed. 2002: McGraw-Hill 1848.

62. Pitkethly, M.J., *Nanomaterials, the driving force.* Materials Today, 2004. 7(12): p. 20 - 29.

63. National Nanotechnology Initiative. [cited 2010 February 2010]; Available from: http://www.nano.gov.

64. Gasparro, F.P., M. Mitchnick, and J.F. Nash, *A review of sunscreen safety and efficacy.* Photochemistry and Photobiology, 1998. 68(3): p. 243-256.

65. Danovaro, R., et al., *Sunscreens cause coral bleaching by promoting viral infections.* Environmental Health Perspectives, 2008. 116(4): p. 441-447.

66. Kamat, P.V. and D. Meisel, *Nanoscience opportunities in environmental remediation.* Comptes Rendus Chimie, 2003. 6(8-10): p. 999-1007.

67. Liu, W.-T., *Nanoparticles and Their Biological and Environmental Applications.* Journal of bioscience and bioengineering, 2006. 102.

68. Nutt, M.O., J.B. Hughes, and S.W. Michael, *Designing Pd-on-Au bimetallic nanoparticle catalysts for trichloroethene hydrodechlorination.* Environ Sci Technol, 2005. 39(5): p. 1346-53.

69. Karn, B., T. Kuiken, and M. Otto, *Nanotechnology and in situ remediation: a review of the benefits and potential risks.* Environ Health Perspect, 2009. 117(12): p. 1813-31.

70. Obare, S.O. and G.J. Meyer, *Nanostructured materials for environmental remediation of organic contaminants in water.* J Environ Sci Health A Tox Hazard Subst Environ Eng, 2004. 39(10): p. 2549-82.

71. Rittner, M., *Nanoparticles - what's now, what's next?* Chemical Engineering Progress, 2003. 99.

72. Reijnders, L., *Cleaner nanotechnology and hazard reduction of manufactured nanoparticles.* Journal of Cleaner Production, 2006. 14(2): p. 124-133.

73. Hart, S.L. and M.B. Milstein, *Creating sustainable value.* Academy of Management Executive, 2003. 17(2): p. 56-67.

74. Seaton, A. and K. Donaldson, *Nanoscience, nanotoxicology, and the need to think small.* Lancet, 2005. 365(9463): p. 923-4.

75. Long, T.C., et al., *Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): Implications for nanoparticle neurotoxicity.* Environmental Science & Technology, 2006. 40(14): p. 4346-4352.

76. Wardak, A., et al., *Environmental regulation of nanotechnology and the TSCA.* Ieee Technology and Society Magazine, 2007. 26(2): p. 48-56.

77. Bell, T., *Understanding Risk Assessment of Nanotechnology*, N.N. Initiative, Editor. 2007.

78. Nel, A., et al., *Toxic potential of materials at the nanolevel.* Science, 2006. 311(5761): p. 622-7.

79. Karakoti, A.S., L.L. Hench, and S. Seal, *The potential toxicity of nanomaterials - The role of surfaces.* Jom, 2006. 58(7): p. 77-82.

80. Handy, R.D., et al., *The ecotoxicology and chemistry of manufactured nanoparticles.* Ecotoxicology, 2008. 17(4): p. 287-314.

81. Morris, J. and J. Willis, *EPA Nanotechnology White Paper* U.S.E.P. Agency, Editor. 2007: Washington p. 120.

82. Fukushi, K. and T. Sato, *Using a Surface Complexation Model To Predict the Nature and Stability of Nanoparticles.* Environmental Science & Technology, 2005. 39(5): p. 1250-1256.

83. Wigginton, N.S., K.L. Haus, and M.F. Hochella, Jr., *Aquatic environmental nanoparticles.* J Environ Monit, 2007. 9(12): p. 1306-16.

84. Revuz, D. and M. Yor, *Continuous martingales and Brownian motion*. 1991: Springer-Verlag.

85. Kung, H.H., *Transition metal oxides: surface chemistry and catalysis*. 1991, Amsterdam: Elsevier.

86. Handy, R.D., R. Owen, and E. Valsami-Jones, *The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs.* Ecotoxicology, 2008. 17(5): p. 315-325.

87. Finnegan, M.P., H.Z. Zhang, and J.F. Banfield, *Phase stability and transformation in titania nanoparticles in aqueous solutions dominated by surface energy.* Journal of Physical Chemistry C, 2007. 111(5): p. 1962-1968.

88. McConnell, W.P., et al., *Electronic and optical properties of chemically modified metal nanoparticles and molecularly bridged nanoparticle arrays.* Journal of Physical Chemistry B, 2000. 104(38): p. 8925-8930.

89. Tagawa, M., et al., *Influence of surface-properties on their adhesion and removal.* Colloid and Polymer Science, 1989. 267(5): p. 434-439.

90. Zhang, Y., et al., *Stability of commercial metal oxide nanoparticles in water.* Water Res, 2008. 42(8-9): p. 2204-12.

91. Adamczyk, Z., et al., *Structure and ordering in localized adsorption of particles.* Journal of Colloid and Interface Science, 1990. 140(1): p. 123-137.

92. Hanarp, P., et al., *Control of nanoparticle film structure for colloidal lithography.* Colloids and Surfaces a-Physicochemical and Engineering Aspects, 2003. 214(1-3): p. 23-36.

93. Semmler, M., et al., *Diffusional deposition of charged latex particles on water-solid interfaces at low ionic strength.* Langmuir, 1998. 14(18): p. 5127-5132.

94. Amal, R., et al., *Structure and kinetics of aggregating colloidal haematite.* Colloids and Surfaces, 1990. 46(1): p. 1-19.

95. Alargova, R.G., S. Deguchi, and K. Tsujii, *Stable colloidal dispersions of fullerenes in polar organic solvents.* Journal of the American Chemical Society, 2001. 123(43): p. 10460-10467.

96. He, F. and D.Y. Zhao, *Preparation and characterization of a new class of starch-stabilized bimetallic nanoparticles for degradation of chlorinated hydrocarbons in water.* Environmental Science & Technology, 2005. 39(9): p. 3314-3320.

97. Li, X.L., et al., *Metal nanoparticles stabilized by cubic silsesquioxanes for catalytic hydrogenations.* Catalysis Letters, 2007. 118(1-2): p. 151-158.

98. Ishikawa, Y., Y. Katoh, and H. Ohshima, *Colloidal stability of aqueous polymeric dispersions: Effect of pH and salt concentration.* Colloids and Surfaces B-Biointerfaces, 2005. 42(1): p. 53-58.

99. Boxall, A.B., K. Tiede, and Q. Chaudhry, *Engineered nanomaterials in soils and water: how do they behave and could they pose a risk to human health?* Nanomedicine (Lond), 2007. 2(6): p. 919-27.

100. Gottschalk, F., et al., *Modeled Environmental Concentrations of Engineered Nanomaterials (TiO2, ZnO, Ag, CNT, Fullerenes) for Different Regions.* Environmental Science & Technology, 2009. 43(24): p. 9216-9222.

101. Mueller, N.C. and B. Nowack, *Exposure modeling of engineered nanoparticles in the environment.* Environ Sci Technol, 2008. 42(12): p. 4447-53.

102. Kiser, M.A., et al., *Titanium nanomaterial removal and release from wastewater treatment plants.* Environ Sci Technol, 2009. 43(17): p. 6757-63.

CHAPTER 2

ANOXIC OXIDATION OF TOLUENE, BENZENE, M-XYLENE, AND *CIS*-DCE IN THE PRESENCE OF BIOLOGICAL SLUDGE

2.1 Abstract

Benzene, toluene, xylene (BTX), and *cis*-1,2-dichloroethene (*cis*-DCE) are common groundwater contaminants as they are used in many industrial processes and household products. The low oxygen concentration found in groundwater makes aerobic degradation less feasible. Technologies based on anaerobic processes have emerged as a low cost alternative for the treatment of contaminated groundwater. These technologies rely in the utilization of alternative electron acceptors, including nitrate (NO_3^-), sulfate (SO_4^{2-}), ferric iron (Fe^{3+}), manganese (Mn^{4+}) and chlorate (ClO_3^-), among others, to promote the degradation of organic molecules. The objective of this work was to evaluate the presence of microorganisms in a total of six different sludge and sediment samples taken from diverse sources, capable of BTX and *cis*-DCE removal under anaerobic conditions in the presence of NO_3^- or ClO_3^-. The final goal was to determine if such microorganisms were ubiquitously present in the sludges and sediments tested in order to consider their use for the anoxic oxidation of BTX and *cis*-DCE. For this purpose batch experiments were set up in which individual BTX and *cis*-DCE compounds were tested

for their biodegradation in microcosms amended with a solution of NO_3^- or ClO_3^-. Non-inoculated and poisoned controls were incubated in parallel to verify that removals by live inocula were due to biological reactions. Degradation was monitored by measuring the contaminant concentration in the headspace. Toluene was found to be the easiest compound to be degraded, as it was readily removed under denitrifying conditions in the majority of inocula tested and in some cases the removal was observed in less than 7 days. NO_3^- analysis during toluene degradation demonstrated that NO_3^--consumption was linked to toluene degradation. Nitrite (NO_2^-) was observed as an intermediate during denitrification. Toluene degradation was not observed under ClO_3^--reducing conditions; whereas in one inoculum, slow degradation of toluene was observed under methanogenic conditions. Benzene was removed in one duplicate after extended incubation in the presence of NO_3^- and ClO_3^- by granular and liquid sludge after a lag time of 100's of days. m-Xylene degradation was only observed when using pond sediments under methanogenic conditions after an initial lag phase of approximately 100 days. Finally, *cis*-DCE was not degraded under any of the conditions investigated. Data suggests that microorganisms suitable for anaerobic degradation of BTX and *cis*-DCE were not present in the inocula tested.

2.2 Introduction

Benzene, toluene, (*o*-, *m*-, *p*-) xylene (BTX), and *cis*-DCE are common contaminants that can reach groundwater mainly from leaking underground fuel storage tanks and leachates escaping landfills. Groundwater contamination by *cis*-DCE occurs not only by leachates containing this pollutant found in waxes, polymers and resins, but also by the anaerobic degradation of highly chlorinated ethenes and ethanes. Table 2.1 summarizes the concentration ranges of these contaminants in landfill leachates reported in the literature. Additionally, BTX compounds constitute an important fraction of fuels, such as gasoline and diesel, and oil. Approximately 480,000 leaking fuel storage tanks are estimated to occur in the US [1]. These contaminants are known to cause adverse effects to human health. Damage to central nervous system, the liver, and kidneys has been associated to long exposure to toluene [2] and (*o*-, *m*-, *p*-) xylene [3]. Likewise, benzene and *cis*-DCE are suspected carcinogens [4, 5].

Table 2.1. Range of BTX and *cis*-DCE concentration found in landfill leachates.

Pollutant	Conc. (µg/L)	Reference
Benzene	0.2 - 1,630	[6]
	2.3 - 38.9	[7]
	0.65 - 3,800	[8]
	3,800	[9]
Toluene	1 - 123,000	[6]
	1.9 – 241	[7]
	0.01 - 41,000	[8]
	41,000	[9]
	0.01 – 1.29	[10]
Xylenes	0.8 - 3,500	[6]
	2 - 2,220	[7]
	4 - 170,000	[8]
	0.03 - 0.208	[10]
cis-1,2-Dichloroethylene	1.4 – 470	[6]
	1.4 -60	[8]

Bioremediation provides an efficient and cost effective alternative for the treatment of groundwater contaminated by landfill leachates and gasoline plumes [11]. Natural attenuation (NA) relies on physical, chemical, and biological processes occurring with no human intervention, and relies in the presence of organisms capable of transforming the contaminants affecting the site and the occurrence of electron acceptors along the path of the contaminated plume [12]. When the contamination cannot be

removed by NA, engineered bioremediation becomes an interesting option. *In situ* treatment is a cost-effective innovative technology that allows remediation without removing the contamination from one place to another. Furthermore, it is less destructive than many other alternatives. Another popular emerging technology is the utilization of permeable reactive barriers (PRBs). PRBs are built below ground by digging a narrow trench, which intercepts the plume, filled with reactive materials. Degradation is promoted by filling the barriers with either electron acceptors or donors, nutrients or any other component lacking in the site, which are slowly released over time. This technology achieves treatment and containment of the plume at relatively low cost [13]. In fact, PRBs are extensively used for the treatment of chlorinated hydrocarbons [14].

Degradation of organic compounds can occur in the presence and absence of oxygen, and ample evidence exists about the biological transformation of BTX under aerobic conditions [15-17]. Aromatic hydrocarbons are known to be aerobically degraded by both prokaryotes and eukaryotes by the incorporation of molecular oxygen into the aromatic molecule by oxygenase enzymes. While eukaryotes generally cometabolize these compounds, prokaryotes use BTX as carbon source and obtain energy from their degradation. In non-ligninolytic fungi, an oxygen atom is incorporated into the aromatic ring via monooxygenase resulting in the formation of an arene oxide which is further oxidized to a dihydrodiol [18]. Bacteria, on the other hand, can perform either a monooxygenase or dioxygenase attack. In unsubstituted aromatics the ring is hydroxylated by diooxygenase enzymes forming a *cis*-dihydrodiol, which is converted to cathechol by dehydrogenase enzymes [19]. The cathechol formed can be cleaved in the

ortho- or *metha*- position to ultimately produce low molecular weight organics that are readily oxidized in the Krebs cycle [20]. Substituted aromatics can be subjected to a monooxygenase attack, where the methyl or ethyl substituents can undergo oxidation prior the activation of the aromatic ring to produce carboxylic acids or substituted pyrocatechols that are further transformed into simpler organic molecules [21]. Aerobic processes are normally faster than those relaying on alternative electron acceptors; however biodegradation of BTX is very sensitive to the oxygen concentration in the groundwater, which is generally limited or absent due to microbial respiration [22]. Utilization of hydrogen peroxide and oxygen releasing compounds (ORCs) has been adopted to overcome this problem; however certain drawbacks to their use have been identified. Hydrogen peroxide can cause complete microbial inhibition at concentrations of 200 mg L^{-1} or higher [23] and ORCs, such as calcium and magnesium peroxide, generate one mol of oxygen per two moles of the ORCs.

Anaerobic degradation for treating groundwater then arises as a viable option. Although degradation rates achieved under anaerobic conditions are lower than those attained in the presence of oxygen, different electron acceptors can be used for the removal of aromatic compounds. Degradation can occur under NO_3^-, SO_4^{2-}, Fe^{3+}, (Mn^{4+}), and methanogenic reducing conditions [24, 25]. The overall degradation pathway involves the transformation of the aromatic molecule, resulting in the formation of a few central intermediates. Benzoyl-coenzyme A (benzoyl-CoA) is the most common aromatic intermediate formed and is further transformed to non-aromatic compounds via hydrolysis. Subsequently reactions allow the formation of acetyl-coenzyme A

(acetyl-CoA) which is readily oxidized in the Krebs cycle. Bacteria belonging to the *Azoracus*, *Geobacter*, *Pseudomonas* and *Thaurea* species are known to successfully metabolize aromatic compounds; however complete mineraliztion is not always achieved, especially in the case of benzene [26-29]. Degradation of toluene is enhanced by the addition of fumarate to the methyl group, forming benzylsuccinate which is converted to benzoyl-CoA by a series of reactions [30]. Likewise, fumarate is incorporated to one of the methyl groups in xylene, resulting in the formation of methylbenzylsuccinate that is further transformed to benzoyl-CoA [28]. Benzene has shown to be the most recalcitrant of the BTX compounds under anaerobic conditions, thus the degradation pathway is not well understood. It has been suggested that benzene can be transformed to phenol, benzoate or toluene as intermediates, which are then transformed to benzoyl-CoA [31].

Chlorinated solvents, such as tetrachloroethene (PCE), trichloroethene (TCE), *cis*-DCE, and vinyl chloride (VC), can be degraded in the absence of oxygen by sequential reductive dechlorination reactions resulting in the formation of ethane and/or ethane [32]. Under these conditions microorganisms transfer the electrons from organic molecules or hydrogen to the chlorinated solvents, which are used as terminal electron acceptors [33]. The rate of the dechlorination reactions decreases as the number of chlorine atoms in the molecules decreases, which might explain the accumulation of *cis*-DCE and VC in anaerobic contaminated sites [34]. However, complete dechlorination has been achieved under methanogenic and SO_4^{2-} reducing conditions [35]. Since *cis*-DCE can be highly recalcitrant to anaerobic reductive dehalogenation, in this work a different approach for its degradation was assessed in the absence of oxygen. The

removal of *cis*-DCE was investigated in anaerobic batch experiments, where it was supplied as substrate. Only a few reports exist of the capability of certain microorganisms to oxidize *cis*-DCE and VC under manganese and VC under iron reducing conditions to CO_2 [36].

The scope of this work was to evaluate the capability of microorganisms in different inocula for degrading of benzene, toluene, m-xylene, and *cis*-DCE, using NO_3^- and ClO_3^-as terminal electron acceptors, to consider their use in PRBs treating contaminated groundwater. NO_3^- and ClO_3^- are convenient for their use in PRBs due to their high solubility. Moreover, reduction of ClO_3^- results in the release of oxygen, which could enhance further degradation of the contaminants.

2.3 Materials and Methods

2.3.1 Inocula sources

Six different inocula were used for the BTX and *cis*-DCE biodegradation experiments. Granular methanogenic sludge from industrial upflow anaerobic sludge blanket (UASB) wastewater treatment plants included: *Nedalco* (distillery, Bergen Op Zoom, Holland), *Aviko* (starch processing, Steederen, Holland), and *Mahou* (brewery, Guadalajara, Spain). Anaerobic digested sludge (ADS) and returned activated sludge (RAS) used in some of the microcosms were obtained from a municipal wastewater plant,

Ina Road facility located in Tucson, Arizona. Finally, sediments employed in the experiments were taken from a local pond (Agua Caliente park, Tucson, Arizona). The selection of the inocula was based on their exposure to a wide diversity of organic compounds, which increases the likelihood of finding BTX and *cis*-DCE degrading bacteria. The sludge was stored in the refrigerator at 4°C.

2.3.2 Microcosm preparation

Biodegradation experiments were performed to determine the presence of microorganisms capable of toluene, benzene, m-xylene or *cis*-DCE degradation. Each experiment consisted of a full treatment (live inocula, alternative electron acceptor, and contaminant), an abiotic control (no inoculum), a killed inoculum (poisoned inoculum) and a methanogenic control (no alternative electron acceptor). Each treatment and corresponding controls were run in duplicate. At first, the biotic control consisted of heat killed inocula. For this purpose the sludge was autoclaved for three consecutive days for 30 min at 120°C. However, this protocol released chemical with surfactant properties into the liquid interfering with the headspace measurements of the BTX and *cis*-DCE. To avoid false readings from the disturbed partitioning of the substrates, killed controls were then arranged by using poison. The killed control contained a final concentration of 0.50 g L^{-1} and 0.25 g L^{-1} of sodium azide (NaN_3) and mercury sulfate ($HgSO_4$), respectively.

Microcosms were set in 160 mL (Wheaton, Millville, NJ, USA) glass bottles of which 50 mL was occupied by the liquid phase and the rest, 110 mL, was comprised by the headspace. According to the treatment, the liquid volume consisted of mineral media, sludge, electron acceptor, poison, and substrate (Table 2.2). The mineral media contained the following chemicals (g L^{-1}): NH_4Cl (0.028), KCl (0.027), K_2HPO_4 (0.017), $CaCl_2$ $2H_2O$ (0.001), $MgCl_2$ $6H_2O$ (0.015), $NaHCO_3$ (5.00), yeast extract (0.002), and trace elements (0.1 mL L^{-1}). The trace elements solution consisted of (g L^{-1}): $FeCl_3$ $4H_2O$ (2.00), $CoCl_2$ $6H_2O$ (2.00), $MnCl_2$ $4H_2O$ (0.50), $AlCl_3$ $6H_2O$ (0.09), $CuCl_2$ $2H_2O$ (0.03), $ZnCl_2$ (0.05), H_3BO_3 (0.05), $(NH_4)_6Mo_7O_2$ $4H_2O$ (0.05), $NaSeO_3$ $5H_2O$ (0.10), $NiCl_2$ $6H_2O$ (0.05), EDTA (1.00), rezasurine (0.20) and HCl 36% (1 mL L^{-1}). The pH of the medium was adjusted between 6.8 to 7.2 before adding the sodium bicarbonate.

The concentration of the granular sludge and pond sediments was set at 1.5 g and 10 g volatile suspended solids per liter (g VSS L^{-1}), respectively; while for the more diluted sludges such as RAS and ADS the concentration was set at 10% (v/v) with respect to the liquid volume. A stock solution of electron acceptors (KNO_3 and $NaClO_3$) was prepared based on the stoichiometric electron equivalence (e^-eq) relationship between the target substrate (BTX or *cis*-DCE) and the electron acceptor. After dilution with the other liquid constituents of the microcosms, NO_3^- and ClO_3^- were in a 2.5 to 5 fold excess to ensure a sufficient concentration of electron acceptor to sustain the degradation of the pollutants.

After filling the microcosms, the glass bottles were sealed using Teflon faced butyl septa (Supelco, San Louis, MO) and aluminum crimps (Fisher Scientific, Pittsburgh, PA). A mixed gas containing 80% N_2 and 20% CO_2 was flushed through the treatments for 6 min to achieve anaerobic conditions. Benzene, toluene and *cis*-DCE were introduced into treatments via concentrated stock solution, which was diluted in the microcosms to achieve a designed total concentration ranging from 100 to 120 mg L^{-1}, depending on the substrate used. Only m-xylene was supplemented directly into treatments to a designed total concentration of 120 mg L^{-1} with a 10 μL gas lock chromatographic syringe (Hamilton, Reno, Nevada) due to its low aqueous solubility, making the preparation of stock solution unfeasible. The total concentration of the substrates in the treatments was calculated by applying Henry's Law; which is a thermodynamic relationship of the partitioning of a compound between the gas and liquid phase. Thus, by knowing the concentration of the contaminant in the either the headspace or liquid phase, it is possible to determine its total concentration. Treatments were incubated at 30°C in the dark in a constant temperature room.

Different stoppers were tested to find the appropriate septa to be used in the microcosm experiments in order to avoid the escaping of the aromatic compounds. For this purpose 160 mL glass bottles, in duplicate, containing a toluene solution at a total concentration ranging from 5 to 100 mg L^{-1}, in 50 mL of liquid, were sealed with rubber, Viton, and Teflon faced butyl stoppers (Supelco, San Louis, MO) and monitored periodically. Treatments were incubated in the dark at 30 °C. After 110 days, bottles sealed with Teflon faced butyl septa showed negligible variation in the total

concentration of toluene, whereas a significant decreased in toluene was observed in all the treatments sealed with rubber and Viton stoppers (data not shown). These results indicated that Teflon faced butyl stoppers were appropriate for their use in the microcosms experiments.

2.3.3 Respikes and transfers

Fresh substrate was added to treatments that showed removal using a chromatographic syringe in an effort to restore the initial concentration. The respiking of the sealed bottles was performed in a fumehood. Microorganisms capable of degradation were transferred to clean glass bottles containing a fresh liquid phase previously flushed with N_2/CO_2 (80% / 20%) and sealed. Transfers were performed in an anaerobic chamber (COY Laboratory Products, Grass Lake, MI) transferring 10% of total granules or 10% of the liquid volume according to the characteristics of the sludge. After decanting the liquid phase of the microcosms subjected to be transferred, the remaining granules were sucked up with a 10 mL syringe and crushed. Then, new substrate was added in the fumehood.

2.3.4 Analytical procedure

Substrate degradation was monitored by taking 100 µL from the headspace with a gas-tight syringe. BTX samples were injected into a gas chromatograph HP5890A Series II (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector (FID) and a 30 m x 0.32 mm GS-GASPRO (J&W Scientific, Palo Alto, CA) column. The carrier gas was helium at a flow rate of 32.45 mL min^{-1}, while the split flow was maintained at 12 mL min^{-1}. Both inlet and detector temperature was set at 250°C and the column temperature was 195°C. *cis*-DCE samples were analyzed in a gas chromatograph HP5890E Series II (Agilent Technologies, Palo Alto, CA) equipped with an electron capture detector (ECD) and a 30 m x 0.32 mm column GS-GASPRO (J & W Scientific, Palo Alto, CA). The flow through the column was 18 mL min^{-1} and the split flow was 17.6 mL min^{-1}. The temperature of the column was set at 195°C, while the temperature of the inlet and the detector was 180°C and 275°C, respectively. The syringe was cleaned after each injection using a syringe cleaner (Hamilton, Reno, NV) to avoid accumulation of previous samples. The headspace concentration of the BTX and *cis*-DCE in the treatments was determined by using standards of known concentrations. Standards were prepared in 160 mL glass bottles and the headspace to water ratio was maintained the same as that set for the treatments. The required concentration was achieved by adding a known volume of a stock solution and amended with Milli-Q water to reach 50 mL of liquid. The total concentration in the treatments was calculated using the Henry's Law constant (H). The H values were obtained at 25°C [2-5] and corrected to for 30°C. The

dimensionless H for benzene, toluene, m-xylene and *cis*-DCE used were 0.279, 0.284, 0.391 and 0.196, respectively.

NO_3^-, NO_2^-, and ClO_3^- were measured by ion chromatography using a Dionex (Sunnyvale, CA) chromatograph equipped with an anion self-regenerating suppressor (ASRS ULTRA II) and an Ionpac AS18 (4 x 250 mm) column. The eluent was KOH (25 mM) at a rate of 1 mL min^{-1}. Concentration in treatments was determined by using external standards previously prepared.

Table 2.2. Description of treatments used in microcosm experiments

Treatment	Medium	Substrate	Sludge	Electron acceptor	NaN_3	$HgSO_4$
Abiotic control	X	X		X		
Killed control	X	X	X	X	X	X
Full treatment	X	X	X	X		
Methanogenic*	X	X	X			

*no e-acceptor control

2.4 Results

2.4.1 Toluene degradation under nitrate reducing conditions

A series of screening experiments were set in using different inoculum sources to determine the presence of toluene degrading bacteria. The expectation was that the biodegradation would be stimulated by using NO_3^- as a terminal electron acceptor. Figure 2.1 shows an example of the time course of toluene degradation by one of the inocula (Nedalco sludge) used in the screening. The toluene concentration decreased with time in the presence of inoculum and the electron acceptor. The treatment lacking NO_3^- displayed very low activity. Toluene concentration remained constant in controls (abiotic and killed) containing NO_3^-; indicating that toluene degradation was the result of a biological reaction in the presence of NO_3^-. In the full treatment, toluene was removed in 6 days. Results indicate that Nedalco inoculum contained bacteria capable of toluene degradation under denitrifying conditions. Some other inocula tested catalyzed toluene removal as well, as shown in Table 2.3. To determine if the activity could be sustained, the full treatments were respike with toluene.

In the microcosms where toluene degradation was observed, pure toluene was spiked into the assays in order to confirm degradation and maintain toluene degrading activity. As shown in Figure 2.1 toluene spiked into the full treatment of Nedalco sludge was removed in about 10 days. The controls and methanogenic treatment showed no decrease in toluene concentration over 18 days, including the 10 days following the

respike in the full treatment. The results demonstrate that the microbial activity could be sustained beyond the five feedings. Figure 2.1 also shows that the total concentration of toluene spiked was 5 times higher than the intended value (100 mg L^{-1}). However, after a few days, the concentration decreased to the intended value; suggesting that the fresh substrate spiked needed a few days to achieve equilibrium with respect to partitioning between headspace, water and free product toluene (the latter eventually dissolving).

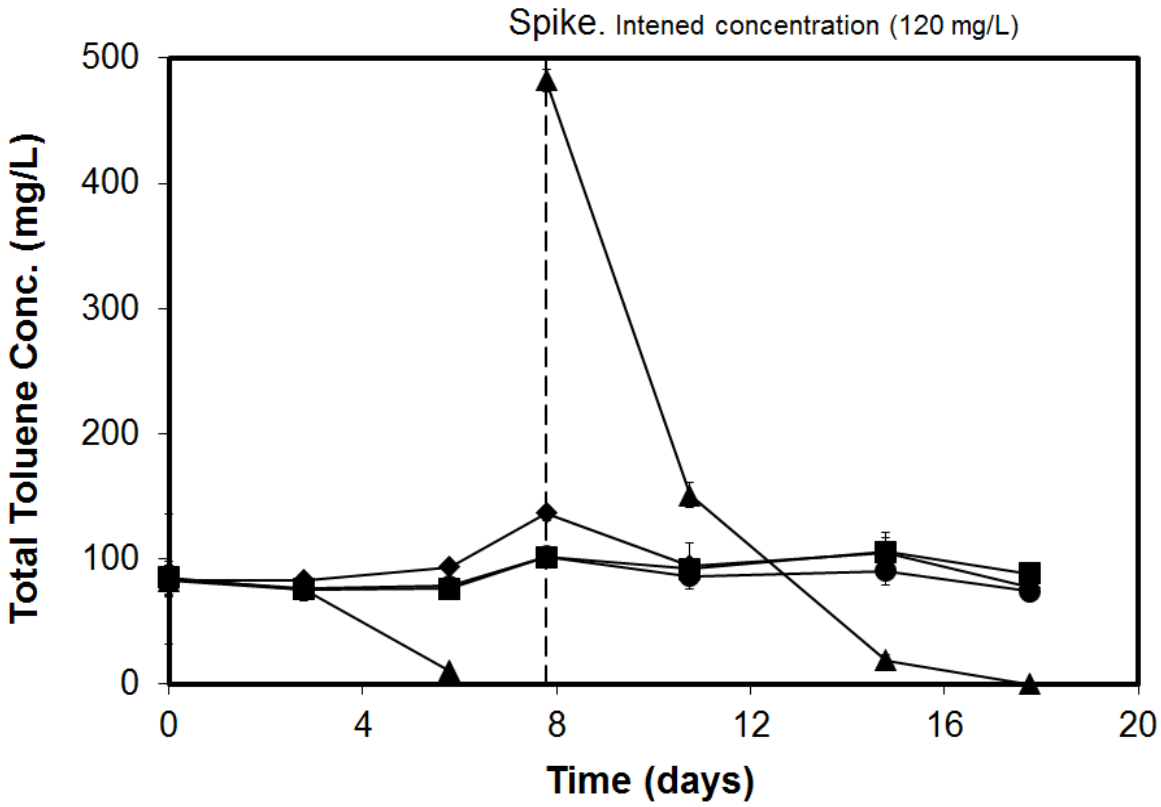

Figure 2.1. Toluene degradation under nitrate reducing conditions using Nedalco sludge. —♦—, Abiotic control; —■—, No NO_3^-; —▲—, NO_3^-; —●—, Poisoned. Error bars represent standard deviation of duplicate assays.

2.4.2 Toluene degradation under chlorate reducing conditions

A screening experiment was also conducted to determine if toluene degradation under ClO_3^- reducing conditions could be observed with any of the several sources of inoculum tested. An example of the screening results is shown in Figure 2.2 with Nedalco sludge as an inoculum source. The figure shows that after 130 days negligible degradation was observed in the full treatment containing ClO_3^- as an electron acceptor. On the other hand, toluene was completely removed under methanogenic conditions after a lag phase of 40 days. Since no degradation was observed in the killed and abiotic controls, the observed reaction is considered to be due to biological activity under methanogenic conditions.

On day 87 and again on day 123, pure toluene was spiked into the methanogenic treatment, to an intended total toluene concentration of 180 mg L^{-1}, in order to determine if the microbial activity could be sustained. After each spike, toluene was allowed to equilibrate and subsequently was removed in time periods of 10 to 20 days. The results suggest that microbial activity in the methanogenic treatment was maintained after several feedings.

Table 2.3 Microcosm experiments results for BTX and *cis*-DCE anaerobic degradation.

Inocula	**Toluene**			**m-Xylene**			**Benzene**			***cis*-DCE**		
	CH_4	NO_3^-	ClO_3^-	CH_4	NO_3^-	ClO_3^-	CH_4	NO_3^-	ClO_3^-	CH_4	NO_3^-	ClO_3^-
Nedalco	+	+++	-	-	-	-	-	+/-**	-	**NT**	**NT**	**NT**
Aviko	-	++	**NT**	-	-	**NT**	-	-	**NT**	**NT**	**NT**	**NT**
Mahou	-	**NT**	-	-	**NT**	-	-	**NT**	+/-*	**NT**	**NT**	**NT**
PS	-	++	**NT**	+/-	-	**NT**	**NT**	**NT**	**NT**	-	-	-
ADS	-	-	-	-	-	-	-	-	+/-*	-	-	-
RAS	-	**NT**	-	-	-	-	-	**NT**	-	-	-	-

+++ loss before 6 days; ++ loss before 13 days (86% removal); + loss before 73 days; +/- loss in one duplicate after 244 d, on the other after 153 day (88.69%); +/-* loss in one duplicate after 413 d; +/-** loss in one duplicate after 716 d; - No degradation after extended incubation; NT not tested.

2.4.3 Transferring of denitrifying cultures

The supernatant from treatments that showed complete toluene removal under NO_3^- reducing conditions was transferred (10% v/v) in an effort to enrich the microbial community. After several unsuccessful attempts it was decided that instead, intact and crushed granules should be transferred (10% of total granules in treatments). Figure 2.3 shows that no lag time was observed for complete toluene removal under nitrate reducing conditions when intact and crushed granules were used. The activity in the full treatments could be sustained.

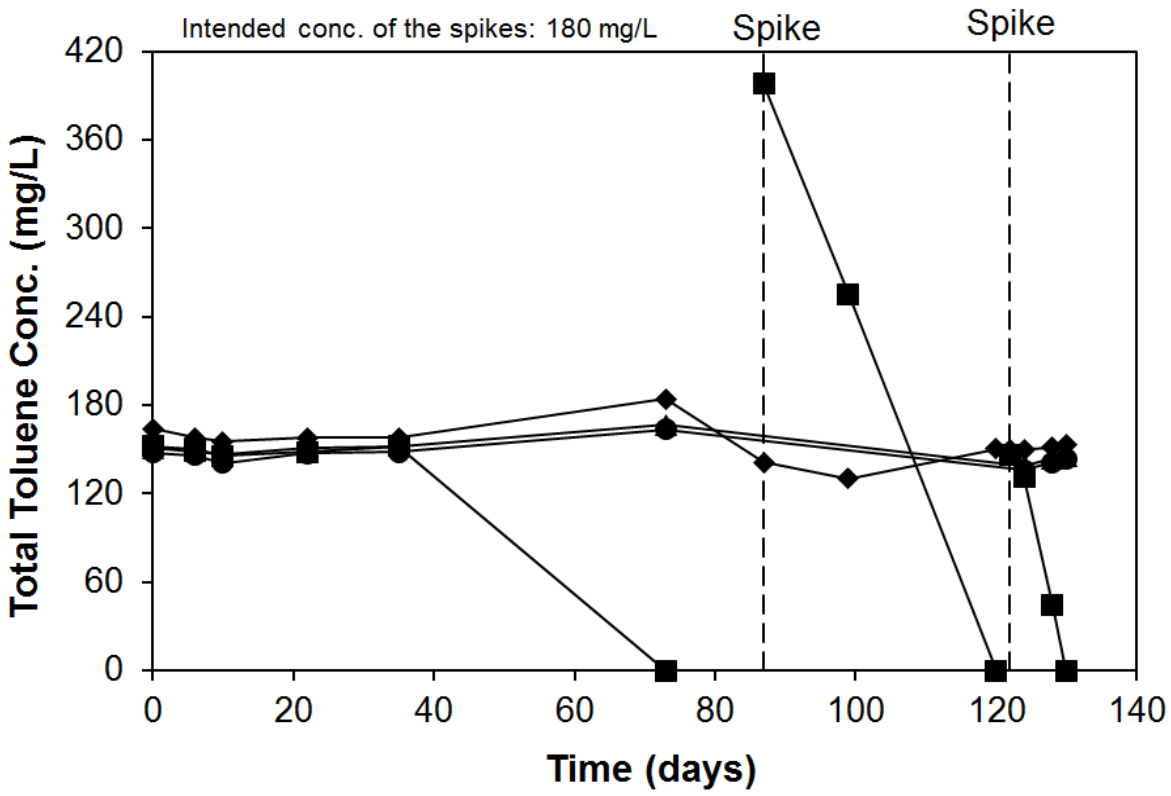

Figure 2.2. Evidence of toluene degradation under methanogenic conditions using Nedalco sludge. —♦—, Abiotic control; —■—, No ClO_3^-; —▲—, ClO_3^-; —●—, Poisoned. Error bars represent standard deviation of duplicate assays.

After the full treatments were respiked and the spike equilibrated, toluene was removed. The rates however were different in the treatments containing intact granules compared to crushed granules, corresponding to 4,117.23 and 7,477.65 mg g $VSS^{-1} \cdot d^{-1}$, respectively; in the time period from day 9 to day 16. Furthermore, complete removal was only achieved with crushed granules by day 30 when the experiment was terminated. The toluene removal reaction seems to be biological since no degradation was observed in both controls, killed and abiotic. These results indicate that bacteria responsible of

toluene removal were embedded in the granule sludge. Figure 2.3 shows that toluene was removed initially in the methanogenic treatments when using crushed granules; however this activity was not sustained since no further degradation was observed in about 20 days after the fresh toluene was respiked.

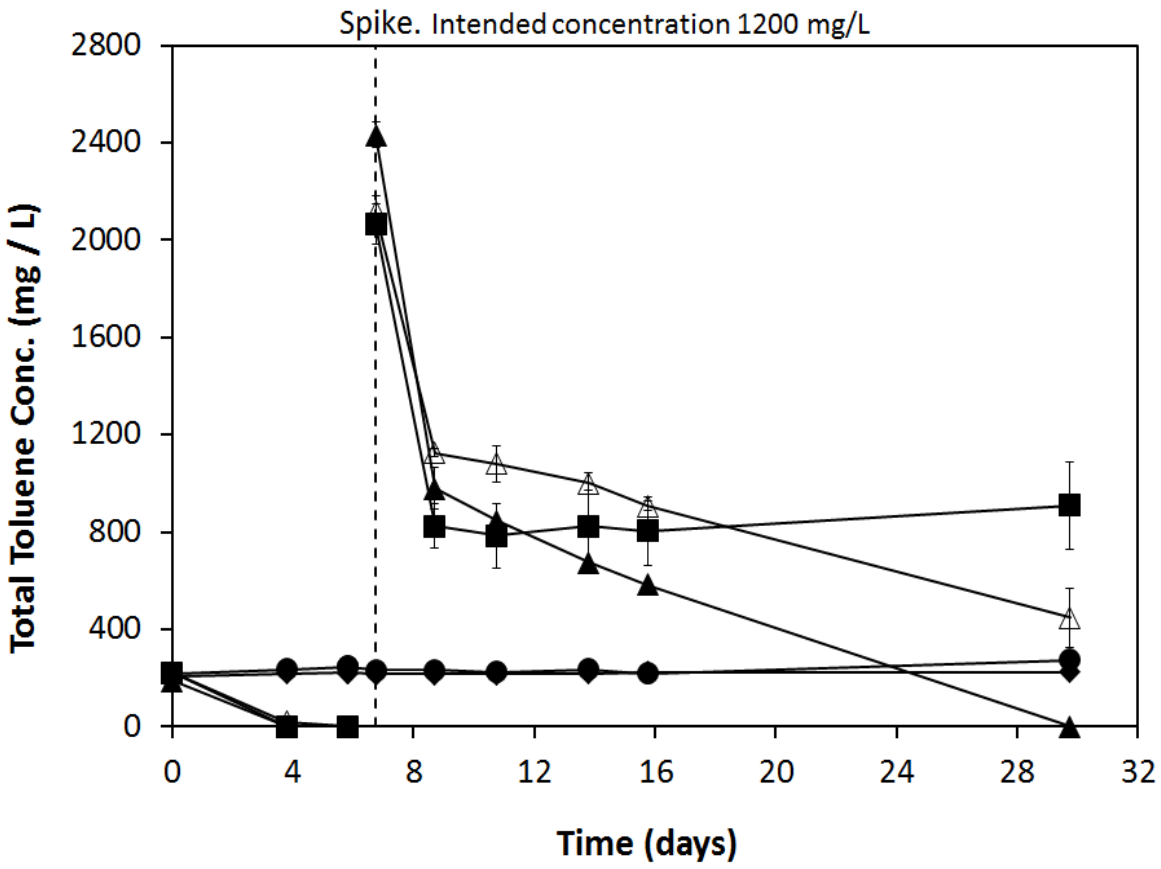

Figure 2.3. Transfer of granular sludge containing toluene degrading microorganisms (Nedalco sludge); intact vs crushed granules. —♦—, Abiotic control; —■—, No NO_3^- (crushed); —▲—, NO_3^- (crushed); —Δ—, NO_3^- (intact); —●—, Poisoned (crushed). Error bars represent standard deviation of duplicate assays.

2.4.4 Transferring of methanogenic cultures

Nedalco granules containing bacteria capable of toluene degradation under methanogenic conditions were crushed and transferred (10% of total granules in treatments) into fresh media in an effort to enrich them. Growth conditions with and without NO_3^- as electron acceptor were compared. Toluene was rapidly degraded in the treatment containing NO_3^-, as can be seen in Figure 2.4. After the initial spike at the beginning of the experiment, all the toluene was already removed after the first measurement on day 3. In order to determine if activity could be sustained pure toluene was respiked into the cultures to an intended total concentration of 200 mg L^{-1}. The microbial activity was clearly sustained since in the nitrate reducing treatments, toluene was removed.

However, the degradation after each respike took longer than the previous one, indicating that no enrichment of toluene degraders was occurring. Removal was also observed in the methanogenic treatment; however the activity was significantly slower compared with the full (NO_3^--amended) treatment. The initial toluene spike at the beginning of the experiment was not completely degraded after 40 days, when the experiment was ended. The results indicate that degradation is biological, since abiotic and killed controls containing NO_3^- presented negligible activity. NO_3^- appears to be an important electron acceptor for the biological degradation of toluene, since its presence dramatically increased the microbial activity towards toluene removal.

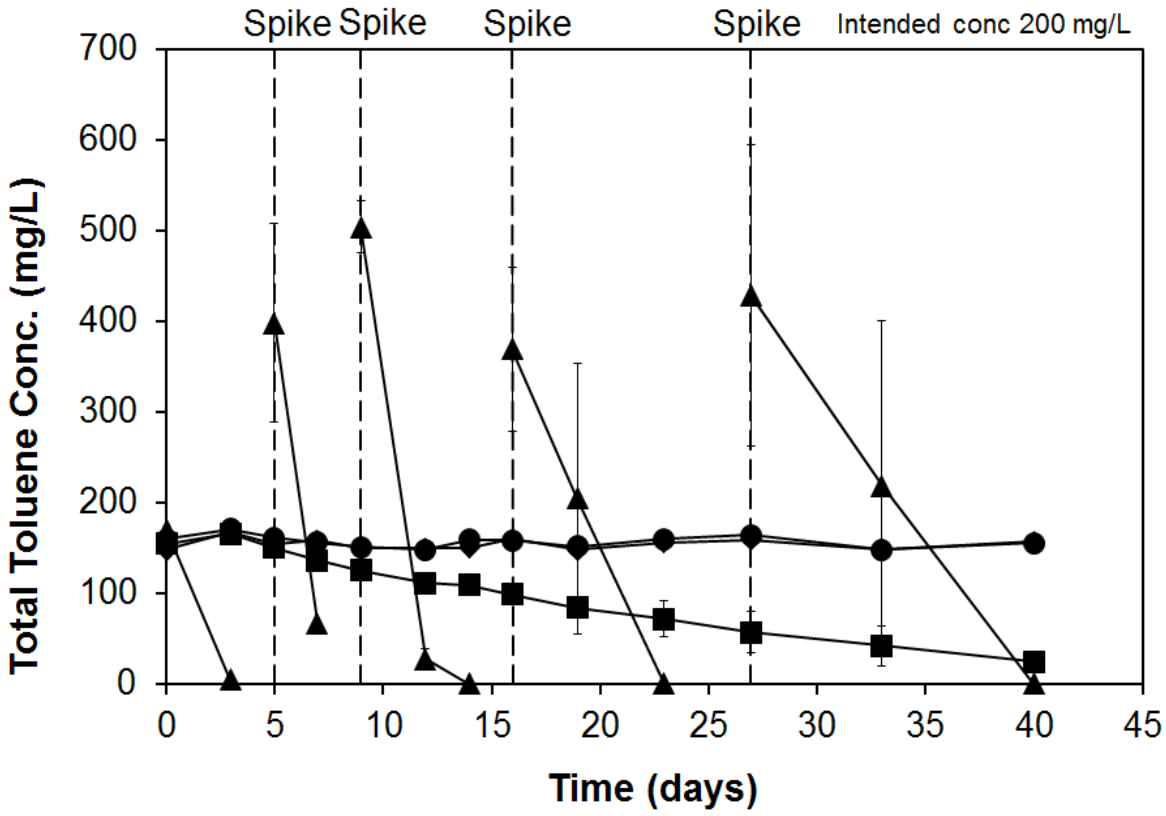

Figure 2.4. Transfer of toluene degrading microorganisms (Nedalco sludge. 10% of total crushed granules previously adapted to methanogenic conditions). —♦—, Abiotic control; —■—, No NO_3^-; —▲—, NO_3^-; —●—, Poisoned. Error bars represent standard deviation of duplicate assays.

2.4.5 Nitrate consumption and nitrite formation linked to toluene degradation

The liquid phase of the controls and treatments shown in Figure 2.4, were sampled to determine the fate of both nitrate and nitrite. Figure 2.5a shows that NO_3^- concentration decreased with time in the full treatment where toluene was rapidly degraded. As expected, no NO_3^- was detected in the methanogenic treatment since that treatment received no added NO_3^-. The NO_3^- concentration in the abiotic and poisoned controls showed very little variation and no evidence of consumption was observed. On day 16, when toluene and NO_3^- were respiked, it can be observed that the NO_3^- concentration continued to drop. NO_3^- consumption was associated with the formation of NO_2^- (Figure 2.5b). An analysis was made to compare the consumption of toluene with the consumption of NO_3^- and formation of NO_2^- over the first 3-days of the experiment, based on the concentration of total electron milliequivalents (e-meq) transformed.

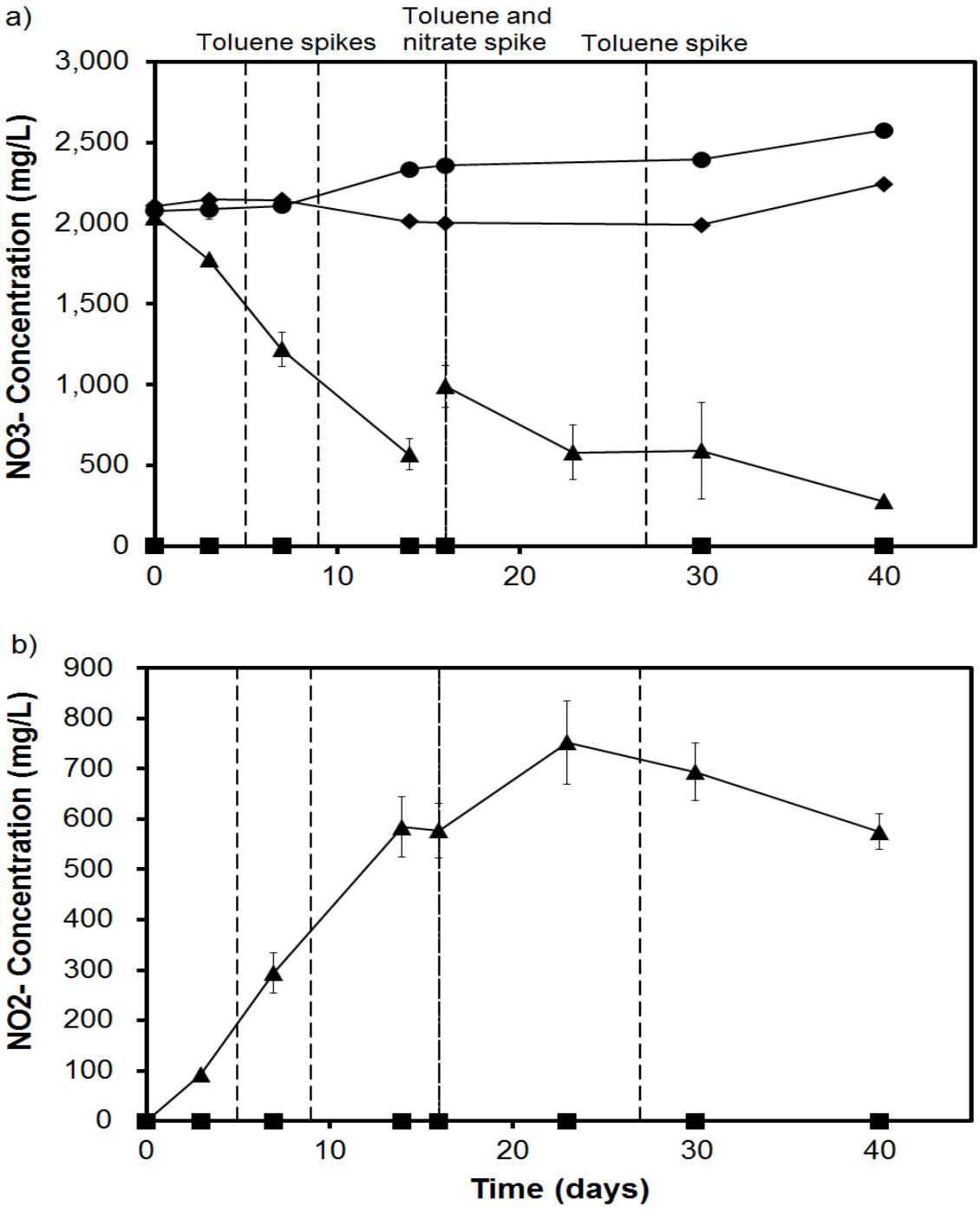

Figure 2.5. Consumption of a) NO_3^- and formation of b) NO_2^- during the anaerobic degradation of toluene. NO_3^- spiked on day 16. Nedalco sludge. —♦—, Abiotic control; —■—, No NO_3^-; —▲—, NO_3^-; —●—, Poisoned. Error bars represent standard deviation of duplicate assays.

Data obtained from samples taken during this period showed that toluene was consumed from an initial total concentration of 165.79 mg L^{-1} (1.80 mM) to levels below the detection limit (0.25 mg L^{-1}), which corresponded to 64.80 e-meq L^{-1}. Simultaneously, 263.55 mg NO_3^- L^{-1} (2.25 mM) were consumed and 92.19 mg NO_2^- L^{-1} (2.00 mM) were generated. As consequence, 4.00 e-meq L^{-1} were involved in the formation of NO_2^- linked to NO_3^-reduction during toluene degradation. The remaining moles of NO_3^- consumed, were assumed to be fully reduced to N_2, corresponding to 11.25 e-meq L^{-1}. This analysis is shown in Table 2.4 and indicates that only 23.5% of electron equivalents in the toluene removed over the first three days could be accounted for by metabolism of NO_x^-, assuming the toluene would have been converted to CO_2 by the denitrifying bacteria.

Table 2.4. Electron-milliequivalent balance over the first 3-days from the transfer experiment shown in Figure 5.

Redox couples	**mM of NO_3^- transformed**	**electron m-eq / L ***	**Total e- meq / L**
Toluene to CO_2		64.80	64.80
NO_3^- to N_2	2.25	11.25	15.25
NO_3^- to NO_2^-	2.00	4.00	

*Milliequivalents of toluene converted to CO_2 (ideally) = 64.80 e-meq L^{-1}. Milliequivalents of NO_3^- converted to N_2 = 11.25 e-meq L^{-1}. Milliequivalents of NO_3^- converted to NO_2^- = 4.00 e-meq L^{-1}. Analysis performed from day 0 to 3.

2.4.6 m-Xylene degradation under nitrate reducing conditions

A series of experiments were conducted to determine the presence of m-xylene degrading bacteria in different inocula. Figure 2.6 shows that no degradation occurred in the NO_3^- containing treatment inoculated with pond sediment. This was also true for all the other inocula tested. On the other hand, complete removal of m-xylene was achieved under methanogenic conditions but only with pond sediment inoculum (Figure 2.6). Degradation commenced after a 100 days of lag phase. Since there was negligible activity in the controls the degradation in the methanogenic treatment was most likely due to a biotic reaction.

In order to determine if the microbial activity could be sustained, a single respike of pure m-xylene was added to the methanogenic treatment on day 485. Figure 2.6 shows that after the respike, m-xylene was degraded. The rate of degradation was greater than during the initial feeding. During the removal of the m-xylene respiked into the methanogenic treatment, there was little removal in the controls and in the NO_3^- treatment. The spiking data demonstrate that the microbial activity was enhanced after exposure to the compound.

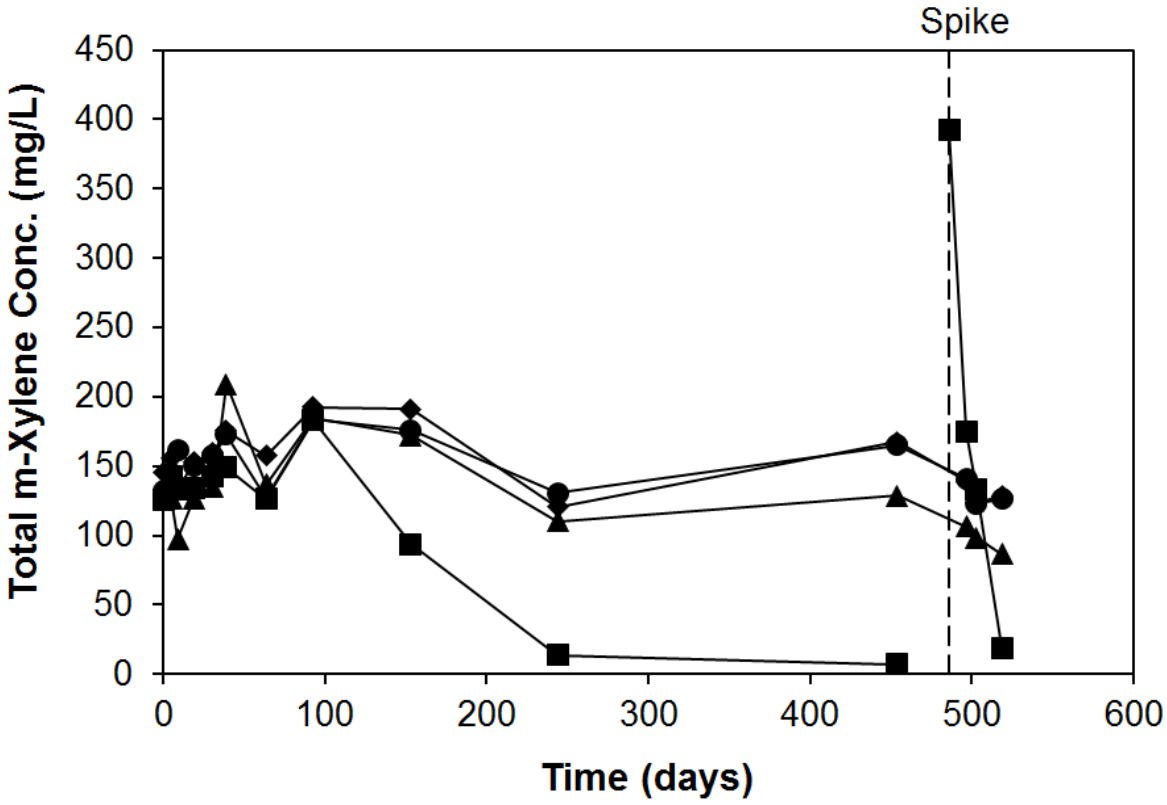

Figure 2.6. Anaerobic degradation of m-xylene under methanogenic conditions. Pond sediments (Agua Caliente park). —♦—, Abiotic control; —■—, No NO_3^-; —▲—, NO_3^-; —●—, Poisoned. Error bars represent standard deviation of duplicate assays, except for —■—.

2.4.7 Benzene degradation under nitrate and chlorate reducing conditions

A series of screening experiments were set up for the degradation of benzene under anaerobic conditions using different inocula and electron acceptors. For most inocula no benzene degradation was observed after extended incubation periods of up to 2 years. However, benzene removal was observed in one duplicate, under nitrate reducing

conditions after 420 days with the Nedalco inoculum (Figure 2.7). The lack of degradation in the methanogenic treatment suggests that NO_3^- was critical as an electron acceptor for the oxidation of benzene. Since negligible activity was observed in the abiotic and poisoned controls, the removal of benzene in the NO_3^- treatment was biological. The data indicate the presence of benzene degrading bacteria in some of the inocula tested.

However, a long time period was required for the population of benzene degraders to become enriched. In two of the inocula tested (ADS and Mahou), benzene removal was observed in one duplicate each when ClO_3^- was used as electron acceptor. Figure 2.8 shows the results with anaerobic digested sludge. After a lag phase of 111 days, the benzene concentration in the full treatment decreased considerably. The degradation reaction was microbially mediated since negligible benzene removal was observed in the controls. The presence of chlorate was a limiting factor since no degradation was observed in the methanogenic treatment after more than 400 day.

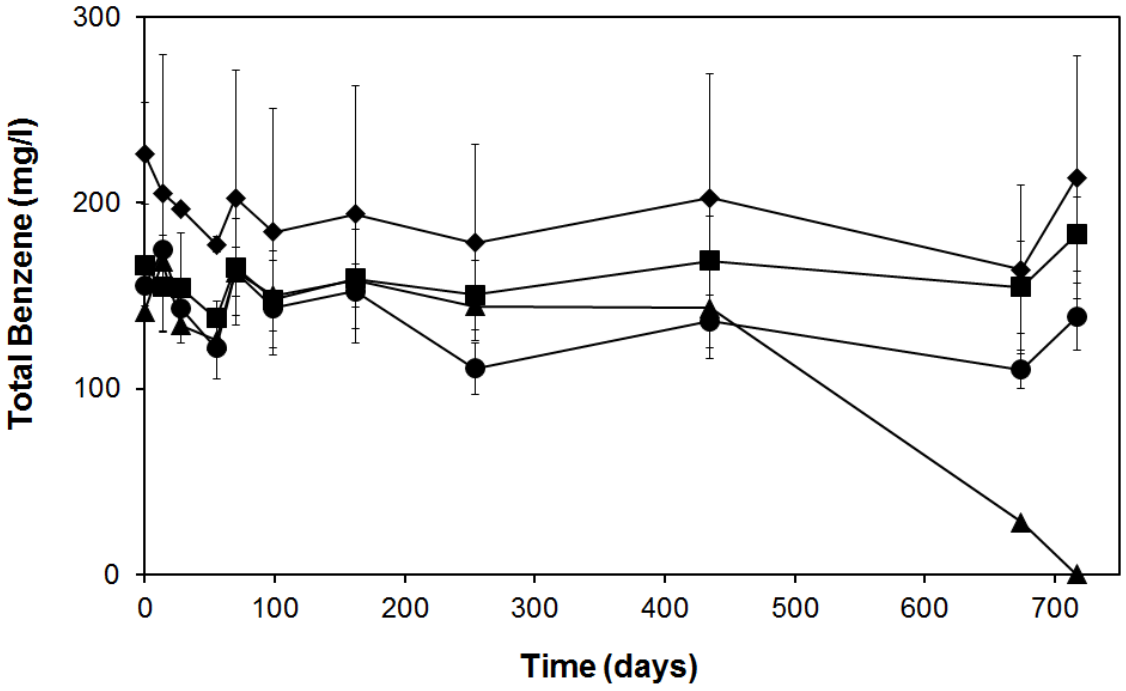

Figure 2.7. Benzene degradation under nitrate reducing conditions. Nedalco sludge. —♦—, Abiotic control; —■—, No NO_3^-; —▲—, NO_3^-; —●—, Poisoned. Error bars represent standard deviation of duplicate assays, except for —▲—.

2.4.8 Experiments summary

Table 2.3 provides an overview of the biodegradation experiments performed and which combinations of inoculum and electron acceptor resulted in biodegradation. Of all the compounds tested, toluene was the easiest to degrade. It was rapidly removed in treatments containing NO_3^- for the majority of the inocula tested. On the other hand, methanogenic degradation was observed after extended incubation in only one inoculum. The use of ClO_3^- as electron acceptor did not help with the degradation of this contaminant. m-Xylene proved to be recalcitrant under NO_3^- and ClO_3^- reducing

condition since no removal was observed. Nonetheless, m-xylene was degraded under methanogenic conditions after a long incubation period but only with pond sediments as inoculum. The trend with benzene was the opposite of that observed for m-xylene, since no degradation was observed under methanogenic conditions. Instead removal linked to NO_3^- reduction was achieved in one of the inocula tested; and removal linked to ClO_3^- reduction was observed in two inocula. However very extended incubation periods were needed to observe benzene degradation. *cis*-DCE was tested in RAS, ADS, and pond sediments and no degradation was observed after extended incubation when using NO_3^- or ClO_3^-. There was also no evidence of *cis*-DCE removal with the methanogenic treatments.

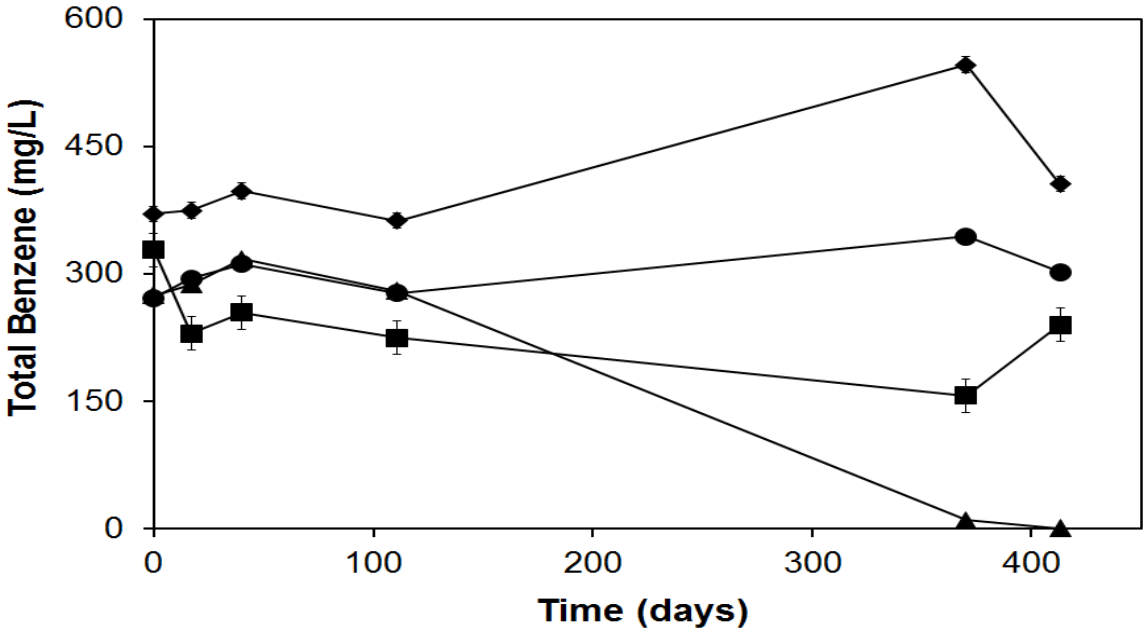

Figure 2.8. Benzene removal using chlorate as terminal electron acceptor. Anaerobic digested sludge. —♦—, Abiotic control; —■—, No ClO_3^-; —▲—, ClO_3^-; —●—, Poisoned. Error bars represent standard deviation of duplicate assays, except for —▲—.

2.5 Discussion

2.5.1 Toluene degradation

In this study toluene, m-xylene, and benzene were shown to be degraded by some anaerobic inocula tested under denitrifying conditions and, in some cases, under methanogenic conditions. The degradation of these BTX compounds only occurred in the presence of unadapted live inocula. There was no removal in killed controls or in non-noculated controls, indicating that degradation was microbially mediated. Thus, some of the inocula tested contained microorganisms capable of BTX degradation under anaerobic conditions.

Toluene was removed under methanogenic conditions after 40 days when using Nedalco sludge. Treatments containing the same inocula were capable of completely removing toluene in 6 days in the presence of NO_3^-. The data demonstrate that although toluene degradation occurs in the absence of external electron acceptor after extended incubation, the presence of an exogenous electron acceptor stimulated the anoxic degradation. The fact that NO_3^- caused an increase of the degradation rate of toluene compared to methanogenic conditions may be due to the higher energy yield of denitrification compared to methanogenesis. Table 2.5 shows the calculated standard delta Gibbs energy ($\Delta G^{0'}$) for different redox reactions. This behavior was also observed by Phelps and Young [37] in polluted sediments from a freshwater site.

Table 2.5. Energy associated to toluene oxidation.

Reaction	ΔG^0 (KJ mol^{-1})
$C_7H_8 + 7.2\ NO_3^- + 0.2\ H^+ \longrightarrow 7\ HCO_3^- + 3.6\ N_2 + 0.6\ H_2O$	-3,547
$C_7H_8 + 18\ NO_3^- + 3\ H_2O \longrightarrow 7\ HCO_3^- + 18\ NO_2^- + 7\ H^+$	-2,456
$C_7H_8 + 12\ NO_2^- + 5\ H^+ \longrightarrow 7\ HCO_3^- + 6\ N_2 + 3\ H_2O$	-4,283
$C_7H_8 + 7.5\ H_2O \longrightarrow 2.5\ HCO_3^- + 4.5\ CH_4 + 2.5\ H^+$	-144

The data for computing the ΔG^0 values of the reactions was taken from Reittmann and McCarty [38] and Thauer et al [39].

Nonetheless, toluene degrading microorganisms were not found in all the inocula tested. Only half of the inocula samples used in the experiments were capable of toluene degradation which is consistent with previous reports that toluene can be recalcitrant under anaerobic conditions in pristine soils [37]. Nonetheless, there are several reports of toluene removal by unpolluted sediments under nitrate reducing conditions [40, 41], as was demonstrated in this study. While toluene oxidation coupled to denitrification has been widely studied, the use of ClO_3^- as electron acceptor has not been evaluated as thoroughly. Extended incubation of the unadapted inocula did not result in an accelerated degradation of toluene linked to ClO_3^- reduction. Therefore microorganisms capable of utilizing chlorate for BTX degradation were not found in the pristine inocula used in this study. Logan and Wu [42] demonstrated, in column experiments inoculated with ADS,

that ClO_3^- can enhance toluene biodegradation; however enrichments were not obtained when using ClO_3^- as the sole electron acceptor.

Treatments that showed degradation were spiked with fresh substrate to evaluate if the microbial activity could be sustained. After injection of new substrate into the bottles, the initial measured concentration was much higher than the intended concentration. A few days later the concentrations approached the intended values. This behavior is attributed to a certain amount of time required for the toluene to equilibrate in concentration between the liquid and headspace. The concentrations measured were based on headspace analysis. After repeated spiking, the norm was an increase in time required for the removal of the fresh substrate after each addition, suggesting loss of required growth factors over time or toluene toxicity.

2.5.2 Transfer of microorganisms

The first attempts to transfer bacteria capable of toluene oxidation consisted in the transfer of microorganisms suspended in the supernatant to fresh media from treatments that showed degradation. However, this approach did not result in degradation of toluene. One possibility for the lack of degradation in the new medium is that toluene degradation may be due to cometabolism, and co-substrates present in the sludge granules were lost by transferring only the supernatant. Cometabolic oxidation of toluene was observed by Rabus and Heider [43] by ethylbenzene adapted bacteria. The lack of degradation could also be attributed to the absence of endogenous organic matter, otherwise present in the

granules, which may have helped increase toluene degradation by generating fumurate during organic matter decomposition. Fumurate is an essential reactant for the initiation of anaerobic toluene degradation [44] and it is known to enhance the degradation. The results observed could indicate as well that microorganisms capable of toluene removal might be attached to granules. To evaluate this concept, both intact and crushed granules were transferred into fresh media.

Toluene removal was observed in both cases; however the degradation rate of crushed granules was higher than that showed by intact granules. accounting possible explanation for this behavior is that crushing the granules might increase the mass transfer of toluene. Microorganisms capable of toluene oxidation might be clustered inside the granules. If so, the difference of the degradation rate could be the result of faster diffusion of toluene to smaller broken up granules. Despite the fact that the first transfer worked in both cases, it was not possible to enrich the cultures for toluene degradation. This could indicate that organic matter present in the granules is not the sole factor needed to sustain toluene degradation. Clearly other factors are important, such as undefined growth factors. Likewise, it is possible that continued exposure to toluene could be toxic to the responsible microorganisms, which is known to have inhibitory effects to certain organisms [45, 46].

2.5.3 Nitrate consumption

A complete analysis for toluene, NO_3^-, and NO_2^- was performed for one the transfer experiments. No NO_2^- was fed into the assay; however toluene removal linked to NO_3^- reduction resulted in formation of NO_2^-. Its formation coincided with NO_3^- consumption indicating incomplete denitrification. NO_2^- accumulated for a few days until it reached a maximum value. Accumulation of NO_2^- from toluene denitrification has been observed previously [47]. The following days the NO_2^- concentration decreased presumably due to complete denitrification to N_2. Evans et al [48] observed NO_2^- reduction during toluene denitrification when the NO_3^- concentration was nearly depleted. In this study an electron equivalent balance was performed for toluene oxidation and NO_3^- reduction from day 0 to day 3. The analysis indicated that 23.5% of the electron equivalents in toluene were accounted for by NO_3^- conversion considering complete oxidation of toluene to CO_2. This low correlation could indicate partial oxidation of toluene in the treatments as observed by Evans et al [49] who found formation of two recalcitrant byproducts, benzylsuccinic acid and benzylfumaric acid, during toluene oxidation linked to denitrification when using strain T1, an anaerobic microorganism capable of toluene oxidation under denitrifying conditions. Another possibility would be the use of toluene or its partially oxidized products by methanogens. Lastly, it should be noted that a greater proportion of the electron equivalents in toluene would be accounted for by NO_3^- reduction, if dissimilatory nitrate reduction to

ammonium (DNRA) had taken place. DNRA is known to occur in methanogenic sludge [50].

2.5.4 m-Xylene

In this study, removal of m-xylene was only observed under methanogenic conditions after an extended incubation time when using non-polluted pond sediments from Agua Caliente Park. There was no degradation in treatments containing neither NO_3^- nor ClO_3^-, indicating that probably bacteria capable of utilizing these compounds as electron acceptors for the oxidation of m-xylene might have not been present in the sediments. It is not clear what factors enhance bioremediation of m-xylene under anaerobic conditions. Although natural attenuation of m-xylene in contaminated sites has been reported [51], anaerobic degradation is not observed in many sediments, sludge, or water samples from both contaminated and pristine sediments. Biotransformation is most likely limited to a few enrichment cultures [52] or bacterial strains [53]. Nontheless, degradation of this contaminant using uncontaminated sediments has been reported by Rabus and Widdel [54] who were able to isolate the denitrifying strain mXyN1, that grows only in the presence of m-xylene, from fresh mud.

2.5.5 Benzene

Benzene is the most recalcitrant of the BTX compounds under anaerobic conditions; the lack of functional groups in the molecule makes it extremely stable which could explain why there was no degradation under methanogenic conditions after extended incubations. However, the use of alternative electron acceptors promoted the removal of this contaminant. In this study, treatments containing NO_3^- and ClO_3^- enhanced the bioremediation after more than 400 days in the fastest treatment (Figure 2.8). Data obtained from batch experiments suggest that microorganisms capable of degradation were found in sludge from municipal and industrial treatment plants as well as natural pond sediments. In general, removal of benzene in anaerobic treatments requires long incubation periods, even when utilizing hydrocarbon contaminated sediments as inoculum. Kazumi et al [55] reported benzene degradation ranging from 180 to 590 days under different redox conditions. Rapid degradation was observed by Botton and Parsons [56] under iron reducing conditions; around 10 μM where removed in about 70 days when using an enrichment culture derived from a contaminated site.

2.5.6 cis-DCE

The low oxidation state of the carbon atoms in lower chlorinated aliphatic compounds makes them recalcitrant under anaerobic environments. The most common approach to biologically degrade these contaminants is via reductive dehalogenation where chlorine atoms are replaced for hydrogen to ideally form ethene. Chlorinated ethenes have been removed from contaminated sediments under anaerobic conditions. Lendvay et al [57] compared the effect of bioaugmentation versus biostimulation. Sediments amended with *Dehalococcoides* did initiated degradation without a lag phase; meanwhile it took three months to start observing degradation in sediments amended with lactate and nutrients but not bioaugmented. Dechlorination can also occur in pristine sediments as demonstrated by He at al. [58] who were able to isolate a strain capable of reductively transforming tetracholoethene to ethene, denominated *Dehalococcoides* sp. strain FL2 from river sediments not contaminated with chlorinated solvents. However, while microbial reduction rates of PCE to *cis*-DCE are high, transformation of *cis*-DCE does not always occurs, and when it does, it is slower.

Some microorganisms can oxidize chlorinated hydrocarbons to carbon dioxide. Chapelle and Bradley [59] documented natural attenuation of chlorinated solvents in contaminated groundwater. They observed reduction of PCE and TCE to cis-DCE, and VC in the SO_4^{2-} reducing conditions zone, which were further oxidized to carbon dioxide and chloride in the iron reducing zone. In this project the anaerobic degradation of cis-DCE was also studied; the approach employed attempted to anoxically oxidize the

contaminant so that it would serve as a carbon and energy source to promote growth of microorganisms. For this purpose, sludge from municipal wastewater treatment plants and pond sediments were used in microcosm experiments. No degradation was observed after extended incubation times in any of the treatments, which included methanogenic, nitrate reducing, and chlorate reducing conditions.

2.6 Conclusions

Microorganisms capable of toluene oxidation under nitrate and methanogenic reducing conditions were found in many of the inocula tested; however no removal was observed when ClO_3^- was used as electron acceptor. m-Xylene and *cis*-DCE proved to be recalcitrant under anoxic environments; bacteria capable of gaining energy from the reduction of NO_3^- and ClO_3^- linked to the oxidation of these contaminants were not found in the inocula studied. However, m-xylene was removed under methanogenic reducing conditions. Benzene, on the other hand, was oxidized in the presence of NO_3^- and ClO_3^-, yet the oxidation was observed only in one of the duplicates in all the cases.

Despite having observed the anoxic oxidation of BTX, microorganisms capable of performing this task were not present in all of uncontaminated inocula tested. Toluene, which was the fastest contaminant to be degraded, was only easily removed by three different sludges. Of the inocula tested, none was suitable for application in PRBs to support the anoxic oxidation of a mixture of aromatic and chlorinated contaminants.

2.7 References

1. EPA, U. *UST/LUST Program Status In Arizona*. 2008 [cited 2009 August 4, 2009].
2. ATSDR, *Toxicological profile for toluene*, U.S.D.O.H.A.H. SERVICES, Editor. 2000: Atlanta.
3. ATSDR, *Toxicological profile for xylene*, U.S.D.O.H.A.H. SERVICES, Editor. 2007: Atlanta.
4. ATSDR, *Toxicological profile for 1,2-dichloroethene*, U.S.D.O.H.A.H. SERVICES, Editor. 1996: Atlanta.
5. ATSDR, *Toxicological profile for benzene*, U.S.D.O.H.A.H. SERVICES, Editor. 2007: Atlanta.
6. Kjeldsen, P.B., M. A.; Rooker, A. P.; Baun, A.; Ledin, A.; and Christensen, T. H., *Present and Long-Term Composition of MSW Landfill Leachate: A Review.* Critical Reviews in Environmental Science and Technology, 2002: p. 297–336.
7. Baun, A., et al., *Xenobiotic organic compounds in leachates from ten Danish MSW landfills--chemical analysis and toxicity tests.* Water Res, 2004. 38(18): p. 3845-58.
8. Jimenez, L.A., R.; and Bayona, J. M., *Determination of organic contaminants in landfill leachates: a review.* Intern. J. Environ. Anal. Chem, 2002. 82: p. 415–430.

9. Sikkema, S.D., E.; Ahlert, R. and Kosson, D., *Identification and Quantification of Volatile Organic Species During Microbial Treatment of Leachate.* Environmental Progress, 1988. 7: p. 77-83.

10. Gray, D., et al., *Spray irrigation of landfill leachate: estimating potential exposures to workers and bystanders using a modified air box model and generalised source term.* Environ Pollut, 2005. 133(3): p. 587-99.

11. Shim, H. and S.T. Yang, *Biodegradation of benzene, toluene, ethylbenzene, and o-xylene by a coculture of Pseudomonas putida and Pseudomonas fluorescens immobilized in a fibrous-bed bioreactor.* J Biotechnol, 1999. 67(2-3): p. 99-112.

12. Takahata, Y., et al., *Rapid intrinsic biodegradation of benzene, toluene, and xylenes at the boundary of a gasoline-contaminated plume under natural attenuation.* Appl Microbiol Biotechnol, 2006. 73(3): p. 713-22.

13. Guerin, T.F., et al., *An application of permeable reactive barrier technology to petroleum hydrocarbon contaminated groundwater.* Water Res, 2002. 36(1): p. 15-24.

14. Scherer, M.M., et al., *Chemistry and microbiology of permeable reactive barriers for in situ groundwater clean up.* Crit Rev Microbiol, 2000. 26(4): p. 221-64.

15. Ridgway, H.F., et al., *Identification and catabolic activity of well-derived gasoline-degrading bacteria from a contaminated aquifer.* Applied and Environmental Microbiology, 1990. 56(11): p. 3565-3575.

16. Thomas, J.M., et al., *Biodegradation of BTEX in subsurface materials contaminated with gasoline - Granger, Indiana.* Water Science and Technology, 1990. 22(6): p. 53-62.

17. Chiang, C.Y., et al., *Aerobic degradation of benzene, toluene, and xylene in a sandy aquifer - data-analysis and computer modeling.* Ground Water, 1989. 27(6): p. 823-834.

18. Beek, B. and W. Reineke, *Aerobic and Anaerobic Biodegradation Potentials of Microorganisms*, in *Biodegradation and Persistance*. 2001, Springer Berlin / Heidelberg. p. 1-161.

19. Fritsche, W. and M. Hofrichter, *Aerobic Degradation by Microorganisms*. Biotechnology. 2008: Wiley-VCH Verlag GmbH. 144-167.

20. Cozzarelli, I.M. and A.L. Baehr, *Volatile fuel hydrocarbons and MTBE in the environment*, in *Environmental geochemistry*, B.S. Lollar, Editor. 2005, Elsevier. p. 433 - 468.

21. Jindrova, E., et al., *Bacterial aerobic degradation of benzene, toluene, ethylbenzene and xylene.* Folia Microbiologica, 2002. 47(2): p. 83-93.

22. Wilson, L.P. and E.J. Bouwer, *Biodegradation of aromatic compounds under mixed oxygen/denitrifying conditions: a review.* J Ind Microbiol Biotechnol, 1997. 18(2-3): p. 116-30.

23. Morgan, P., S.T. Lewis, and R.J. Watkinson, *Biodegradation of benzene, toluene, ethylbenzene and xylenes in gas-condensate-contaminated ground-water.* Environ Pollut, 1993. 82(2): p. 181-90.

24. Roychoudhury, A.N. and G.L. Merrett, *Redox pathways in a petroleum contaminated shallow sandy aquifer: Iron and sulfate reductions.* Sci Total Environ, 2006. 366(1): p. 262-74.

25. Langenhoff, A.A.M., et al., *Microbial reduction of manganese coupled to toluene oxidation.* Fems Microbiology Ecology, 1997. 22(2): p. 119-127.

26. Boll, M., G. Fuchs, and J. Heider, *Anaerobic oxidation of aromatic compounds and hydrocarbons.* Curr Opin Chem Biol, 2002. 6(5): p. 604-11.

27. Farhadian, M., et al., *In situ bioremediation of monoaromatic pollutants in groundwater: a review.* Bioresour Technol, 2008. 99(13): p. 5296-308.

28. Chakraborty, R., *Anaerobic degradation of monoaromatic hydrocarbons.* Appl Microbial Biotechnol, 2004. 64: p. 437-446.

29. Evans, W.C. and G. Fuchs, *Anaerobic degradation of aromatic compounds.* Annu Rev Microbiol, 1988. 42: p. 289-317.

30. Krieger, C.J., et al., *A stable organic free radical in anaerobic benzylsuccinate synthase of Azoarcus sp strain T.* Journal of Biological Chemistry, 2001. 276(16): p. 12924-12927.

31. Coates, J.D., et al., *Anaerobic benzene oxidation coupled to nitrate reduction in pure culture by two strains of Dechloromonas.* Nature, 2001. 411(6841): p. 1039-43.

32. Shim, H. and T.K. Wood, *Aerobic degradation of mixtures of chlorinated aliphatics by cloned toluene-o-xylene monooxygenase and toluene o-monooxygenase in resting cells.* Biotechnol Bioeng, 2000. 70(6): p. 693-8.

33. Distefano, T.D., J.M. Gossett, and S.H. Zinder, *Hydrogen as an electron-donor for dechlorination of tetrachloroethene by an anerobic mixed culture.* Applied and Environmental Microbiology, 1992. 58(11): p. 3622-3629.

34. Pant, P. and S. Pant, *A review: advances in microbial remediation of trichloroethylene (TCE).* J Environ Sci (China), 2009. 22(1): p. 116-26.

35. Kuchovsky, T. and O. Sracek, *Natural attenuation of chlorinated solvents: a comparative study.* Environmental Geology, 2007. 53(1): p. 147-157.

36. Bradley, P.M., *Microbial degradation of chloroethenes in groundwater systems.* Hydrogeology Journal 2000: p. 104-111.

37. Phelps, C.D. and L.Y. Young, *Anaerobic biodegradation of BTEX and gasoline in various aquatic sediments.* Biodegradation, 1999. 10(1): p. 15-25.

38. Rittmann, B.E. and P.L. McCarty, *Environmental biotechnology : principles and applications*. 2001, Boston: McGraw-Hill.

39. Thauer, R.K., K. Jungermann, and K. Decker, *Energy-conservation in chemotrophic anaerobic bacteria.* Bacteriological Reviews, 1977. 41(1): p. 100-180.

40. Fries, M.R., et al., *Isolation, characterization, and distribution of denitrifying toluene degraders from a variety of habitats.* Appl Environ Microbiol, 1994. 60(8): p. 2802-10.

41. Hutchins, S.R.S., G. W.; Kovacst, D. A.; and Smith, G. A., *Biodegradation of Aromatic Hydrocarbons by Aquifer Microorganisms under Denitrifying Conditions.* Environ. Sci. Technol., 1991: p. 68-76.

42. Logan, B.E. and J. Wu, *Enhanced Toluene Degradation Under Chlorate-Reducing Conditions by Bioaugmentation of Sand Columns with Chlorate- and Toluene-Degrading Enrichments* Bioremediation Journal, 2002. 6(2): p. 87-95.

43. Rabus, J.H.a.R., *Initial reactions of anaerobic metabolism of alkylbenzenes in denitrifying and sulfate-reducing bacteria.* Arch Microbiol, 1998: p. 377–384.

44. Leuthner, B., et al., *Biochemical and genetic characterization of benzylsuccinate synthase from Thauera aromatica: a new glycyl radical enzyme catalysing the first step in anaerobic toluene metabolism.* Mol Microbiol, 1998. 28(3): p. 615-28.

45. Playne, M.J. and B.R. Smith, *Toxicity of organic extraction reagents to anaerobic-bacteria.* Biotechnology and Bioengineering, 1983. 25(5): p. 1251-1265.

46. Alagappan, G. and R. Cowan, *Substrate inhibition kinetics for toluene and benzene degrading pure cultures and a method for collection and analysis of respirometric data for strongly inhibited cultures.* Biotechnology and Bioengineering, 2003. 83(7): p. 798-809.

47. Elmen, J., et al., *Kinetics of toluene degradation by a nitrate-reducing bacterium isolated from a groundwater aquifer.* Biotechnol Bioeng, 1997. 55(1): p. 82-90.

48. Evans, P.J., et al., *Metabolites formed during anaerobic transformation of toluene and o-xylene and their proposed relationship to the initial steps of toluene mineralization.* Appl Environ Microbiol, 1992. 58(2): p. 496-501.

49. Evans, P.J., et al., *Quantification of denitrification by strain T1 during anaerobic degradation of toluene.* Appl Microbiol Biotechnol, 1992. 37(1): p. 136-40.

50. Akunna, J.C., C. Bizeau, and R. Moletta, *Nitrate reduction by anaerobic sludge using glucose at various nitrate concentrations - ammonification, denitrification and methanogenic activities.* Environmental Technology, 1994. 15(1): p. 41-49.

51. Reinhard, M.H., G. D.; Steinle-Darling, E.; and LeBron, C. A., *In Situ Biotransformation of BTEX Compounds Under Methanogenic Conditions.* Groud Water Monitoring and Remediation 2005: p. 50-59.

52. Evans, P.J., D.T. Mang, and L.Y. Young, *Degradation of toluene and m-xylene and transformation of o-xylene by denitrifying enrichment cultures.* Appl Environ Microbiol, 1991. 57(2): p. 450-4.

53. Seyfried, B., et al., *Initial reactions in the anaerobic oxidation of toluene and m-xylene by denitrifying bacteria.* Appl Environ Microbiol, 1994. 60(11): p. 4047-52.

54. Rabus, R. and F. Widdel, *Anaerobic degradation of ethylbenzene and other aromatic hydrocarbons by new denitrifying bacteria.* Arch Microbiol, 1995. 163(2): p. 96-103.

55. Kazumi, J.C., M. E.; Suflita, J. M.; Lovley, D. R.; and Young, L. Y., *Anaerobic Degradation of Benzene in Diverse Anoxic Environments.* Environ. Sci. Technol., 1997: p. 813-818.

56. Botton, S. and J.R. Parsons, *Degradation of BTX by dissimilatory iron-reducing cultures.* Biodegradation, 2007. 18(3): p. 371-81.

57. Lendvay, J.M.L., et al., *Bioreactive Barriers: A Comparison of Bioaugmentation and Biostimulation for Chlorinated Solvent Remediation.* Environ. Sci. Technol, 2003: p. 1422-1431.

58. He, J., et al., *Isolation and characterization of Dehalococcoides sp. strain FL2, a trichloroethene (TCE)- and 1,2-dichloroethene-respiring anaerobe.* Environ Microbiol, 2005. 7(9): p. 1442-50.

59. Chapell, F.H.B., P. M., *Selecting Remediation Goals by Assessing the Natural Attenuation Capacity of Groundwater Systems.* Bioremediation Journal, 1998: p. 227-238.

CHAPTER 3

BIOLOGICAL TREATMENT OF A SYNTHETIC LANDFILL LEACHATE IN A COMBINED ANAEROBIC – AEROBIC SYSTEM

3.1 Abstract

Landfilling is the preferred alternative worldwide for disposing household waste due to its simplicity and low cost, compared to other options. However, landfills pose a risk to surrounding water bodies due to the formation of leachates that can contaminate surface water and groundwater. Landfill leachates can contain numerous organic and inorganic contaminants since they are formed as percolating water mixes with chemicals leached out from the buried waste. The objective of this work was to evaluate the performance of a system, consisting of an upflow anaerobic sludge blanket (UASB) reactor and a down flow hanging sponge (DHS) column, for the treatment of a synthetic landfill leachate with a chemical oxygen demand (COD) and ammonia (NH_4^+) content comparable to those found in real leachates. The organic contaminants in the synthetic leachate, comprised by acetic, propionic, butyric and valeric acid, phenol, and p-cresol, were successfully removed during the anaerobic treatment in the UASB reactor. The hydraulic retention time (HRT) in the reactor was decreased after 31 days from 48 to 24 h, which resulted in an increase of the organic loading rate (OLR) from an average of

1.04 $\pm$ 0.46 to 2.3 $\pm$ 0.56 g COD-CH_4 L^{-1} d^{-1}. This change did not seem to affect the removal of the pollutants since the efficiency of the reactor remained constant. Mass balance calculations showed good correlation between the COD removed and the amount of methane (CH_4) gas formed in the reactor. COD removal increased with time, reaching up to 98% elimination. No NH_4^+ removal was observed in the UASB reactor. The aerobic treatment was conducted in a DHS column, started 41 days after the UASB. High nitrification rates were observed during the operation of the reactor. The average loading rate of NH_4^+ to the DHS, which operated at an HRT of 1 day, was 180.9 $\pm$ 41.6 mg NH_4^+-N L^{-1} d^{-1}. During the last 79 days of operation, an average 82% of the NH_4^+ entering the reactor was converted to nitrate (NO_3^-). Nitrite (NO_2^-) was formed during the oxidation of NH_4^+ as it was frequently detected in the effluent, suggesting that partial nitrification could be achieved by controlling the aeration rate. The UASB-DHS system proved to be adequate for the removal of organic contaminants and nitrification of the synthetic leachate.

3.2 Introduction

The increment of waste generation is strongly linked to the progress of the modern lifestyle. Nowadays, Americans generate about three times more solid waste than in the 1960's and the most common practice is to discard it by disposal into municipal solid waste (MSW) landfills. Of the 251 million tons of refuse generated in 2006, approximately 12.5% was burned and 55% landfilled, and the rest, 32.5%, was recycled [1]. Landfilling is so widely used that it is estimated that around the world 70% of household waste is disposed of in landfills [2]. Despite the extensive use of landfills, they have been recognized to pose a high threat to air quality [3] as well as surface- and groundwater quality [4]. Water contamination has been associated with the generation of leachates, which result from the mixing of percolating water with the chemicals present in the landfilled waste. Although engineered landfills are utilized to minimize this risk, they can be subject to failure. High density polyethylene and geotextile liners can become deteriorated by the high temperatures reached inside the landfill [5]. Moreover, old landfills were built without liners and collection systems [6], increasing the likelihood of adverse environmental impacts to surrounding waters. Different studies have demonstrated the negative effects of landfill leachates to groundwater [7-9]. Leachates are generally high in pH and rich in COD and NH_4^+. They are known to cause eutrophication and saprobibation to receiving waters [10] and they proved to be toxic to certain living organisms [11, 12].

The composition of the leachates depends on the characteristics of the landfill, composition of the buried waste, and the environmental conditions of the location [13]. Contaminants in leachates can be grouped into four major categories, consisting in dissolved organic matter (DOM), inorganic macrocomponents, heavy metals, and xenobiotic organic compounds [14]. Table 3.1 shows the concentration ranges of pollutants commonly found in landfill leachates. Volatile fatty acids (VFA) are normally present at the highest concentration among organic compounds, as they are generated during the decomposition of proteins, lipids, and carbohydrates [15]. Other organic pollutants commonly present in leachates are low molecular weight (LMW) alcohols and amines, phenols, aliphatic and aromatic hydrocarbons and volatile esters [16]. Occurrence of phenols in leachates results from their presence in certain household and hazardous products and from the degradation of the lining [17]. Toxic chemicals from plasticizers, pharmaceuticals, solvents, and oils have been identified in landfill leachates as well [18]. Different studies have proved the toxicity of leachates to certain test organisms, including aerobic bacteria (*Salmonella typhimurium* and *Photobacterium phosphorium*), zooplankton (*Daphnia magna* and *Daphnia pulex*), green algae (*Selenastrum capricornutum*) and rainbow trout [11, 12, 19]. Given the negative effects of these contaminants on public health and the environment, treatment of landfill leachates is an important matter. Many techniques have been applied for the treatment of the leachates, including aerobic and anaerobic biological processes, adsorption onto activated carbon, flocculation-coagulation, recirculation, ozonation, and new technologies such as reverse osmosis and filtration [20, 21]. In general, biological

processes have shown to be effective in removing organic compounds and nitrogen (N) in young leachates at a low cost [22]. Compared to anaerobic processes, aerobic treatment requires more energy and generates more waste sludge [23]. During anaerobic digestion complex molecules are converted to carbon dioxide (CO_2), methane (CH_4), and NH_4^+ after multiple intermediate steps [24].

Table 3.1. Concentration range of contaminants commonly found in landfill leachates.

Group	Contaminant	Concentration range (mg/L)	Reference
Organic matter	Biological Oxygen Demand (BOD5)	20 - 570,000	[6]
		100 - 90,000	[25]
		3 - 25,000	[22]
	Chemical Oxygen Demand (COD)	140 - 152,000	[6]
		150 - 100,000	[25]
		556 - 70,900	[22]
		1,700	[26]
Inorganic components	Ammonium-N	50 - 2,200	[6]
		1 - 1,500	[25]
		0.2 - 13,000	[22]
		85	[26]
Organic components ($\mu g\ L^{-1}$)	Phenol	0.6 – 1,200	[6]
		0.6 – 2.2	[27]
		30 – 17,000	[5]
	Cresols	1 – 2,100	[6]
		0.12 – 60.9	[27]
		2,100	[5]

Short chain VFAs such as propionate, butyrate, and valerate are converted by acetogenic bacteria to acetate, hydrogen gas (H_2), and CO_2 depending on the parent compound. A low partial pressure of H_2 is essential for the acetogenic process [28]. Finally CH_4 is produced from acetate by acetoclastic bacteria and from CO_2 and H_2 by reductive methanogenesis [29]. Evidence exist that most of the CH_4 generated during anaerobic digestion is produced from acetate reduction [30]. Phenol can be degraded anaerobically as well, and its biodegradation by a methanogenic consortia was reported many years ago [31]. Complete mineralization of phenol and p-cresol was observed in sewage sludge and their transformation resulted in generation of CH_4 [32]. Benzoate and acetate were detected as intermediate products during phenol degradation which were further transformed to CH_4 and CO_2 [33].

The utilization of anaerobic processes for wastewater treatment has gained importance due to the development of the upflow anaerobic sludge blanket (UASB) reactor, which allows treatment of industrial and domestic wastewater at low cost [34]. The system relies in a high sludge concentration in the reactor that results in capability for treating high organic loadings [35]. Moreover, the sludge biomass has a high settleability preventing loss of biomass due to washout and produced biogas that can be used to generate energy. Due to their simplicity, efficiency and cost, full scale UASBs have been successfully implemented in Mexico, Colombia, Brazil and Uruguay, among others [36]. Alternatively, the utilization of the UASB reactor for treating landfill leachate has been investigated. Kennedy [37] studied the efficiency of batch and continuous UASB reactors. Although performance was similar at low to medium OLRs,

continuous UASB performed better at high OLRs than the sequencing batch treatment. The performance of the reactors for COD removal varied between 71 and 92% depending on the OLR and the concentration of the leachate. UASB reactors treating industrial and domestic wastewater are normally operated at 30 to 35^{o}C in order to achieve high efficiencies. A pilot UASB treating a municipal landfill leachate showed the dependence of the efficiency of the reactor with temperature; where the COD removal reported between 18 to 23^{o}C was 65-75% COD whereas that at 13-14^{o}C only 50-55% [25]. Although effluents from UASB reactors contain low organic concentrations, their N content remains fairly constant during treatment and rarely comply with discharging standards [38], and require pos-treatment.

Methanogenic conditions are not appropriate for removing NH_4^+. For this purpose, aerobic processes are desired after anaerobic degradation. Activated sludge, trickling filters or rotating biological contactors are commonly used as post-treatment alternatives [22]. The downflow hanging sponge (DHS) reactor has recently emerged as a simple and low cost option for aerobic post-treatment. The DHS reactor was designed to treat UASB effluents from municipal wastewater treatment plants (WWTPs) and consists of polyurethane rectangles as packing material where bacteria can attach and grow [39]. Machdar et al [40] installed a DHS reactor fed with treated wastewater from a UASB in a sewage treatment site in Japan. The combined system, with an overall HRT of 8 h, achieved 81-84% total COD removal; however the nitrification was not as efficient reaching only a maximum NH_4^+-N elimination of 61%. A similar arrangement system was tested in a municipal wastewater treatment plant in Japan, achieving higher quality of

the treated effluent [41]. The average total COD removal was equal to 90% at an overall HRT of 10.7 h, whereas up to 86% elimination of NH_4^+ was achieved in the DHS reactor. Moreover, Agrawal et al [42] documented up to 84% N removal in a DHS connected to the effluent of an UASB treating raw sewage after post-denitrification. In this case, N elimination resulted from the combined activity of nitrification and denitrification.

The objective of this work was to study the COD and NH_4^+ removal achieved by a system of laboratory-scale reactors consisting of an UASB followed by a DHS fed with a synthetic media rich in VFA, NH_4^+, and phenolic compounds. The role of a DHS reactor to polish the COD of the UASB effluent and nitrify NH4+-N was also evaluated.

3.3 Materials and Methods

Two laboratoty-scale reactors, one anaerobic and one aerobic, were set up in series to study the removal of COD and NH_4^+ from a synthetic landfill leachate. Different analyses were performed to evaluate the treatability of the leachate and the efficiency of both reactors.

3.3.1 Anaerobic reactor

The anaerobic UASB reactor, having a total and working volume of 420 and 401 mL, respectively, was made of glass (Figure 3.1). The maximum diameter and height were 52 mm and 338 mm, respectively, with the effluent port located 209 mm above the

base. An opening located at the top of the reactor was used for collecting the gases. The reactor was packed with methanogenic granular sludge obtained from a distillery wastewater treatment plant located at Nedalco distillery in Bergen Op Zoom, Holland. The total mass of sludge, which was determined by its volatile suspended solids (VSS) content, was equal to 121.8 g and once packed occupied less than 1/3 of the reactor's working volume. The UASB was operated with a flow rate corresponding to a 48 h HRT for the first 31 days of operation, at which time the HRT was decreased to 24 h. The OLR increased from 1.04 $\pm$ 0.46 to 2.30 $\pm$ 0.56 g COD-CH_4 L^{-1} d^{-1}.

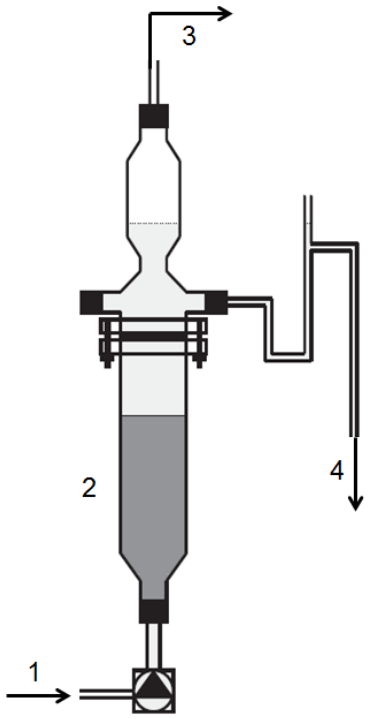

Figure 3.1. Diagram of the upflow anaerobic sludge blanket reactor. (1) Influent leachate, (2) sludge blanket, (3) gas effluent, (4) treated effluent.

3.3.2 Aerobic reactor

The aerobic reactor was started up 41 days after the starting of the UASB. It consisted of a DHS column made of acrylic, having a total and working volume of 350 and 300 mL, respectively; with a height of 268 mm and a 50.8 mm diameter (Figure 3.2). The influent was evenly distributed by a shower, located at the top of the reactor, made of the same material as the reactor. Polyurethane sponge was cut in small rectangles of 1 x 0.5 cm and packed into the reactor until completely filling the working volume. Aerobic returned activated sludge (RAS) from a local wastewater treatment plant (Roger Road. Tucson, AZ) was used for seeding the reactor at a 10% (v/v) ratio. For this purpose a sample of 100 mL of sludge, prior homogenization, was placed in a clean graduated cylinder and allowed to sediment for 1 h. The supernatant liquid was decanted and the sponge rectangles were mixed with 30 mL of the remaining precipitated material. A down-flow stream of humidified air, at a constant flow rate of 5 mL s^{-1}, was supplied from a port located 2 cm from the top. The air was pumped by a commercial pump (Aqua Culture, Walmart) through a clean Erlenmeyer beaker containing Milli-Q water. Another port located at the bottom of the reactor was used for effluent recirculation at a flow set 6 times higher than the influent. The retention time of the reactor was maintained at 24 h.

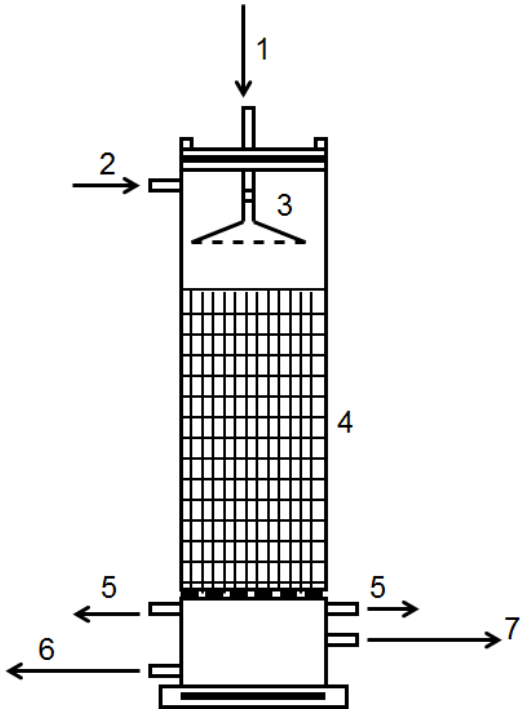

Figure 3.2. Schematic diagram of the upflow hanging sponge reactor used in the experiments. (1) Pre-treated leachate influent, (2) humidified air influent, (3) shower, (4) polyurethane sponge, (5) humidified air exit, (6) recirculation, (7) treated effluent.

3.3.3. Synthetic leachate

The synthetic media resembled the COD and NH_4^+ concentrations normally found in real landfill leachates; it contained as well macro- and micro-components needed for bacterial growth. The organic components of the leachate were (g L^{-1}): yeast extract (0.020), acetic acid (0.585), propionic acid (0.217), butyric acid (0.500), valeric acid (0.164), and phenol and p-cresol which concentration was increase from an average 0.03 to 0.90 g L^{-1} after 103 days of first being added into the leachate. The inorganic

fraction of the media consisted of (g L^{-1}): $CaCl_2$ $2H_2O$ (0.010), KH_2PO_4 (0.037), $MgSO_4$ $7H_2O$ (0.015), $MgCl_2$ $6H_2O$ (0.078), NH_4Cl (0.700), $NaHCO_3$ (2.00), and trace elements (1 mL L^{-1}). The trace elements solution contained (g L^{-1}): $FeCl_3$ $4H_2O$ (2.00), $CoCl_2$ $6H_2O$ (2.00), $MnCl_2$ $4H_2O$ (0.500), $AlCl_3$ $6H_2O$ (0.090), $CuCl_2$ $2H_2O$ (0.030), $ZnCl_2$ (0.050), H_3BO_3 (0.050), $(NH_4)_6Mo_7O_2$ $4H_2O$ (0.050), $NaSeO_3$ $5H_2O$ (0.100), $NiCl_2$ $6H_2O$ (0.050), EDTA (1.00), rezasurine (0.200) and HCl 36% (1 mL L^{-1}). Chemicals were purchased from Sigma-Aldrich (St. Louis, MO). The pH of the synthetic leachate was adjusted between 6.8 to 7.2 before adding the sodium bicarbonate. The media was flushed with N_2 gas for 30 min and then connected to a N_2 bag to ensure anaerobic conditions. The influent was kept at 4ºC for no longer than 4 days to avoid degradation of the organic components.

3.3.4 Analytical procedure

Samples from three different locations were taken to monitor the efficiency of the reactors. The influent was sampled directly from the leachate container and the effluent of both reactors was sampled from the effluent lines.

In order to monitor the concentrations of the VFAs, phenol, and p-cresol, 1.5 mL of each sample were centrifuged at 10,000 rpm for 15 min. Then, 1 mL of the supernatant was transferred into 1.6 mL glass vials (Fisher Scientific, Pittsburgh, PA) and amended with 10 µL formic acid (Sigma-Aldrich, St. Louis, MO). A volume of 0.5 µL of the prepared samples were injected, with help of an autosampler, into a gas chromatograph

(Agilent Technologies 7890A, Palo Alto, CA) equipped with a flame ionization detector (FID) and a 30 m x 0.53 mm column (Restek Stabilwax-DA, Bellefonte, PA). The carrier gas was helium at a flow rate of 115 mL min^{-1} with a split ratio equal to 6:1. The inlet temperature was set at 250ºC while the detector temperature was 275ºC. Standards containing known concentrations of VFA, phenol, and p-cresol, amended with 10 µL formic acid, were measured together with the samples.

The soluble COD fraction was determined by colorimetric analysis. 20 mL Kimax test tubes (Fisher Scientific, Pittsburgh, PA), in duplicate, were filled with 2.5 mL of sample previously passed through 200 nm filters (Fisher Scientific, Pittsburgh, PA) and amended with 1.5 mL of digestion solution and 3 mL of concentrated sulfuric acid. The digestion solution contained (g L^{-1}): $H_2Cr_2O_7$ (10.22), Hg_2SO_4 (33.30), and H_2SO_4 (167 mL L^{-1}). The sulfuric acid solution was prepared by dissolving 10.25 g of Ag_2SO_4 in 1 L H_2SO_4. Tubes then were caped and placed in the oven for 2 h at 150ºC. Chemicals used for COD measurements were purchased from Fisher Scientific (Pittsburgh, PA). Two tubes containing only 2.5 mL of Milli-Q water, used as blanks, were prepared along with the samples. Once at room temperature, the blanks and samples were analyzed in a DU 530spectrophotometer (Beckman-Coulter, Fullerton, CA) at a wavelength of 600 nm.

NH_4^+ was measured using a DC 218-NH_4 NH_4^+ probe (Mettler Toledo, Columbus, OH). For this purpose 2 mL of sample, prior centrifugation at 10,000 rpm for 15 min, were spiked with 40 µL of an ionic strength adjustor (ISA) buffer solution (Mettler Toledo, Columbus, OH). The concentration read by the probe was based on a

calibration set before the analysis, where the standards were prepared following the same procedure as that for the samples. The pH was measure using an Orion pH probe (Fisher Scientific, Pittsburgh, PA). The probe was calibrated using standards (Thermo Scientific, Waltham, MA) before measuring the samples.

NO_3^- and NO_2^- were measured by ion chromatography using a Dionex IC-300 system (Sunnyvale, CA) chromatograph equipped with an anion self-regenerating suppressor (ASRS ULTRA II) and an Ionpac AS18 (4 x 250 mm) column. The eluent concentration was 25 mM of KOH at a rate of 1 mL min^{-1}. The instrument was calibrated by using external standards.

CH_4 production in the anaerobic reactor was monitored by liquid displacement. The exhaust open was connected with plastic tubing to a 2% NaOH solution dyed with methylene blue (Fisher Scientific, Pittsburgh, PA). The displaced liquid was captured in a plastic container and weighted to determine the CH_4 formation after several mathematical calculations.

Finally, the flow of the anaerobic reactor was determined by measuring the weight loss in the leachate container every delta time. The aerobic reactor flow was monitored by measuring the effluent container every delta time.

3.4 Results

A UASB reactor packed with methanogenic granular sludge was set up to evaluate the feasibility of removing COD of a synthetic landfill leachate composed of a mixture of VFA and phenols. A DHS column was connected in series to the UASB to treat the NH_4^+-rich effluent. The fate of the organic and inorganic components was examined to evaluate the efficiency of both reactors.

3.4.1 UASB reactor

Initially, the UASB reactor was operated at a HRT of 48 h (period I), which was decreased to 24 h on day 31 (period II). As a consequence, the OLR was increased from an average of 1.04 $\pm$ 0.46 to 2.3 $\pm$ 0.56 g COD-CH_4 L^{-1} d^{-1} as shown in Table 3.2, together with the influent pH of the synthetic leachate and the volumetric CH_4 production.

The COD removal indicted by difference in influent and effluent samples demonstrated that the UASB was highly efficient for removing the organic content from the media, achieving an average elimination above 88% and 98% during period I and II, respectively. Figure 3.3 shows the concentration of COD measured in the samples. The average COD during period I was 2.54 $\pm$ 0.17 g COD L^{-1} in the influent and 0.30 $\pm$ 0.10 g COD L^{-1} in the effluent; whereas for the second period an average of 2.43 $\pm$ 0.43 and 0.05 $\pm$ 0.01 g COD L^{-1} were measured in the influent and effluent of the

reactor, respectively. The decrease of the HRT caused an slight increase of COD in the treated effluent as observed in Figure 3.3. However, the COD spike in the effluent was negligible and microorganisms in the reactor adapted to the higher OLR in approximately 15 days. Moreover, extended operation time resulted in higher COD removal efficiencies, as higher removal was achieved after day 73.

Generation of CH_4 in the UASB was expected as it is the final product of the methanogenic degradation of organic compounds, and the OLR as function of CH_4 formed in the reactor is presented in Figure 3.4. The average volumetric CH_4 production obtained from CH_4 measurements were higher than those obtained from direct COD readings (Table 3.2), indicating effective conversion of organics in the media, as previously discussed, and possible degradation of organic content in the sludge itself. CH_4 production increased as a more organic-rich leachate was fed to the reactor after day 124. Further analysis of individual contaminants were followed as well.

Table 3.2. pH, organic loading rate (OLR), and volumetric CH_4 production in the UASB.

Period	HRT (h)	pH In	pH Out	OLR (g COD / L d)	Volumetric CH_4 production (g CH_4-COD / L d)
I	48	8.23 $\pm$ 0.08	7.81 $\pm$ 0.18	1.02 $\pm$ 0.46	1.11 $\pm$ 0.22
II	24	8.43 $\pm$ 0.20	8.16 $\pm$ 0.24	2.17 $\pm$ 0.56	2.47 $\pm$ 0.49

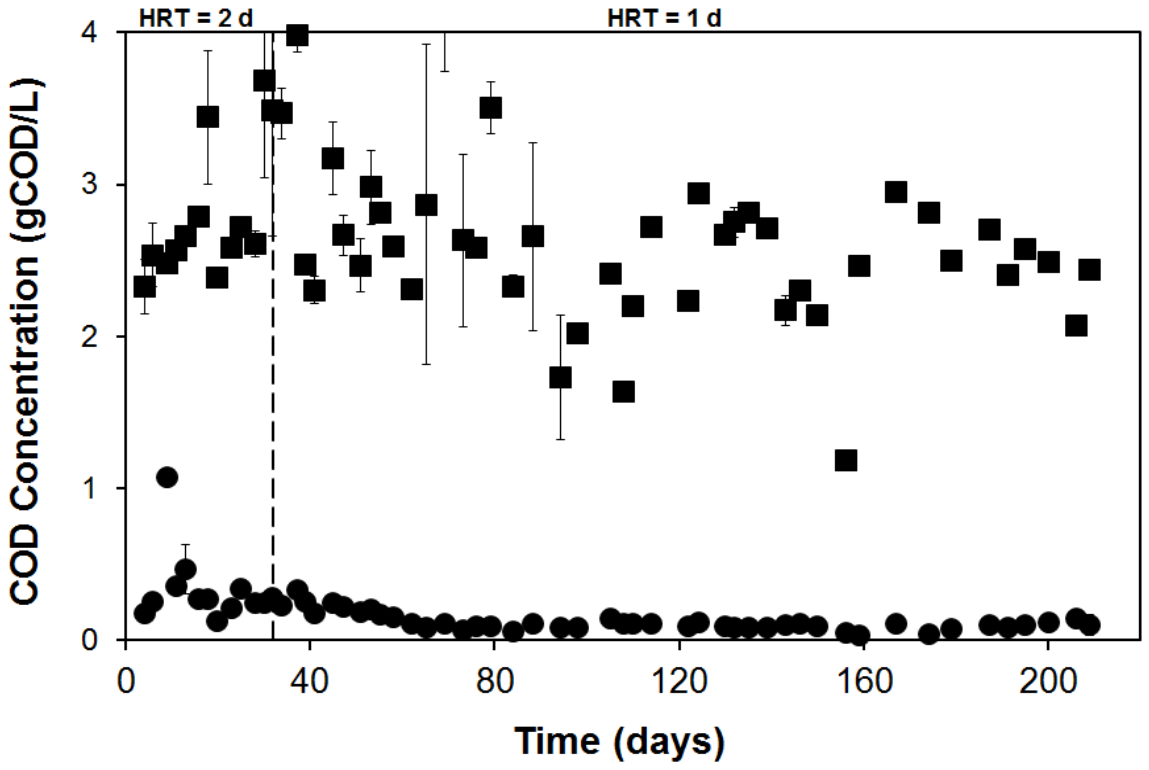

Figure 3.3. Fate of COD during anaerobic treatment in the UASB reactor, where (■) represents the influent and (●) the effluent.

Influent and effluent samples were used to measure the fate of the VFA mixture, phenol, and p-cresol. Figure 3.5 shows that regardless of the fluctuations of VFAs concentration in the influent, the content in the UASB effluent was low. Moreover, it can be observed that the effluent VFA measurements followed the same trend observed with the COD measurements, where higher removal was documented after day 76. The increase in the OLR from day 31 until the end of the experiment did not seem to have any effect over the efficiency of the UASB reactor for removing the organic acids.

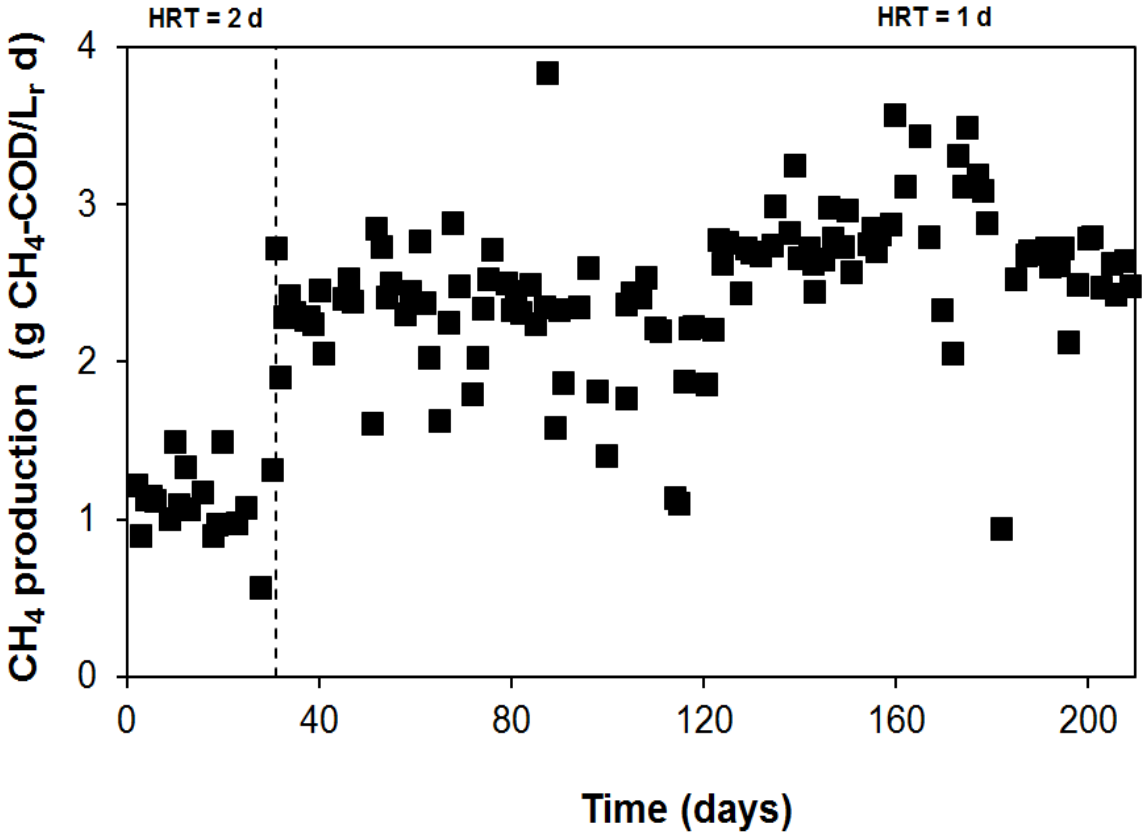

Figure 3.4. CH_4 generation linked to the anaerobic degradation of the organic components in the synthetic leachate during anaerobic treatment in the UASB reactor during periods I and II.

The average VFA concentration in the influent during the operation of the reactor was 2.16 $\pm$ 0.45 g VFA-COD L^{-1}, whereas that of the effluent was 0.88 $\pm$ 0.22 g VFA-COD L^{-1}. Thus, the overall average elimination of VFA reached 95%. Analysis of each individual VFA (data not shown), indicated that mainly acetic acid was present in the effluent. The synthetic leachate had an average measured concentration of acetate of 633 $\pm$ 97.2 mg L^{-1} and the average level detected in the effluent was 58.9 $\pm$ 14.1 mg L^{-1}, which represents approximately 90% elimination. Propionic, butyric,

and valeric acid were intermittently found in the treated effluent throughout the operation of the reactor. Concentration of these acids in effluent samples containing these compounds was higher in samples collected during the first weeks of reactor operation, showing that microbial adaptation was important for the elimination of such contaminants.

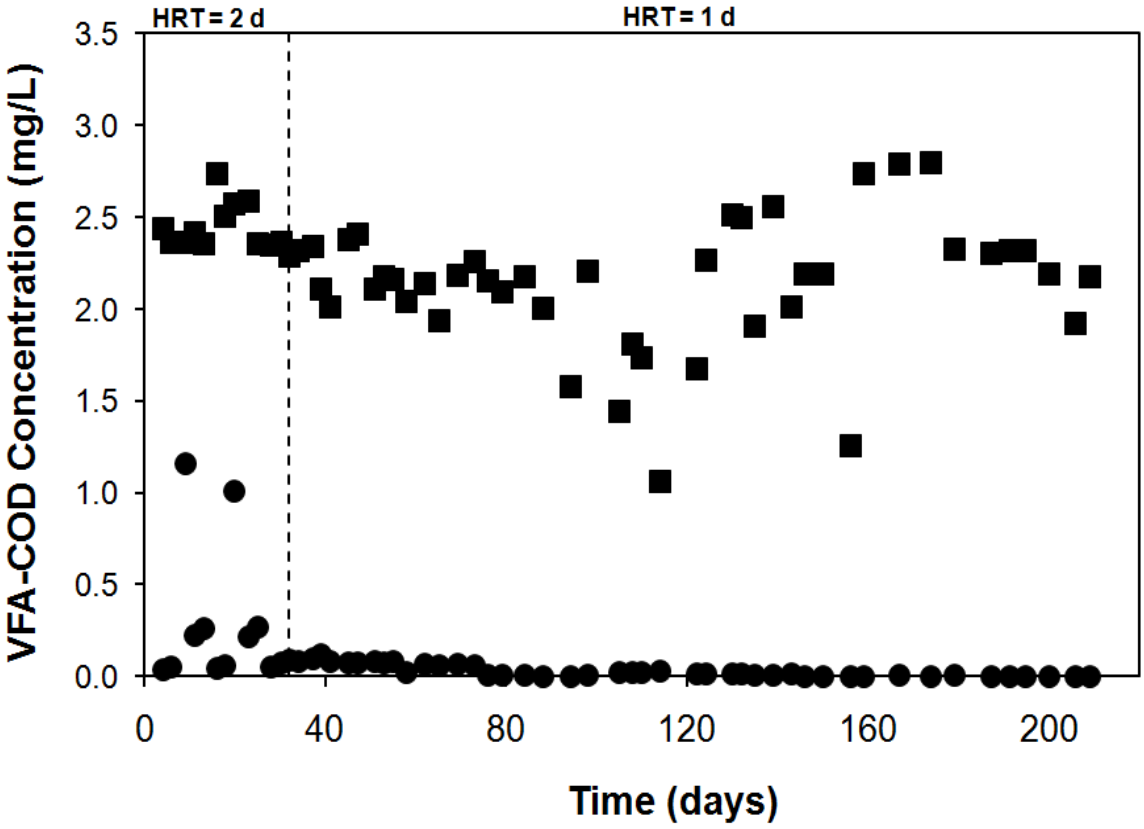

Figure 3.5. VFA concentration in the influent (■) and effluent (●) of the UASB reactor as function of operation time.

Phenol and p-cresol were amended, via stock solution, to the synthetic leachate after 20 days of operation. The concentration of both contaminants was then doubled after day 124. Figure 3.6a shows that the bacteria in the sludge bed readily became enriched with degraders of phenol since high removal efficiencies were observed immediately after this compound was introduced into feed. It can be observed as well that the reactor performance increased with time. Doubling the COD OLR, which occurred at day 31, did not seem to have a significant effect over elimination of phenol as the effluent concentration, 11.5 $\pm$ 2.21 mg L^{-1}, remained fairly constant from day 25 to day 37 achieving approximately 74% removal. Phenol removal gradually increased during the following 30 days reaching up to 92% elimination, and from day 69 its concentration in the treated UASB effluent was below the detection limit, which was found to be 0.25 mg L^{-1}. More importantly, increasing the concentration of phenol from 41.2 $\pm$ 6.47 to 90.6 $\pm$ 8.42 mg L^{-1} on day 124, did not affect the removal efficiency of the rector and microorganisms were capable of acclimatizing to the higher concentration. The fate of p-cresol is shown in Figure 3.6b. Adaptation of bacteria to this contaminant resulted slightly more difficult than to phenol. During the first 17 days after addition of p-cresol, the effluent concentration steadily increased until reaching a maximum value, 34.6 $\pm$ 0.24 mg L^{-1}, at day 37. After increasing the COD OLR, the overall p-cresol removal from day 33 to day 40 was greatly impacted, resulting in an average elimination of only 57%. However, during the next following 28 days, the efficiency gradually increased and during this period the removal efficiency averaged 90%. From day 73 until the end of the experiment the concentration of p-cresol in the effluent samples was below the detection

limit of 0.5 mg L^{-1}. As observed with phenol, increasing the average p-cresol concentration in the leachate from 39.6 $\pm$ 8.94 to 85.8 $\pm$ 6.52 mg L^{-1} did not impact the performance of the UASB reactor.

The anaerobic treatment proved to be suitable for treating the organic content of the synthetic leachate. However, as shown in Figure 3.7, NH_4^+ remained in the treated effluent, since methanogenic processes are not appropriate for nitrification. In order to achieve NH_4^+ removal, the effluent of the UASB was sent to an aerobic DHS reactor. The DHS was started on day 41 of the UASB operation. The HRT was set to 24 h and the effluent of this reactor was recirculated back to the top at a rate 6 times higher than the influent. Samples were taken from the influent and effluent to monitor the fate of NH_4^+, NO_3^-, and NO_2^-, as well as the COD. A 11-day period was required for nitrifying microorganisms to enrich to a point where they could remove most of the NH_4^+ in the pre-treated leachate, as shown in Figure 3.8a. During the following 40 days the effluent concentration remained quite low. The influent and effluent average concentration was equal to 165.8 $\pm$ 50.4 and 13.7 $\pm$ 11.7 mg NH_4^+-N L^{-1}, respectively, achieving an average removal of approximately 91%. However, the efficiency of the reactor decreased considerably from day 105 to day 122. There were no apparent reasons for this shift as the operational conditions, pH, and OLR did not change during this period. High NH_4^+ elimination was observed again from day 130 until the end of the experiment, reaching an average of approximately 96% removal, as the average influent concentration, 154.8 $\pm$ 20.8 mg NH_4^+-N L^{-1}, was decreased to 5.08 $\pm$ 1.08 mg NH_4^+-N L^{-1}. Nitrification in the reactor was monitored by measuring NO_3^- and NO_2^-.

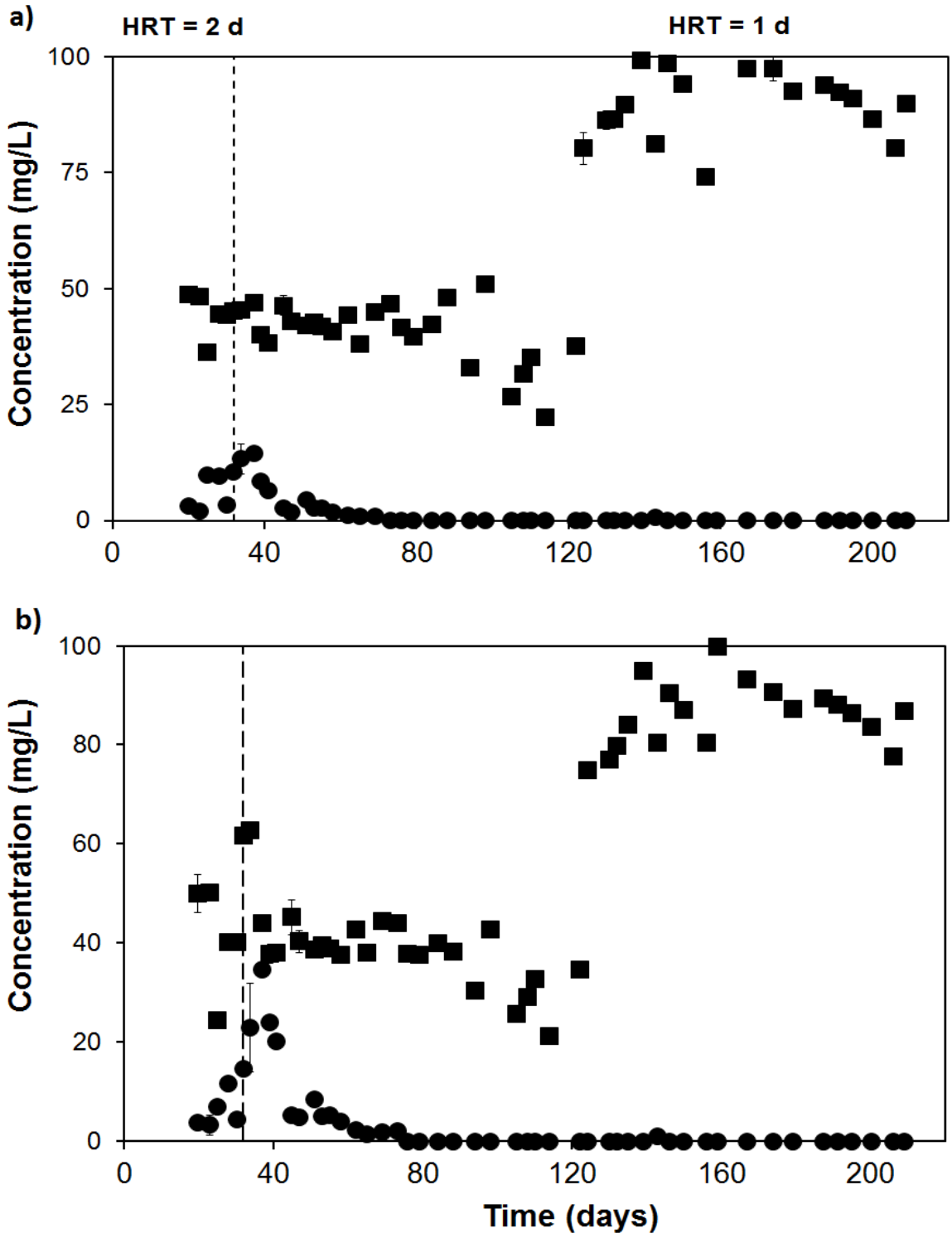

Figure 3.6. Influent and (■) and effluent (●) concentration of a) phenol and b) p-cresol in the UASB reactor as function of time.

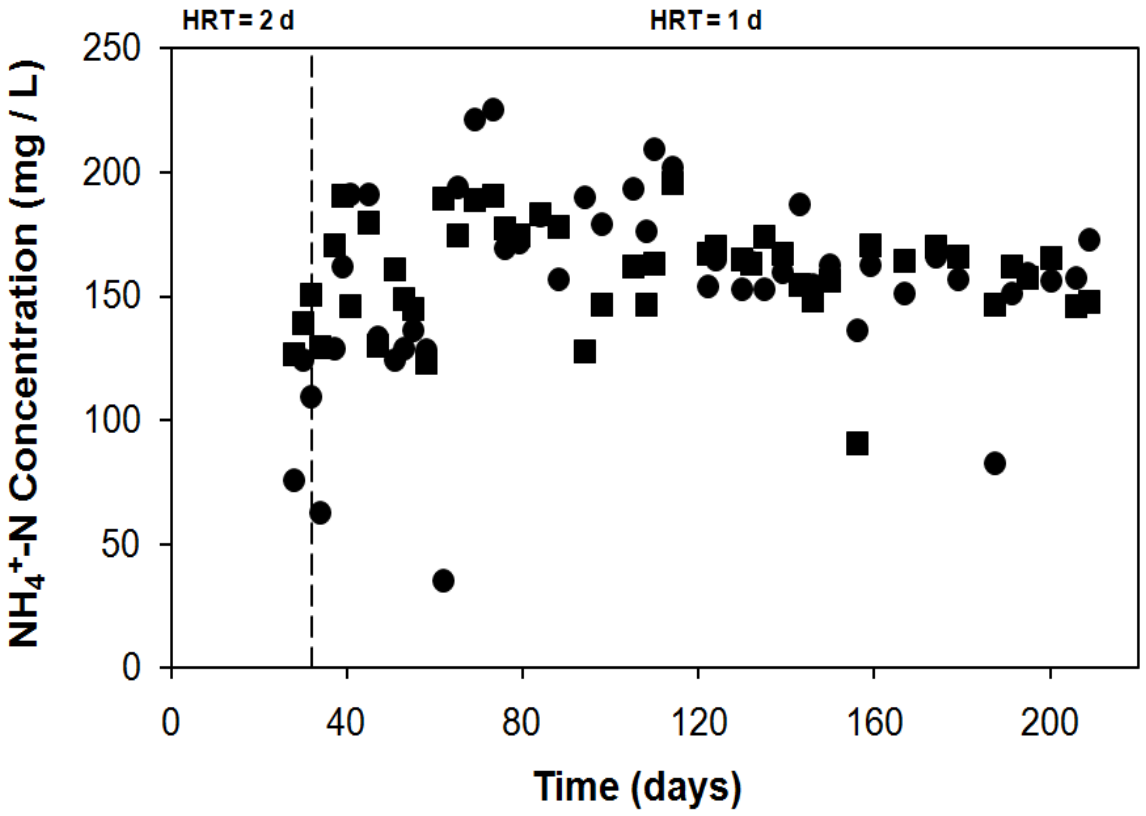

Figure 3.7. Fate of NH_4^+ during anaerobic treatment in the UASB reactor as function of time, where the influent is indicated by (■) and the effluent by (●).

3.4.2 DHS reactor

Figures 3.8b and 3.8c show that NO_3^- and NO_2^- were present only in the DHS effluent and not in the UASB effluent (fed to DHS). For the first 40 days, low NO_3^- content was observed in the effluent, and the average concentration was 11.3 $\pm$ 4.88 mg NO_3^--N L^{-1}. From day 90, NO_3^- formation started to increase until reaching a maximum during the last 30 days of operation of the reactor, with an average

concentration of 145.0 $\pm$ 5.70 mg NO_3^--N L^{-1}. NO_2^- showed the opposite behavior as observed in Figure 3.8c. High concentrations of NO_2^- were detected in the samples during the first 30 days; then NO_2^- content gradually decreased until day 134. After this point, NO_2^- was intermittently detected in the treated effluent at an average concentration equal to 1.45 $\pm$ 0.32 mg NO_2^--N L^{-1}. These data suggest that NO_2^- was the primary product of NH_4^+oxidation in the reactor; however there was a shift in the products of nitrification, with NO_3^- becoming the dominant the species produced over time. Table 3.3 shows the N balance for the DHS reactor. The table is divided in three periods of time based on the NH_4^+ removal efficiency of the reactor. The 11-day lag phase was not taken into account for this analysis. During the first period, from day 55 to day 98, NO_2^- was the main product detected of the nitrification. From day 105 to day 124, the main product shifted to NO_3^-. In the last period, from day 130 to day 209, the efficiency of the reactor remained fairly constant and NO_3^- was present at higher concentrations than NO_2^-. The overall mass balance indicated N loses during treatment as the content was always higher in the influent than in the treated effluent. This could have resulted from analytical errors or the presence of other byproducts, not measured in the samples. A small fraction of this difference could be attributed to the formation of anaerobic niches within the packed sponges which could have enhanced the complete denitrification of NO_3^- to nitrogen gas (N_2). The average COD concentration in the influent samples was found to be 0.05 $\pm$ 0.01 g COD L^{-1} which could have resulted in the formation of up to 8.75 mg N_2-N L^{-1}, assuming 100% efficiency of the denitrification process.

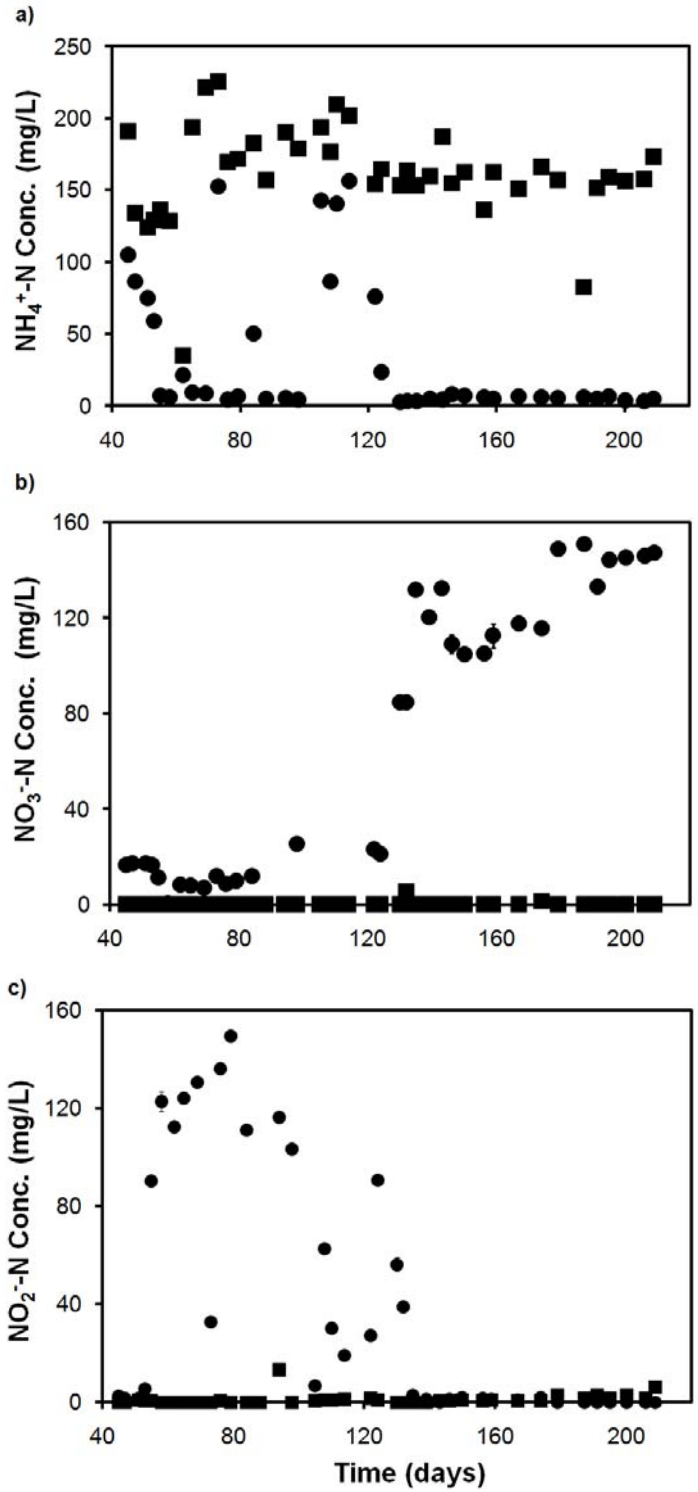

Figure 3.8. Fate of a) NH_4^+-N, b) NO_3^--N and c) NO_2^--N in the DHS reactor where the influent and effluent concentrations are indicated by (■) and (●), respectively.

Table 3.3. Nitrogen balance in the DHS reactor.

Day	***55 – 98***	***105 - 124***	***130 – 209***
N total In (mmol)	172.0	120.2	327.5
N total Out (mmol)	135.7	97.7	292.0
Removal of $NH4^+$ (%)	89.8 $\pm$ 1.7	36.6 $\pm$ 1.3	96.5 $\pm$ 0.1
Conversion to NO_3^- (%)	7.6 $\pm$ 2.3	14.1 $\pm$ 1.1	82.9 $\pm$ 8.8
Conversion to NO_2^- (%)	77.4 $\pm$ 7.9	20.1 $\pm$ 1.2	4.14 $\pm$ 1.2

3.5 Discussion

3.5.1 UASB reactor

The methanogenic reactor exhibited a high COD removal efficiency throughout the experiment despite the decrease in the HRT at day 31. Most of the organic load in the influent was converted to CH_4 gas, as indicated by CH_4 measurements and corroborated by mass balances (Table 3.2). Formation of CH_4 would have resulted from the conversion of VFA, phenol, and p-cresol to CH_4 by the combined activity of acetogenic and methanogenic microorganisms [43]. Conversion of phenol, and p-cresol, also present in

the synthetic leachate, would have depended on phenolic compound degrading bacteria converting the phenols to VFA and H_2 [44]. A small fraction of the CH_4 formed in the reactor could have resulted from the endogenous decay of sludge biomass. The COD data show that the organic contaminants in the leachate were efficiently removed. The capability of the UASB for removing the pollutants added to the synthetic leachate could be explained by the wide diversity of microorganisms present in anaerobic methanogenic sludges [45-47]. The increase of the OLR, from 1.04 $\pm$0.46 to 2.3 $\pm$ 0.56 g COD L^{-1} d^{-1}, did not seem to have an effect in the performance of the reactor since the average COD removal during the experiment was up to 95%, which demonstrates the capability of the UASB for treating the synthetic media. High COD elimination in laboratory-scale UASB reactors treating raw landfill leachate has been observed at similar ORLs, 2.1 and 2.3 g COD L^{-1} d^{-1} [48, 49] and at ORLs as high as 2.3 g COD L^{-1} d^{-1} [50].

The fate of the VFAs was monitored to obtain information about the behavior of each individual fatty acid during treatment. Figure 3.5 shows that, after an adaptation period, the concentration in the effluent decreased to very low values. In fact, some data points were below the detection limit of the analysis. The overall average VFA removal achieved in the UASB was 95% and the individual analysis of each of the VFA added to the synthetic leachate (not shown) demonstrated that most of the VFA content in the leachate after treatment corresponded to acetic acid. The presence of acetate could be the result of an inefficient degradation of the organic acid or a partial fermentation of the organic components of the leachate since acetate has been identified as a fermentation byproduct of low molecular weight organic molecules, including higher fatty acids,

fumarate, succinate, glycerol, lactate among others [51]. Propionic acid was intermittently observed in the samples which could indicate partial removal of the propionic acid or an incomplete acetogenesis of certain VFA in the synthetic media. However, its average concentration in the treated media was lower than 5 mg L^{-1}. Finally, valeric and butyric acid were below the detection limit in the effluent of the reactor.

The data obtained show that microorganisms in the reactor were capable of rapidly adapting to the presence of the phenolic compounds. Moreover, increasing the average phenol concentration in the influent from 41.2 $\pm$6.47 to 90.6 $\pm$ 8.43 mg L^{-1} and the p-cresol content from 39.6 $\pm$ 8.94 to 85.8 $\pm$ 6.52 mg L^{-1} did not impact the removal of such contaminants. In general, the degradability of phenol and p-cresol could be linked to the presence of the hydroxyl group in the molecule, as observed by Battersby [52].. Degradation of phenolics contaminants by digested sludge has been previously documented in batch experiments [32, 53-55]. Removal under continuous feeding conditions has been observed as well. High elimination of phenol and p-cresol in UASB reactors has been documented [56-58]. Data shows that after introducing the phenol and p-cresol into the treatment, bacteria enriched faster to the presence of phenol, as higher removal efficiencies were achieved for this compound after the first days. Interestingly, neither of these compounds were detected above the detection limit in the treated effluent after day 73, indicating a possible use of p-cresol as co-substrate after the feeding of these contaminants.

Lastly, the NH_4^+ concentration was monitored in influent and effluent of the UASB (Figure 3.7). The content of NH_4^+ in the samples remained fairly constant, indicating there is no mechanism of anaerobic removal of NH_4^+ in the absence of O_2 or NO_x^-. The minor fluctuations observed in the NH_4^+ content of the treated media could have resulted from the endogenous decay of biomass, which results in releasing of NH_4^+, or from the uptake of NH_4^+ due to cell yield, which would have caused a small increase in the effluent. The effluents of UASB reactors have been recommended to undergo post-treatment to achieve good nutrient removal [59].

3.5.2 DHS reactor

The effluent of the UASB was low in COD but rich in NH_4^+, thus it was treated in the DHS reactor with the main objective of oxidizing the high concentration of NH_4^+ present in the anaerobically pre-treated synthetic leachate. The data show that nitrifying microorganisms enriched rapidly since NH_4^+removal occurred quickly without much of a lag period before achieving excellent removal efficiency. The overall average elimination of NH_4^+ was up to 96% and the main by-products of the oxidation were NO_2^- and NO_3^-. These results coincide with those obtained by Tandukara et al [60], which demonstrated high NH_4^+ oxidation in a similar reactor. A N mass balance performed with the data obtained from the influent and effluent samples, demonstrated that after adaptation most of the NH_4^+ was partially oxidized to NO_2^- for a period of 43 days, indicating that NH_4^+ oxidizing microorganisms dominated during the first days of operation of the reactor. The

conversion of NH_4^+ decreased considerably in the following 26 days, but after day 130 high removal was obtained again. At this point NO_3^- was found at high concentrations in the effluent, whereas NO_2^- was hardly detected. The decrease in the performance of the DHS reactor at the middle of the experiment could have resulted from a switching in the bacterial communities that dominated in the reactor. *Nitrosomonas* and *Nitrobacter* species had been identified in sponges used in DHS reactors used for NH_4^+ removal [61]. Uemura et al [62] observed a succession of microorganisms related to *Nitrosomonas* and *Nitrobacter* in a DHS treating wastewater rich in nitrogen.

The activity of the nitrite-oxidizing bacteria increased with time, indicating the dependence of these organisms to ammonia-oxidizing bacteria. Evidence exists that nitrite-oxidizing organisms grow around ammonium-oxidizers in biofilms formed from wastewater [63]. To achieve complete nitrification, the NO_2^- formed from the oxidation of NH_4^+ must be further oxidized to NO_3^-. However, this is not always achieved and NO_2^- can accumulate. Partial nitrification to NO_2^- can result in the removal of N at reduced operational cost, since less O_2 would be required in the process. The presence of NH_4^+ and NO_2^- at low O_2 concentrations could enhance the anaerobic ammonium oxidation (ANAMMOX), which involves the utilization of NO_2^- as electron acceptor by microorganisms to promote the conversion of NH_4^+ to N_2 gas [64]. ANAMMOX results in N elimination without a denitrification post-treatment. DHS reactors could attain N removal by controlling the air flow to promote the formation of NO_2^-, instead of NO_3^-. Chuang et al [65] achieved partial nitrification by controlling the O_2 concentration a DHS reactor; however, a slight change in the supply of O_2 promoted complete nitrification.

Due to the extremely low organic carbon present in the influent media, carbon needed by the microorganisms must have been fixed from the bicarbonate in the leachate.

3.6 Conclusions

The anaerobic-aerobic system used in this study was suitable for treating a syntheting landfill leachate containing a high COD concentration, in the form of VFAs, phenol, and p-cresol, and high NH_4^+concentration. The organic loading feeding the UASB was efficiently eliminated from the beginning of the experiment, and was recovered in the form of CH_4 gas. The low concentrations of VFAs detected in the effluent corresponded mainly to acetate, probably resulting as a fermentation byproduct. However, the values were so low that removal exceeded 95%. Phenol and p-cresol proved to be degradable by methanogenic sludge and no toxic effects were observed. Microorganisms were capable to rapidly enrich to oxidize these pollutants enabling high efficiency from the start. Additionally, NH_4^+ was effectively removed in the DHS reactor. Its operation was divided in two periods, one dominated by ammonia oxidizers and another dominated by both ammonia and nitrite oxidizer microorganisms; however the efficiency during the whole operation time was high.

3.7 References

1. EPA. *Municipal Solid Waste (MSW) in the United States*. 2009 [cited 2010; Available from: http://www.epa.gov/epawaste/nonhaz/municipal/msw99.htm.
2. Slack, R.J., J.R. Gronow, and N. Voulvoulis, *Household hazardous waste in municipal landfills: contaminants in leachate.* Sci Total Environ, 2005. 337(1-3): p. 119-37.
3. Wilkins, K., *Volatile organic compounds from household waste.* Chemosphere, 1994. 29(1): p. 47-53.
4. Christensen, T.H., et al., *Biogeochemistry of landfill leachate plumes.* Applied Geochemistry, 2001. 16(7-8): p. 659-718.
5. Jimenez, L., R. Alzaga, and J.M. Bayona, *Determination of organic contaminants in landfill leachates: A review.* International Journal of Environmental Analytical Chemistry, 2002. 82(7): p. 415-430.
6. Kjeldsen, P., et al., *Present and long-term composition of MSW landfill leachate: A review.* Critical Reviews in Environmental Science and Technology, 2002. 32(4): p. 297-336.
7. Schwarzbauer, J., et al., *Occurrence and alteration of organic contaminants in seepage and leakage water from a waste deposit landfill.* Water Res, 2002. 36(9): p. 2275-87.

8. Fatta, D., A. Papadopoulos, and M. Loizidou, *A study on the landfill leachate and its impact on the groundwater quality of the greater area.* Environmental Geochemistry and Health, 1999. 21(2): p. 175-190.

9. Mor, S., et al., *Leachate characterization and assessment of groundwater pollution near municipal solid waste landfill site.* Environ Monit Assess, 2006. 118(1-3): p. 435-56.

10. Haarstad, K. and T. Maehlum, *Important aspects of long-term production and treatment of municipal solid waste leachate.* Waste Management & Research, 1999. 17(6): p. 470-477.

11. Plotkin, S. and N.M. Ram, *Multiple bioassays to assess the toxicity of a sanitary landfill leachate.* Arch Environ Contam Toxicol, 1984. 13(2): p. 197-206.

12. Atwater, J.W., et al., *Experiments using Daphnia to measure landfill leachate toxicity.* Water Research, 1983. 17(12): p. 1855-1861.

13. Mangimbulude, J.C., et al., *Seasonal dynamics in leachate hydrochemistry and natural attenuation in surface run-off water from a tropical landfill.* Waste Manag, 2009. 29(2): p. 829-38.

14. Christensen, T.H., et al., *Attenuation of landfill leachate pollutants in aquifers.* Critical Reviews in Environmental Science and Technology, 1994. 24(2): p. 119-202.

15. Reinhart, D.R. and F.G. Pohland, *The assimilation of organic hazardous wastes by municipal solid-waste landfills.* Journal of Industrial Microbiology, 1991. 8(3): p. 193-200.

16. Albaiges, J., F. Casado, and F. Ventura, *Organic indicators of groundwater pollution by a sanitary-landfill.* Water Research, 1986. 20(9): p. 1153-1159.

17. Reinhart, D.R., *A review of recent studies on the sources of hazardous compounds emitted from solid-waste landfills - a United-States experience.* Waste Management & Research, 1993. 11(3): p. 257-268.

18. Murray, H.E. and J.N. Beck, *Concentrations of Synthetic organic chemicals in leachate from a municipal landfill.* Environ Pollut, 1990. 67(3): p. 195-203.

19. Baun, A., et al., *Toxicity testing of organic chemicals in groundwater polluted with landfill leachate.* Environmental Toxicology and Chemistry, 1999. 18(9): p. 2046-2053.

20. Salem, Z., et al., *Evaluation of landfill leachate pollution and treatment.* Desalination, 2008. 220(1-3): p. 108-114.

21. Wiszniowski, J., et al., *Landfill leachate treatment methods: A review.* Environmental Chemistry Letters, 2006. 4(1): p. 51-61.

22. Renou, S., et al., *Landfill leachate treatment: Review and opportunity.* Journal of Hazardous Materials, 2008. 150(3): p. 468-493.

23. Alkalay, D., et al., *Anaerobic treatment of municipal sanitary landfill leachates: the problem of refractory and toxic components.* World Journal of Microbiology & Biotechnology, 1998. 14(3): p. 309-320.

24. Pavlostathis, S.G. and E. Giraldogomez, *Kinetics of anaerobic treatment - A critical review.* Critical Reviews in Environmental Control, 1991. 21(5-6): p. 411-490.

25. Kettunen, R.H. and J.A. Rintala, *Performance of an on-site UASB-reactor treating leachate at low temperature.* Water Research, 1998. 32(3): p. 537-546.

26. Sikkema, S.D., E.; Ahlert, R. and Kosson, D., *Identification and Quantification of Volatile Organic Species During Microbial Treatment of Leachate.* Environmental Progress, 1988. 7: p. 77-83.

27. Baun, A., et al., *Xenobiotic organic compounds in leachates from ten Danish MSW landfills - chemical analysis and toxicity tests.* Water Research, 2004. 38(18): p. 3845-3858.

28. Bryant, M.P., *Microbial methane production - theoretical aspects.* Journal of Animal Science, 1979. 48(1): p. 193-201.

29. Zinder, S.H., *Physiological Ecology of Methanogens*, in *Methanogensis: Ecology, Physiology, Biochemistry & Genetics* J.G. Ferry, Editor. 1993, Chapman and Hall, Inc.: New York. p. 128 - 205.

30. Smith, P.H. and R.A. Mah, *Kinetics of acetate metabolism during sludge digstion.* Applied Microbiology, 1966. 14(3): p. 368-&.

31. Healy, J.B. and L.Y. Young, *Cathecol and phenol degradation by a methanogenic population of bacteria.* Applied and Environmental Microbiology, 1978. 35(1): p. 216-218.

32. Boyd, S.A., et al., *Anaerobic biodegradation of phenolic-compounds in digested-sludge.* Applied and Environmental Microbiology, 1983. 46(1): p. 50-54.

33. Knoll, G. and J. Winter, *Anaerobic degradation of phenol in sewage-sludge - Benzoate formation from phenol and CO2 in the presence of hydrogen.* Applied Microbiology and Biotechnology, 1987. 25(4): p. 384-391.

34. Foresti, E., *Anaerobic treatment of domestic sewage: established technologies and perspectives.* Water Science and Technology, 2002. 45(10): p. 181-186.

35. Lettinga, G., et al., *Use of the upflow sludge blanket (USB) reactor concept for biological wastewater-treatment, especially for anaerobic treatment.* Biotechnology and Bioengineering, 1980. 22(4): p. 699-734.

36. Aiyuk, S., et al., *Anaerobic and complementary treatment of domestic sewage in regions with hot climates - A review.* Bioresource Technology, 2006. 97(17): p. 2225-2241.

37. Kennedy, E.J. and E.M. Lentz, *Treatment of landfill leachate using sequencing batch and continuous flow upflow anaerobic sludge blanket (UASB) reactors.* Water Research, 2000. 34(14): p. 3640-3656.

38. Verstraete, W. and P. Vandevivere, *New and broader applications of anaerobic digestion.* Critical Reviews in Environmental Science and Technology, 1999. 29(2): p. 151-173.

39. Tandukar, M., et al., *A low-cost municipal sewage treatment system with a combination of UASB and the "fourth-generation" downflow hanging sponge reactors.* Water Sci Technol, 2005. 52(1-2): p. 323-9.

40. Machdar, I., et al., *Combination of a UASB reactor and a curtain type DHS (downflow hanging sponge) reactor as a cost-effective sewage treatment system for developing countries.* Water Science and Technology, 2000. 42(3-4): p. 83-88.

41. Tawfik, A., A. Ohashi, and H. Harada, *Sewage treatment in a combined up-flow anaerobic sludge blanket (UASB)-down-flow hanging sponge (DHS) system.* Biochemical Engineering Journal, 2006. 29(3): p. 210-219.

42. Agrawal, L.K., et al., *Treatment of raw sewage in a temperate climate using a UASB reactor and the hanging sponge cubes process.* Water Science and Technology, 1997. 36(6-7): p. 433-440.

43. Stams, A.J.M., *Metabolic interactions between anaerobic-bacteria in methanogenic environments.* Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology, 1994. 66(1-3): p. 271-294.

44. Evans, W.C. and G. Fuchs, *Anaerobic degradation of aromatic-compounds.* Annual Review of Microbiology, 1988. 42: p. 289-317.

45. Fernandez, N., et al., *Analysis of microbial community during biofilm development in an anaerobic wastewater treatment reactor.* Microbial Ecology, 2008. 56(1): p. 121-132.

46. Diaz, E.E., et al., *Phenotypic properties and microbial diversity of methanogenic granules from a full-scale upflow anaerobic sludge bed reactor treating brewery wastewater.* Applied and Environmental Microbiology, 2006. 72(7): p. 4942-4949.

47. Macleod, F.A., S.R. Guiot, and J.W. Costerton, *Layered structure of bacterial aggregates produced in an upflow anaerobic sludge bed and filter reactor.* Applied and Environmental Microbiology, 1990. 56(6): p. 1598-1607.

48. Berrueta, J. and L. Castrillon, *Anaerobic treatment of leachates in UASB reactors.* Journal of Chemical Technology and Biotechnology, 1992. 54(1): p. 33-37.

49. Wang, P., et al., *Landfill leachate treatment with combined UASB and Fenton coagulation.* Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering, 2000. 35(10): p. 1981-1988.

50. Garcia, H., J.L. Rico, and P.A. Garcia, *Comparison of anaerobic treatment of leachates from an urban-solid-waste landfill at ambient temperature and at 35 degrees C.* Bioresource Technology, 1996. 58(3): p. 273-277.

51. Schink, B., *Energetics of syntrophic cooperation in methanogenic degradation.* Microbiology and Molecular Biology Reviews, 1997. 61(2): p. 262-&.

52. Battersby, N.S. and V. Wilson, *Survey of the anaerobic biodegradation potential of organic chemicals in digesting sludge.* Appl Environ Microbiol, 1989. 55(2): p. 433-9.

53. Young, L.Y. and M.D. Rivera, *Methanogenic degradation of 4 phenolic-compounds - Reply.* Water Research, 1987. 21(7): p. 859-861.

54. Szewzyk, U., R. Szewzyk, and B. Schink, *Methanogenic degradation of hydroquinone and catechol via reductive dehydroxylation to phenol.* Fems Microbiology Ecology, 1985. 31(2): p. 79-87.

55. Healy, J.B. and L.Y. Young, *Anaerobic biodegradation of eleven aromatic compounds to methane.* Appl Environ Microbiol, 1979. 38(1): p. 84-9.

56. Fang, H.H.P. and G.M. Zhou, *Degradation of phenol and p-cresol in reactors.* Water Science and Technology, 2000. 42(5-6): p. 237-244.

57. Chang, Y.J., N. Nishio, and S. Nagai, *Characteristics of granular methanogenic sludge grown on phenol synthetic medium and methanogenic fermentation of phenolic waste-water in a UASB reactor.* Journal of Fermentation and Bioengineering, 1995. 79(4): p. 348-353.

58. Kennes, C., R. Mendez, and J.M. Lema, *Methanogenic degradation of p-cresol in batch and in continuous UASB reactors.* Water Research, 1997. 31(7): p. 1549-1554.

59. Lettinga, G. and L.W.H. Pol, *UASB-process design for various types of wastewaters.* Water Science and Technology, 1991. 24(8): p. 87-107.

60. Tandukara, M., O. Akiyoshib, and H. Hidekia, *Nitrogen removal by down-flow hanging-sponge reactor operated as a post-treatment unit for a UASB treating domestic sewage.* Proceedings of the Water Environment Federation, 2007: p. 5296-5303.

61. Araki, N., et al., *Behaviors of nitrifiers in a novel biofilm reactor employing hanging sponge-cubes as attachment site.* Water Science and Technology, 1999. 39(7): p. 23-31.

62. Uemura, S., et al., *Removal of organic substances and oxidation of ammonium nitrogen by a down-flow hanging sponge (DHS) reactor under high salinity conditions.* Bioresource Technology, 2010. 101(14): p. 5180-5185.

63. Okabe, S., H. Satoh, and Y. Watanabe, *In situ analysis of nitrifying biofilms as determined by in situ hybridization and the use of microelectrodes.* Applied and Environmental Microbiology, 1999. 65(7): p. 3182-3191.

64. Strous, M., et al., *Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (Anammox) process in different reactor configurations.* Water Research, 1997. 31(8): p. 1955-1962.

65. Chuang, H.P., et al., *Effective partial nitrification to nitrite by down-flow hanging sponge reactor under limited oxygen condition.* Water Research, 2007. 41(2): p. 295-302.

CHAPTER 4

FATE OF CERIUM OXIDE (CeO_2) NANOPARTICLES DURING WASTEWATER TREATMENT

4.1 Abstract

Nanoparticles (NPs) are materials with at least one dimension of 100 nm, as established by the National Nanotechnology Initiative (NNI). Nano-sized inorganic oxides such as silica (SiO_2), ceria (CeO_2), and alumina (Al_2O_3) are used in many industrial processes, including catalysis, polymers, coatings and semiconductor manufacturing. These three inorganic oxides are on the Organization for Economic Co-operation and Development (OECD) list of priority nanomaterials for immediate testing. Little is known about the behavior of these contaminants in the environment and information on the stability of NPs in wastewaters is very scarce. The objective of this research was to investigate the fate of CeO_2 NPs during municipal wastewater treatment and elucidate the main mechanisms contributing to their removal. The study was conducted in a laboratory-scale activated sludge system (A/S) fed with municipal wastewater collected from a local treatment facility and a nano-sized CeO_2 dispersion (average primary particle size = 50 nm). Continuous bioreactor experiments were also conducted with a well-defined synthetic wastewater to study the contribution of specific wastewater components to NP (de)stabilization. The organic matter removal efficiency

was determined by monitoring the total as well as the soluble chemical oxygen demand (COD) in the influent and effluent. The effect of pH and organic matter on the aggregation, and possible sedimentation, of the particles was evaluated in batch experiments by dispersing the NPs in different matrices. Finally, the contribution of the biomass for the removal of CeO_2 during treatment was investigated in batch treatments in the presence and absence of biomass.

4.2 Introduction

The NNI defines NPs as materials with at least one dimension of 100 nm or less. In this size range, particles have physicochemical characteristics that differ from their bigger counterparts. Probably the most important feature of NPs is their large specific surface area and increased quantum effects [1]. As a consequence of these unique properties, NPs are being incorporated into numerous products and industrial processes. Cosmetics, health care products, food packing materials, clothing, tires, and many other consumer goods contain NPs. In 2006, 212 nano-enable consumer products were identified in a survey; three years later the number exceeded 1,000 and the trend continues to increase [2]. Despite the ever increasing commercial and industrial applications of NPs, little is known about their possible toxic effects and fate in the environment.

A significant fraction of engineered NPs can be expected to reach municipal wastewater treatment plants (MWWTPs). This was recently demonstrated based on a model that considered the fate of these materials once released from consumer products or industrial processes [3]. Evidence of the occurrence of NPs in treatment plants includes the finding of silver sulfide NPs in the final stage sludge from a full scale MWWTP [4]. The question to be asked is whether or not MWWTPs are suitable for removing these materials during treatment. Current MWWTPs were not designed to treat these emerging contaminants [5] and the detection of non-manufactured NPs, i.e. biogenic colloids, in treated wastewater could suggest the incapability of MWWTPs for removing such materials [6]. Additionally, titanium dioxide (TiO_2) NPs were detected in the treated effluent from several MWWTPs located in Arizona, California, Colorado, Iowa, Maryland, and New York [7]. The characteristics of the wastewater will have a great effect on the behavior of the NPs in the treatment plant. Many NPs do not dissolve in water; rather they form thermodynamically unstable colloidal dispersions whose stability depends on the pH and ionic strength (IS) of the wastewater.

Sorption onto primary and secondary sludge and entrapment into microbial floccules have been suggested as additional mechanisms of NP removal in MWWTP [8]. Extracellular polymeric substances (EPS) are the most important constituents of microbial floccules and they can behave as ligands, enhancing the attraction of charged particles [9]. However, [10] in one study reduced aggregation of TiO_2, ZnO, and CeO_2 NPs in the presence of naturally occurring organic matter (OM) was observed, and this finding was independent of the pH [11]. Certain NPs can bind to OM in the wastewater

[12, 13] due to their overall negative charge from the presence of carboxylic and phenolic functional groups.

Cerium (Ce) is the most abundant of the rare earths elements at a concentration of 60 parts per million in the Earth's crust [14]. It is a very strong oxidizing agent that becomes stabilized when bonded to oxygen [15]. The adverse effects of CeO_2 are still not well known. CeO_2 NPs showed limited toxicity to human mesothelioma and rodent fibroblast cell lines when incubated for 6 days at a concentration of 30 mg CeO_2 L^{-1} [16]. They caused 50% inhibition after a three day incubation period with such cells; yet activity remained fairly constant during the next following 3 days. Evidence of significant cytotoxicity and oxidative stress to human lung cancer cells by 20 nm CeO_2 NPs was documented by Lin et al [17]. Oxidative species were generated at all concentrations tested, ranging from 3.5 to 23.3 μg CeO_2 mL^{-1}, causing lipid peroxidation and cell membrane damage. However, Schubert et al [18] found that CeO_2 NPs could act as antioxidants, protecting nerve cells from oxidative stress. Antioxidant effects could result from special crystal defect structure. Hence, more research is needed to understand the toxicity of CeO_2 NPs to living organisms.

Exposure to Ce may occur mainly from cerium oxide (CeO_2) containing products, which include glasses, ceramics, televisions tubes, semiconductors, gasoline, etc. Incorporation of CeO_2 NPs in diesel engines can reduce particulate matter emissions [19]. CeO_2 NPs are used as an oxygen donating catalyst for the oxidation of carbon monoxide (CO) and hydrocarbons [20]. In the semiconductor industry CeO_2 NPs, together with aluminum oxide (Al_2O_3) and silicon dioxide (SiO_2) NPs, are employed for the

chemical-mechanical polishing (CMP) of wafers. Smooth wafer surfaces free of defect are achieved by CMP processes [21]. In 2003 the slurry market for CMP was estimated to be $400 million [22], and the annual average growth rate of the CMP global market during 2008 was 14.2% [23]. CeO_2 NPs are expected to be present in municipal and industrial wastewater since most of the engineered NPs are released to sewer systems [24].

Only a few studies exist related to the fate of NPs during wastewater treatment. Removal of bare and coated SiO_2 NPs in a laboratory-scale primary treatment was studied using unscreened and screened real domestic wastewater [25]. Coagulation and further sedimentation was only observed in Tween-coated particles. Limbach et al reported up to 94% removal of CeO_2 NPs during simulated secondary treatment when dispersing the material in synthetic wastewater [26]. Moreover NPs proved to be highly stabilized in the synthetic media. Elimination of TiO_2 NPs in sequencing batch reactor simulating secondary treatment was studied by Kiser et al [7]. An average elimination of 88% was observed when treating synthetic wastewater containing the NPs. Although these results provide a general understanding about the behavior of NPs during treatment, their short duration and the utilization of synthetic wastewater, instead of real domestic wastewater, might not represent accurately the conditions NPs would encounter under real conditions.

The scope of this work was to assess the behavior of CeO_2 NPs during secondary treatment in a bench-scale aerated activated sludge system over long term experiments with real domestic wastewater. CeO_2 was selected for this project due its low background concentration in wastewater, which facilitates its monitoring without interference of natural or anthropogenic occurring CeO_2. Stability of CeO_2 NPs in water and wastewater, as well as their sorption to biomass, was studied to insight on the dominant mechanisms of CeO2 removal.

4.3 Materials and Methods

The NPs were obtained as a fine powder with an average particle size (APS) of 50 nm in diameter according to the manufacturer (Sigma-Aldrich, St Louis, MO). However a transmission electron microscope image of the powder (Figure 4.1) shows particles as small as 35 nm in diameter.

4.3.1 CeO_2 stability in aqueous dispersions

The impact of pH and wastewater composition on the stability of CeO_2 NP dispersions was tested based on measuring particle aggregation and sedimentation. A two-step process was used to prepare the CeO_2 stock suspension. A concentrated stock suspension containing 4.07 g Ce L^{-1} (5.00 g CeO_2 L^{-1}) was prepared by dispersing the

NPs in 1 mM HCl and applying sonication for 10 min at 70% intensity to achieve a uniform dispersion (Daigger GEX130, 130W, Cole-Parmer Instruments, Vernon Hills, IL). Then, the stock was diluted in 1 mM HCl to 0.81 g Ce L^{-1} (1.00 g CeO_2 L^{-1}) and a pH of 3.10 $\pm$ 0.20. The diluted stock suspensions were diluted 11-fold to an intended concentration of 74.0 mg Ce L^{-1} (90.9 mg CeO_2 L^{-1}) with real domestic wastewater or synthetic wastewater adjusting the pH to 7.10, which was the original pH of the wastewater, as well as 11-fold dilution with Milli-Q water at pH 3.11 or 7.06 in 15 mL falcon tubes (BD Biosciences, Bedford, MA) in duplicate. Both real and synthetic wastewater samples were passed through 25 nm filters (Millipore.Billerica, MA) to avoid interference of suspended materials. A 1 mL sample was taken from the initial 11 mL content from the treatment containing Milli-Q water at pH 3.11 after vortex mixing the aqueous dispersions to determine average particle size (APS) and zeta-potential at the beginning of the experiment. Then the tubes were incubated for 24 h without any mechanical mixing. After incubation, 1 mL sample was taken from the upper 15% of supernatant of each assay to measure CeO_2 recovery. The Ce content in the supernatant was analyzed after digesting the samples.

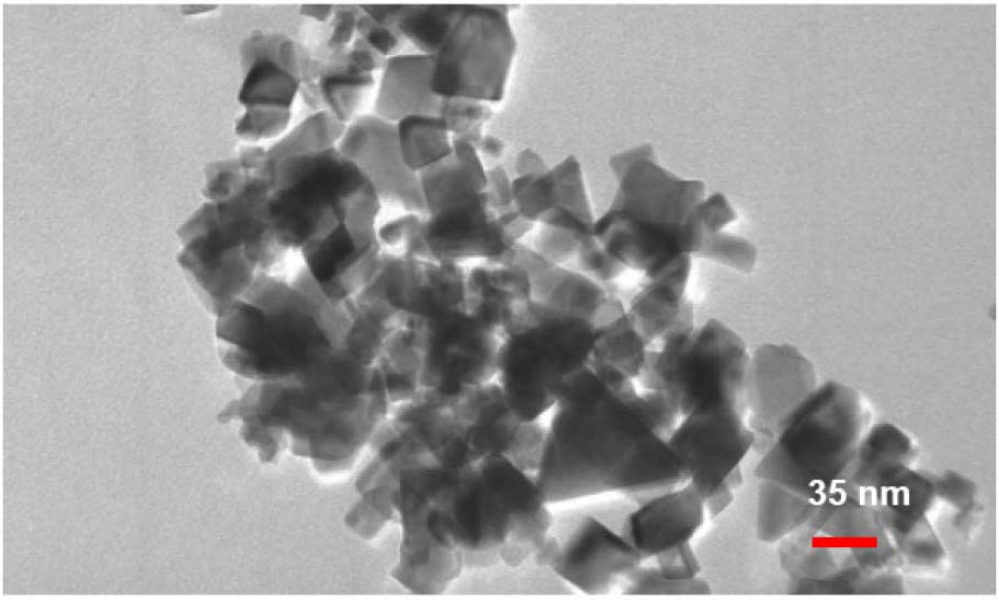

Figure 4.1. Transmission electron microscope image of nano-sized ceria with an average particle size 50 nm.

4.3.2 Laboratory-scale secondary treatment

The bench-scale aerobic activated sludge treatment system, constructed with clear acrylic plastic, consisted of an aeration tank connected to a settler with a working volume of 1.19 and 0.66 L, respectively (Figure 4.2). The laboratory-scale secondary treatment system operated at 27.5 ºC and was supplied with wastewater at a steady volumetric flow rate set to 2.77 $\pm$ 0.09 L d^{-1} corresponding to a hydraulic retention time (HRT) of 10.3 $\pm$ 0.32 h in the aeration tank. The average flow leaving the settler was 2.66 $\pm$ 0.21 L d^{-1} equal to an HRT of 6.50 $\pm$ 0.92 h (Table 4.1). The flow rate was

measured by collecting a defined volume of influent and effluent during a determined time. The aeration tank was seeded with returned activated sludge (RAS) at a volatile suspended solids (VSS) concentration of 3.50 g VSS L^{-1}. Two air pumps (Aqua, Walmart) provided air at a rate of 430 L d^{-1} and mixing for the aeration tank and recirculation of the settled sludge. The laboratory-scale secondary treatment system was operated in two different experiments. In the first experiment the reactor system was fed with synthetic wastewater for 28 days (experiment I). CeO_2 NPs were introduced into the influent after 13 days of operation and they were supplied until the end of the experiment, 13 days more. The synthetic wastewater consisted of (g L^{-1}): peptone (0.220), meat extract (0.150), urea (0.010), K_2HPO_4 (0.008), $NaHCO_3$ (0.400). The pH was adjusted to 7.00 $\pm$ 0.10 before adding the sodium bicarbonate. The average pH of the wastewater during the operation of the reactor was 7.68 $\pm$ 0.15 and the total chemical oxygen demand (COD) content was equal to 371.7 $\pm$ 33.03 mg COD L^{-1}. In the second experiment the reactor was fed with real domestic wastewater for 70 days (experiment II). In this case the feeding of the NPs lasted for 57 days, as they were added at day 7. Real domestic wastewater was collected after primary sedimentation from Roger Road, a local MWWTP located in Tucson, AZ. The average wastewater pH was found to be 7.71 $\pm$ 0.14. The wastewater contained an average COD concentration of 247.4 $\pm$ 50.39 mg COD L^{-1}. The CeO_2 NPs were introduced into the system via diluted stock dispersion at a concentration equal to 0.81 g Ce L^{-1} (1 g CeO_2 L^{-1}). The stock was continuously stirred to avoid agglomeration of the NPs and combined with wastewater at a volumetric ratio of 1:10 at the entrance of the aeration tank.

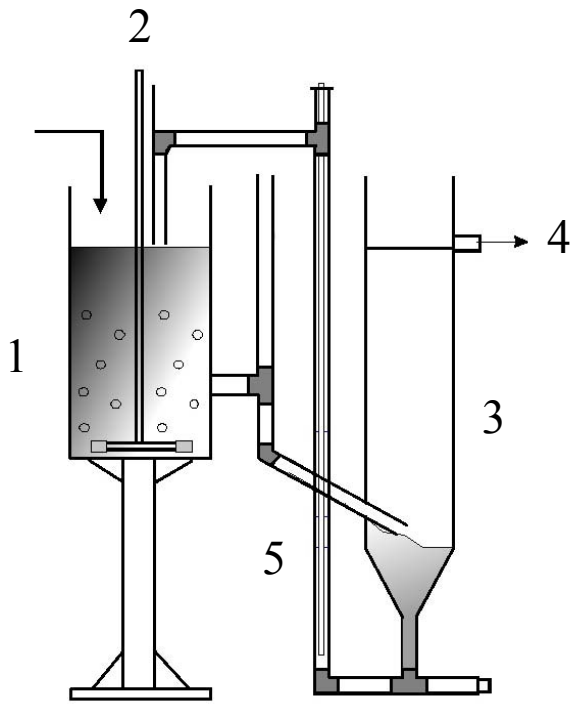

Figure 4.2. Schematic of the laboratory-scale secondary treatment system used. (1) Aeration tank, (2) air, (3) settler, (4) effluent, (5) recycled sludge.

Table 4.1. Operation parameters of the laboratory-scale activated sludge system fed with synthetic and real wastewater.

Parameter	Synthetic wastewater	Real wastewater
Operation time (days)	27	65
Θ in the aeration tank (h)	10.58 (± 0.54)	10.11 (± 0.96)
Average pH In	7.68 (± 0.14)	7.41 (± 0.14)
Average pH Out	7.05 (± 0.63)	7.05 (± 0.42)
Mixed liquor volatile suspended solids concentration (g/L)	3.13 (± 0.74)	3.23 (± 0.24)

Samples were taken from the influent and effluent of the settler to follow the removal of the organic content of the wastewater by measuring the total and soluble COD and acetate concentration. The pH of the samples was monitored as well as it is an important variable that can affect the microbial activity in the secondary treatment. To maintain a fairly constant biomass concentration in the reactor 90 mL of sludge were taken from the aeration tank every 3 days, which were replaced with wastewater. Biomass concentration was determined by measuring the VSS content of the samples. The fate of CeO_2 NPs during treatment was followed by measuring the total and filtered Ce concentration in the samples, including the diluted stock dispersion. Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) analysis were performed to confirm the presence and absence of Ce in selected samples.

4.3.3 Batch adsorption experiments

The contribution of the sludge biomass to the removal of NPs was studied in a sorption experiment. For this purpose, treatments were set up at pH 3.0 and 6.0 in duplicated 50-mL falcon tubes. The treatments consisted of MilliQ-water alone, MilliQ-water with washed sludge at a VSS concentration of 3.50 g VSS L^{-1}, as well as the supernatant from the MilliQ-water with washed sludge. The sludge was washed four times by mixing it with Milli-Q water and centrifuging the mixture afterwards at 4000 rpm for 30 min. The intended Ce concentration added to the treatments was set to 81.40 mg Ce L^{-1} (100.0 mg CeO_2 L^{-1}). The treatment containing of Milli-Q water was

sampled right after diluting the stock to establish the initial dispersed Ce concentration. The tubes were continuously mixed for 15.23 h and then incubated for 4.83 h under stationary conditions to emulate the secondary treatment (10.3 and 6.50 h HRT in aeration basin and settler, respectively). Finally the supernatant from each treatment was sampled and digested to measure the total Ce concentration remaining in suspension. CeO_2 sorbed onto sludge was estimated by comparing the Ce content in the supernatant of each treatment.

4.3.4 Analytical procedure

Total Ce concentration was measured in the samples taken from the stability test, the laboratory-scale secondary treatment, and sludge biomass sorption experiment. For this purpose samples were digested in an automated microwave system (MDS2100, CEM Corp., Matthews, NC) in a solution containing 8 mL of HNO_3 (69%) and 2 mL of H_2O_2 (30%) for 30 min at 70 PSI. After digestion, samples were diluted in Milli-Q water and analyzed in an inductively coupled plasma – optical emission spectrometer (ICP-OES, Optima 2100 DV, Perkin Elmer, Waltham, MA) at 413 nm wavelength. Filtered samples were passed through 200 nm (Whatman. Piscataway, NJ) and 25 nm (Millipore.Billerica, MA) filters and then amended with a drop of HNO_3 concentrated to preserve the samples. The resulting filtrate was diluted with 4% HNO_3 (v/v) before analysis in an ICP-OES.

Particle size (PS) and zeta-potential were measured by light scattering using a Zeta Sizer Nano ZS (Malvern, Inc., Westborough, MA). For this purpose a capillary

vessel was filled with 1 mL sample, carefully removing any air bubble trapped in the capillary. The temperature of the analysis was 25^{o}C and Milli-Q water was used as dispersant. The refractive index of CeO_2 was set equal to 1.828.

The pH of the samples and stock dispersions were measured using a pH probe (Orion-Thermo Scientific, Cincinnati, OH) calibrated before measurements with certified standards (Orion-Thermo Scientific, Cincinnati, OH). Ce and acetate determinations were quantified by analyzing standards of known concentrations together with the samples.

4.3.5 Electron microscopy

Preparation and analysis of the samples taken from the aeration tank after being exposed to CeO_2 was performed at the University spectroscopy and imaging facilities (USIF) at the University of Arizona. The samples were provided to USIF in aqueous solution and without addition of any preservative. Formaldehyde was added as a fixative at 50% (v/v) ratio 4 h before the preparation of the samples. Later the specimens were vacuum dried and hand broken into small pieces forming a powder-like material, which was placed and glued in a metallic base. Finally, the samples were coated with gold (Au) and palladium (Pd). The coating serves as a protective barrier from the electron beam at which the samples are subjected when using these techniques. Electron dispersive spectroscopy (EDS) was used to prove the presence of Ce in the samples.

4.3.6 Organic content of the wastewater and biomass concentration

The total COD concentration in the liquid samples was measured by mixing 2.5 mL of sample with 1.5 mL of digestion solution and 3.5 mL of a sulfuric acid (H_2SO_4) solution. The digestion solution contained (g L^{-1}): $H_2Cr_2O_7$ (10.27), Hg_2SO_4 (33.30), and H_2SO_4 (167 mL L^{-1}). The H_2SO_4 solution was prepared by dissolving10.25 g of Ag_2SO_4 in 1 L of concentrated acid. The resulting mixture was digested for 2 h at 150 ºC. Lastly, the absorbance of the digested samples was analyzed in a spectrophotometer (Beckman-Coulter, Fullerton, CA) at a 600 nm wavelength. The soluble COD fraction was determined following the same procedure after centrifuging the samples for 10 min at 10,000 rpm.

The fate of acetate in the system was monitored as well. After centrifugation of the influent and effluent samples, 1 mL of the supernatant was transferred into 1.5 mL glass vials and amended with 0.5 μL of concentrated formic acid. The acetate content of the samples was measured in a 7890A Agilent Technologies gas chromatograph (GC) (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector (FID) and a 30 m x 0.53 mm Stabilwax-DA column (Restek, Bellefonte, PA). The temperature at the inlet was set to 250ºC and 275ºC for the detector. The initial temperature in the oven was gradually increased from 90ºC to 140ºC at a rate of 10ºC per minute. Samples were injected using an autosampler applying a 6:1 split ratio. Ultra high purity helium was used as carrier gas at a flow rate of 16 mL min^{-1}.

Biomass content in the reactor was determined by measuring the VSS concentration of the samples. For this purpose 20 mL of the sample were filtered through 450 nm glass fiber filters (Whatman. Piscataway, NJ) applying vacuum. Filters containing the retentate were placed in aluminum vessels (Fisher Scientific, Pittsburgh, PA) and dried overnight at 105^{o}C. Finally, the dried material was ashed in a muffle furnace at 550^{o}C for 4 h.

4.4 Results

4.4.1 Stability of CeO_2 NPs

The stability of CeO_2 NPs was studied in aqueous dispersions at different pH values and organic composition. Figure 4.3a shows the APS of the NP dispersions in the assays before and after 24 h incubation without mixing. Initial samples were taken from the bulk after diluting the NP stock in the aqueous treatments. After incubation, samples were taken from the overlying supernatant. At pH 3.11, NPs were stable as demonstrated by the small average particle size of the suspension, which remained constant over time. The average hydrodynamic diameter of the particles measured at the beginning and end of the test was 147.7 $\pm$ 2.17 nm. Destabilization was observed at circumneutral pH. Aggregation of NPs in Milli-Q water at pH 7.06 was evident.

The APS measured after dilution of the stock, 782.7 ± 263.2 nm, was up to five times greater than that obtained at pH 3.11. The diameter of the particles that remained suspended in the supernatant after incubation was smaller than those present in the bulk at the beginning of the experiment. Surprisingly, the effect of pH on CeO_2 NPs was overcome when diluted in synthetic wastewater. In the presence of the synthetic wastewater, the particles behaved similarly as those in Milli-Q water at low pH. The suspension was very stable since the small average hydrodynamic particle size of 157.8 ± 2.62 nm was sustained over time. In contrast, the presence of real wastewater caused the NPs to readily aggregate and the measured APS increased considerably to the micron range. The average diameter of the particles exposed to real wastewater was 2,457 ± 211.7 nm.

The zeta-potential exhibited by the particles in the different treatments is presented in Figure 4.3b. The data obtained show that at higher absolute values (either positive or negative), dispersions become more stable. The average zeta-potential over the 24 h period in the treatment consisting of Milli-Q water at pH 3.11 was equal to 45.8 ± 2.88 mV, whereas that of the synthetic wastewater was -32.6 ± 0.30 mV.

Agglomeration was enhanced as the absolute value of the zeta-potential of the particles decreased, as demonstrated by the increased aggregation observed in the real domestic wastewater and Milli-Q at pH 7.06 treatments, which showed an average zeta-potential closer to zero. Results indicated that the charge of the particles play a major role over stability. Nevertheless, other factors may affect the agglomeration of the particles as

the APS was up to four times higher in real domestic wastewater compared to the Milli-Q water treatment at pH 7.06.

The residual CeO_2 NPs was monitored by measuring the Ce in the supernatant of each assay after incubation (Figure4.4). The designed total Ce concentration in the treatments was 74.0 mg Ce L^{-1} (90.9 mg CeO_2 L^{-1}). The treatment consisting of Milli-Q water at pH 3.11 was sampled from the bulk at the beginning of the experiment and digested in order to determine the maximum Ce content that could be expected in the supernatant of each treatment, which was found to be 71.8 $\pm$ 0.53 mg Ce L^{-1} (88.2 CeO_2 L^{-1}). This measurement was set as the control. The same treatment was sampled 24 h later and found that NPs remained in suspension over time as indicated by the Ce content in the supernatant, 74.0 $\pm$ 8.82 mg Ce L^{-1} (90.9 $\pm$ 10.8 mg CeO_2 L^{-1}), which matched the one obtained for the controls (Figure 4.4). Neutral pH values promoted the sedimentation of the particles suspended in clean water. The Ce concentration in the supernatant of Milli-Q water at pH 7.06 after incubation decreased significantly when compared to the Milli-Q treatment at pH 3.11. The average Ce concentration in the supernatant was 25.0 $\pm$ 6.14 mg Ce L^{-1} (30.7 $\pm$ 7.54 mg CeO_2 L^{-1}), indicating that the majority of the particles, approximately 65.5%, settled during the test. However the effect of the pH was overcome to some extent when CeO_2 NPs were diluted in synthetic wastewater at pH 7.10 as was observed by the high content of Ce in the samples. The residual Ce concentration in that treatment was 90.2% based on measuring 65.8 $\pm$ 2.16 mg Ce L^{-1} (80.8 $\pm$ 2.65 mg CeO_2 L^{-1}) in the supernatant.

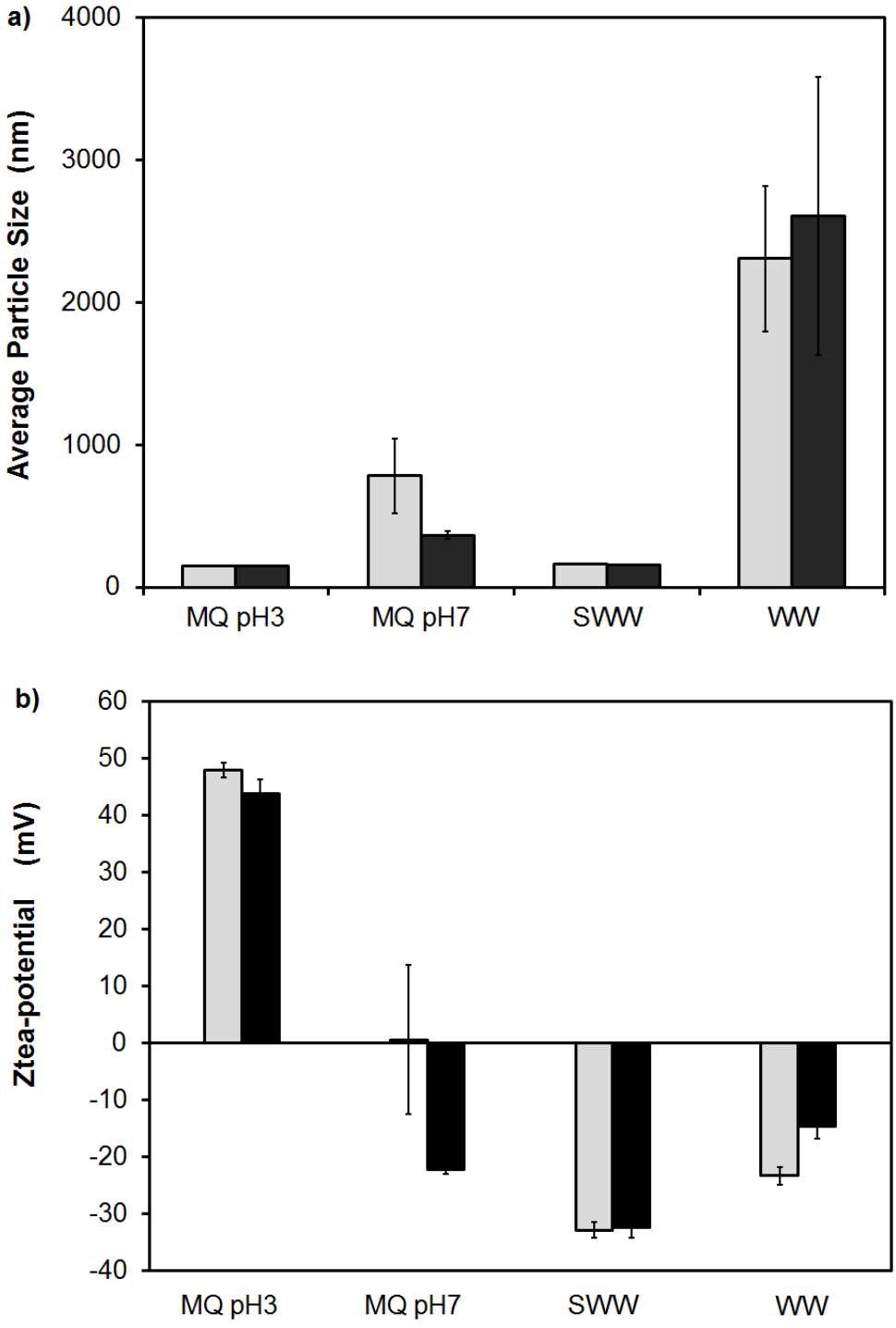

Figure 4.3. a) Particle size distribution and b) zeta-potential of CeO_2 in different matrices at t=0 (■) and t=24 h (■) incubation in MQ = Milli-Q water (pH 3.11 and 7.06); SWW = Synthetic wastewater (pH 7.10); WW = Real wastewater (pH 7.09).

In the case of real wastewater most of the aggregates formed readily sedimented rather than stay in suspension. Only a small fraction from the total Ce initial concentration, 5.70%, did not sediment, as the concentration of Ce measured in the samples was equal to an average 4.15 $\pm$ 3.38 mg Ce L^{-1} (5.10 $\pm$ 4.15 mg CeO_2 L^{-1}). In general, the residual Ce concentration in the supernatant followed the behavior observed in terms of particle size for each treatment. Suspensions with smaller aggregate sizes resulted in higher stability of the Ce concentration during incubation.

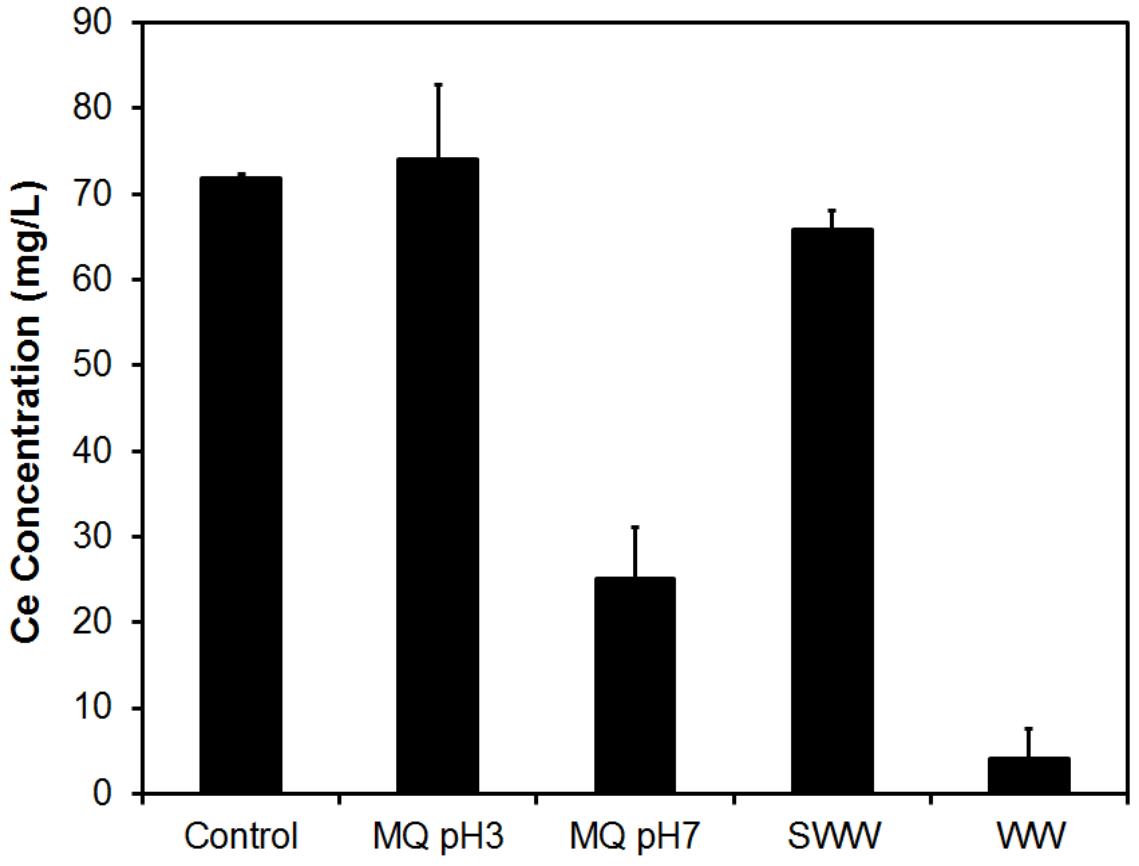

Figure 4.4 Ce concentration in the supernatant from different matrices after t =24 h incubation. Control = Milli-Q water at t=0 (pH 3.11); MQ = Milli-Q water (pH 3.11 and 7.06); SWW = Synthetic wastewater (pH 7.10); WW = Real wastewater (pH 7.09).

4.4.2 Removal of CeO_2 NPs from synthetic wastewater during activated sludge treatment

The fate of CeO_2 NPs during secondary treatment was studied using a laboratory-scale activated sludge system. The reactor was fed with synthetic wastewater for 13 days without adding CeO_2 to allow stabilization of the system. Afterwards NPs were continuously amended to the influent wastewater to achieve a designed CeO_2 NP concentration of 90.9 mg L^{-1} (74.0 mg Ce L^{-1}). NPs were fed for the following 13 days, from day 13 to day 26. Figure 4.5a shows the fate of total Ce in the reactor. The total average Ce concentration measured in the influent was equal to 69.9 $\pm$ 20.3 mg Ce L^{-1} whereas the average concentration detected in the effluent was 3.94 $\pm$ 1.73 mg Ce L^{-1}. The average removal of the total Ce during secondary treatment was 94.4%, indicating that the activated sludge treatment was effective in removing the majority of the CeO_2 supplied to the system. The efficiency of the treatment for removing small particles was studied by filtering the samples using 200 nm filters. Figure 4.5b illustrates that only a small fraction of the suspended CeO_2 particles entering the reactor were smaller than 200 nm. Their average concentration in the influent was 0.64 $\pm$ 0.40 mg Ce L^{-1} (0.78 $\pm$ 0.49 mg CeO_2 L^{-1}), representing less than 1.0% of the CeO_2 entering the reactor. After treatment, the content of these particles in the effluent was found to be 0.13 $\pm$ 0.08 mg Ce L^{-1}, which represents an overall removal of 78.9% of Ce in particles $<$ 200 nm. Although the $<$ 200 nm size fraction of CeO_2 particles were significantly removed in the bench-scale secondary treatment system, an appreciable fraction of around 21.1% did

escape the treatment. The synthetic wastewater may represent the COD concentration encountered in real domestic wastewater; however it does not properly represent the complex OM and other suspended solids that could potentially affect the behavior of NPs in the activated sludge treatment, as was observed during the stability test.

4.4.3 Fate of CeO_2 NPs during activated sludge treatment fed with municipal wastewater

In order to assess the fate of the CeO_2 NPs in the secondary treatment with complex OM, the reactor was stopped, seeded with fresh sludge, and fed with real domestic wastewater for 65 days. The designed concentration of CeO_2 NPs in the influent wastewater was set to 90.9 mg CeO_2 L^{-1} (74.0 mg Ce L^{-1}) as when done feeding synthetic wastewater. Figure 4.6a shows that the influent contained an average total concentration of 54.9 $\pm$ 12.8 mg Ce L^{-1} (67.5 $\pm$ 15.7 mg CeO_2 L^{-1}). The discrepancy between the designed and measured Ce content could be attributed to sedimentation of CeO_2 in the influent line. Most of the particles that reached the secondary treatment were removed from the wastewater during treatment. The average concentration of total Ce leaving the settler was 1.83 $\pm$ 1.42 mg Ce L^{-1}, achieving an average removal of approximately 96.6%, providing evidence that the activated sludge system was suitable for treating real wastewater containing CeO_2. The fate of the particles in size fractions smaller than 200 nm and 25 nm was monitored by serial filtrations, to study the efficiency of the treatment for eliminating the small aggregates and primary particles. The fraction of the

total CeO_2 particles passing through the 200 nm membrane filter in the influent was 14.4%. Figure 4.6b shows their behavior in the treatment. The average removal achieved was equal to 98.6% based on the influent and effluent concentrations of < 200 nm CeO_2 size fraction which corresponded to 7.94 ± 5.94 and 0.12 ± 0.11 mg Ce L^{-1}, respectively. Most of these particles were actually smaller than 25 nm as they accounted for up to 94.2% of the < 200 nm CeO_2. Hence, the content of < 25 nm CeO_2 NPs in the samples was very similar to that observed for the < 200 nm particles (Figure 4.6b). The average removal achieved for these NPs was 92.3%, which shows that the secondary treatment system was highly efficient for eliminating such small materials. A sludge sample taken from the aeration tank after being exposed to CeO_2 for 56 days was imaged using SEM (Figure 4.7a). The image shows the presence of bacteria, protozoa, and extracellular products. The presence of Ce in the sludge sample was confirmed by EDS analysis (Figure 4.7b) of the white square marked in Figure 4.7a. The spectrum also shows high counts of oxygen and carbon which could be attributable to the presence of microbial biomass in the sludge.

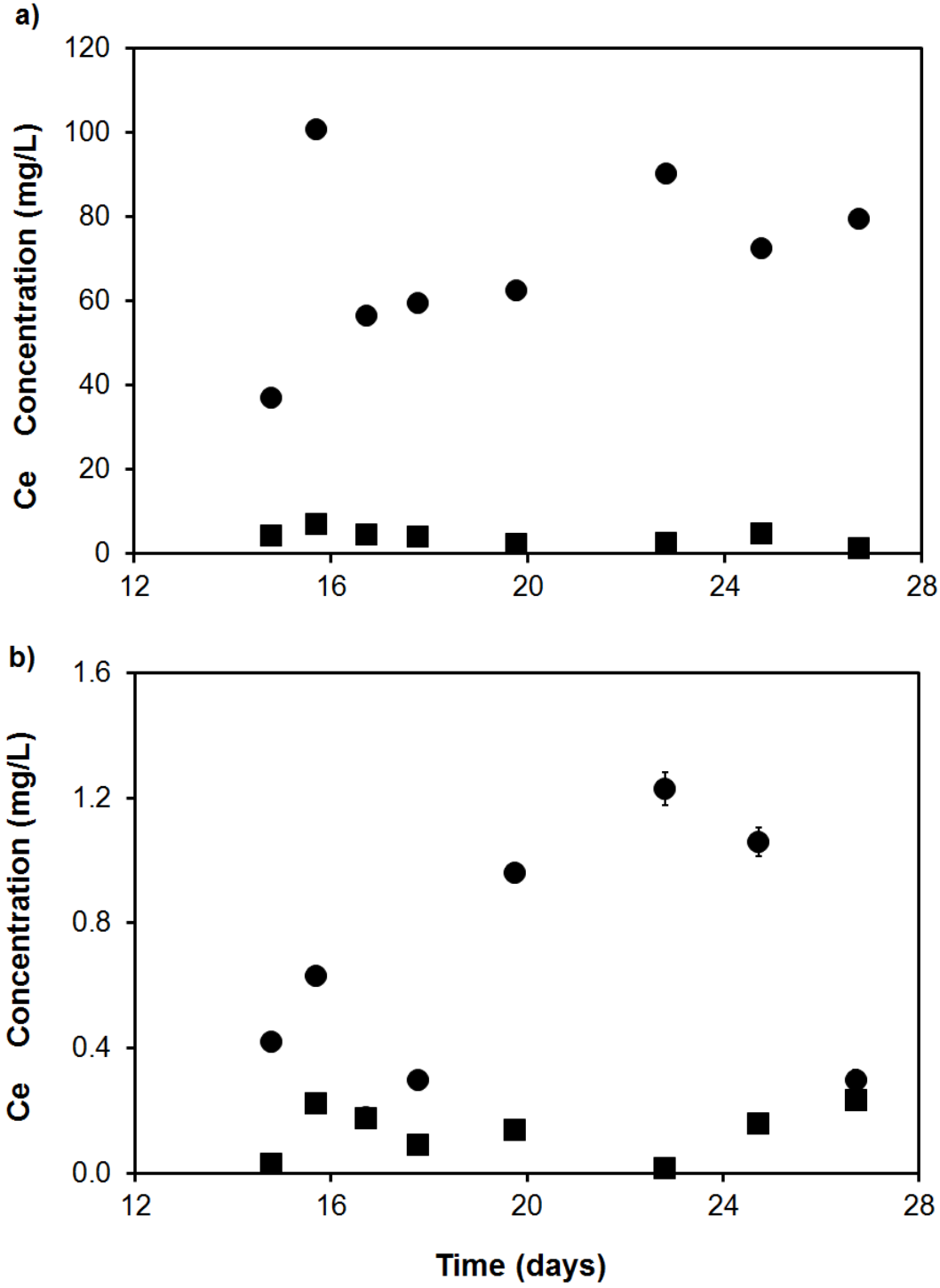

Figure 4.5. Fate a) total and b) < 200 nm Ce in the influent (●) and (■) effluent samples when feeding synthetic wastewater to the secondary treatment.

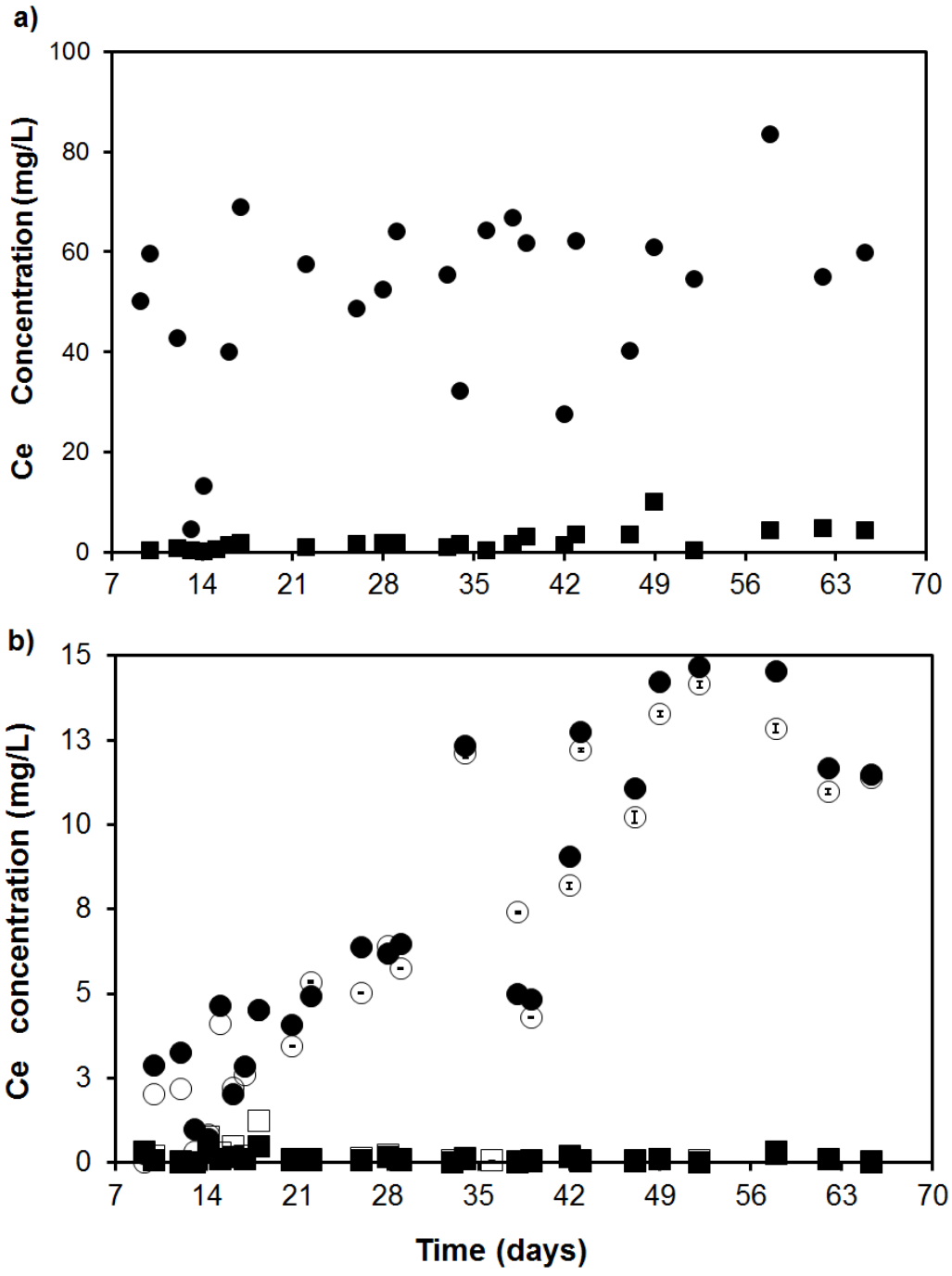

Figure 4.6. Concentration of a) total unfiltered and b) filtered Ce in the (●) influent and (■) effluent of activated sludge treatment operated with real domestic wastewater. Bold markers in second panel indicate CeO_2 < 200 nm, while empty markers refer to CeO_2 < 25 nm.

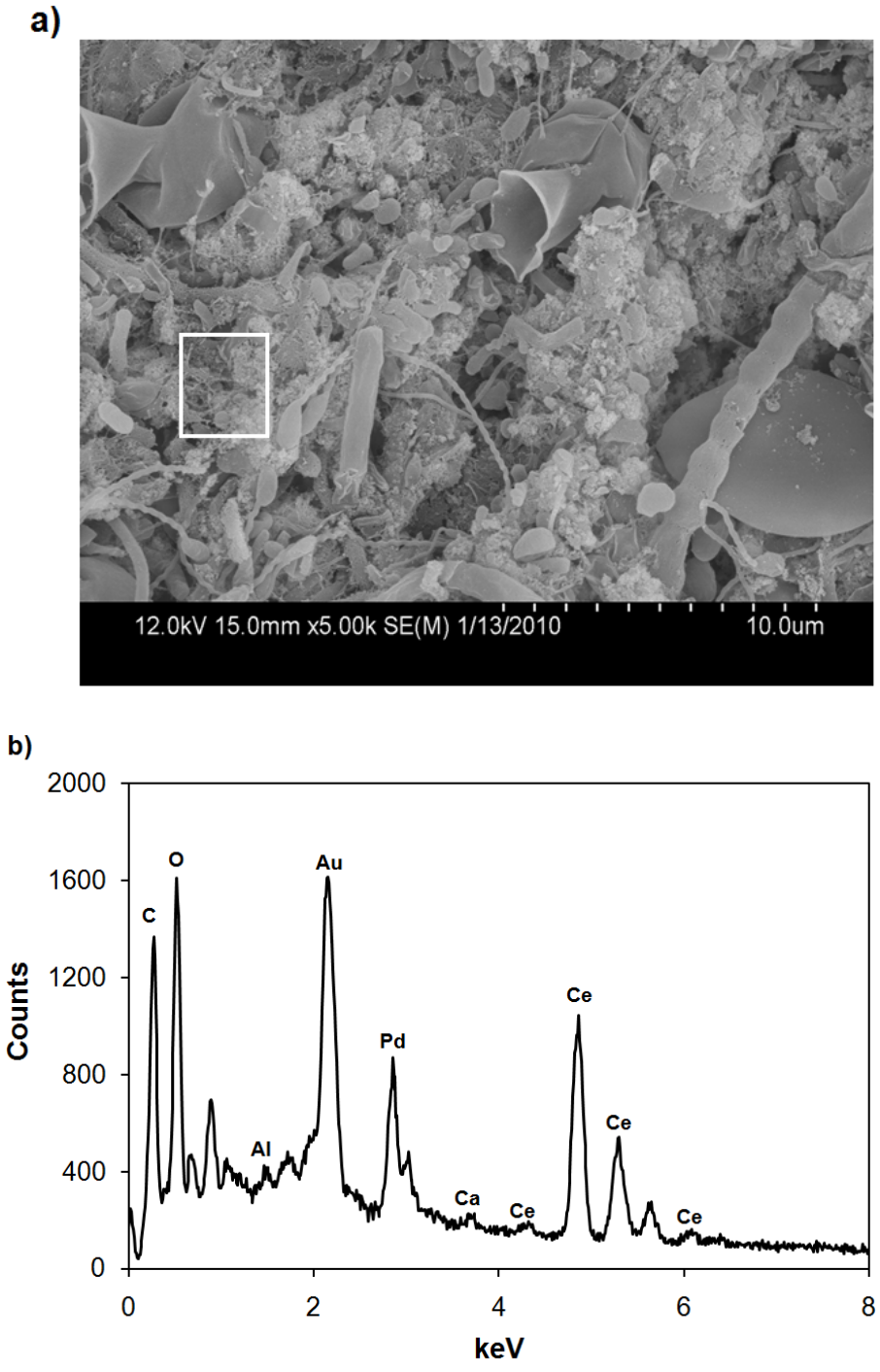

Figure 4.7. a) SEM image of a sample from the aeration tank after being fed with CeO_2 for 2 months and b) EDS analysis of the sample. The white rectangle in the image indicates the section of the sample where the EDS analysis was performed. Accelerating voltage: 12.0 kV.

4.4.4 Contribution of biomass to CeO_2 removal during activated sludge secondary treatment

The role of sludge biomass on the removal of CeO_2 NPs was studied, at low and neutral pH, in batch experiments that resembled the mixing and settling conditions of the laboratory-scale secondary treatment (Figure 4.8). Samples taken from the treatments with Milli-Q water at pH 3.05 and 6.02 after dispersing the CeO_2 stock were used as controls to determine the maximum Ce content expected in the supernatant of the treatments at the end of the experiment. Ce concentration from these samples was found to be 87.6 $\pm$ 0.11 mg Ce L^{-1} at pH 3.05, and 81.6 $\pm$ 2.97 mg Ce L^{-1} at pH 6.02 which was close to the designed concentration of 81.4 mg Ce L^{-1}. The results in the figure indicate that NPs remained stable over time at low pH and nearly half sedimented at circumneutral pH when dispersed in Milli-Q water, which coincided with the behavior observed in the stability test. The presence of washed sludge biomass overcame the stabilizing effects of pH, as contact with CeO_2 NPs resulted in large and significant decrease in Ce concentration in the supernatant. A similar residual Ce content in the supernatant was measured in this treatment at pH 3.03 (2.50 $\pm$ 0.66 mg Ce L^{-1}) and at pH 5.9 (4.93 $\pm$ 1.11 mg Ce L^{-1}). This may be due to sorption onto biomass; however a large decrease in Ce content can also be caused by the destabilizing effect of soluble organics released from the sludge on the nano-dispersion, as observed in the treatments containing only sludge supernatant. The contribution of the sludge supernatant to the agglomeration of the particles also increased with pH, as shown in Figure 4.8.

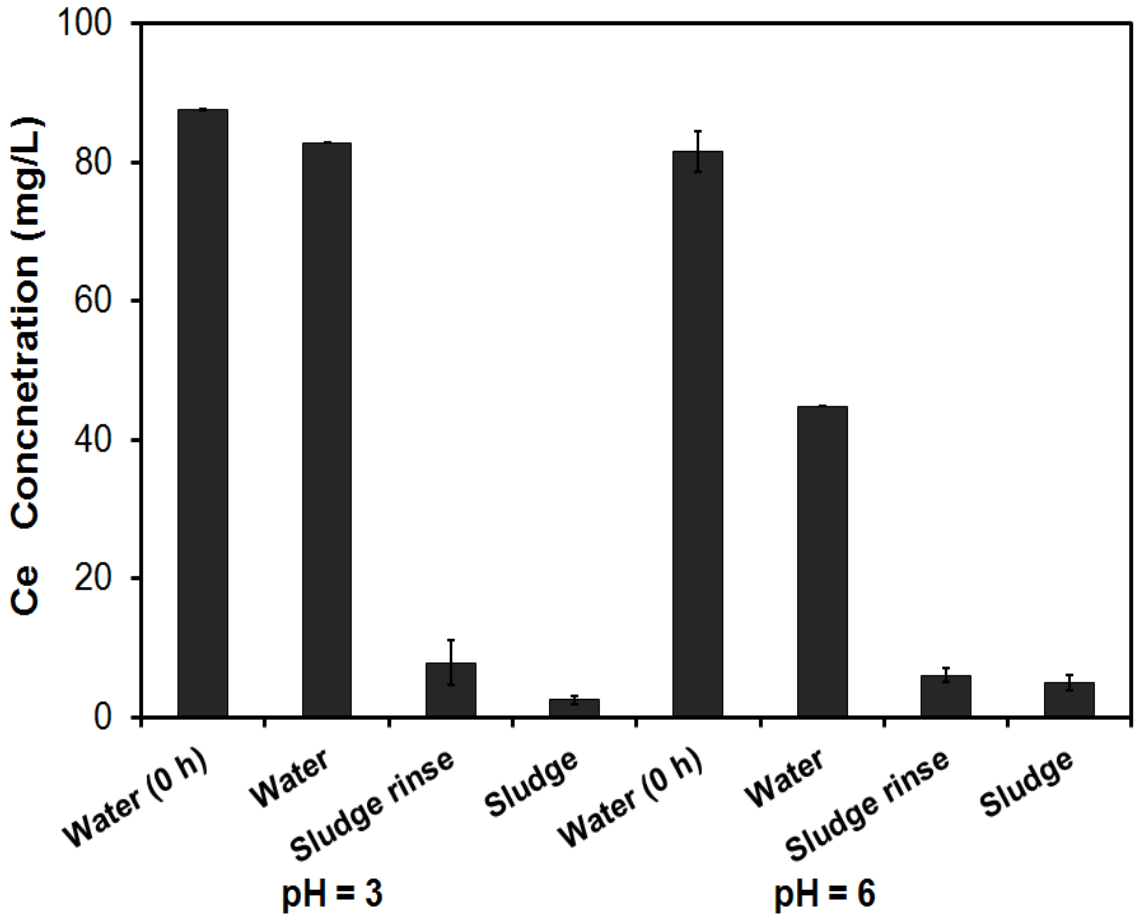

Figure 4.8. Sorption of CeO_2 to biomass. Total unfiltered Ce was measured from the supernatant of each assay after 20 h. Two pH values were considered for this test. Treatments consisted of Milli-Q water at pH 3.0 and 6.0, sludge biomass at pH 3.0 and 5.9, and rinse sludge at pH 2.7 and 6.0. The Milli-Q water treatment was sampled at the beginning of the experiment (Water 0 h) to be used as a control.

4.4.5 COD removal during secondary treatment

The performance of the activated sludge treatment was monitored with COD measurements of the influent and effluent. In both experiments the secondary treatment was first operated by feeding wastewater without addition of NPs. This step was done to obtain baseline information about the average COD elimination in absence of CeO_2. Figure 4.9 shows the fate of total COD concentration when using synthetic wastewater. The average COD removal before the feeding of CeO_2 was 92.2%. The efficiency was maintained as NPs were introduced into the treatment when the average COD removal was 88.2%. The performance of the activated sludge treatment, when utilizing real domestic wastewater, is presented in Figure 4.10. The soluble fraction of the total COD in the influent wastewater was found to be 64.6%. The total and soluble COD elimination achieved before addition of CeO_2 NPs was 81.1 and 83.5%, respectively. After feeding the CeO_2 NPs there was a slight decrease in both soluble and total COD removal. The efficiency observed during the feeding of CeO_2 NPs was 65.4 and 65.9% for the soluble fraction and total COD, respectively. The efficiency drop off after adding CeO_2 was not due to a deterioration in effluent quality but rather it was more related to a general decrease in the average influent COD.

The fate of acetate during treatment was monitored when feeding real domestic wastewater (Figure 4.11). Although the influent concentrations fluctuated considerably, acetate was consistently highly removed in the activated sludge system. There was no noticeable effect of the presence of CeO_2 to acetate removal as its concentration in the

effluent was below the detection limit during the first 16 days of operation. Although acetate was detected in samples taken from day 17 until the end of the experiment, its average concentration during this period was only 2.02 $\pm$ 1.60 mg L^{-1} which represents an average net removal of 93.4%.

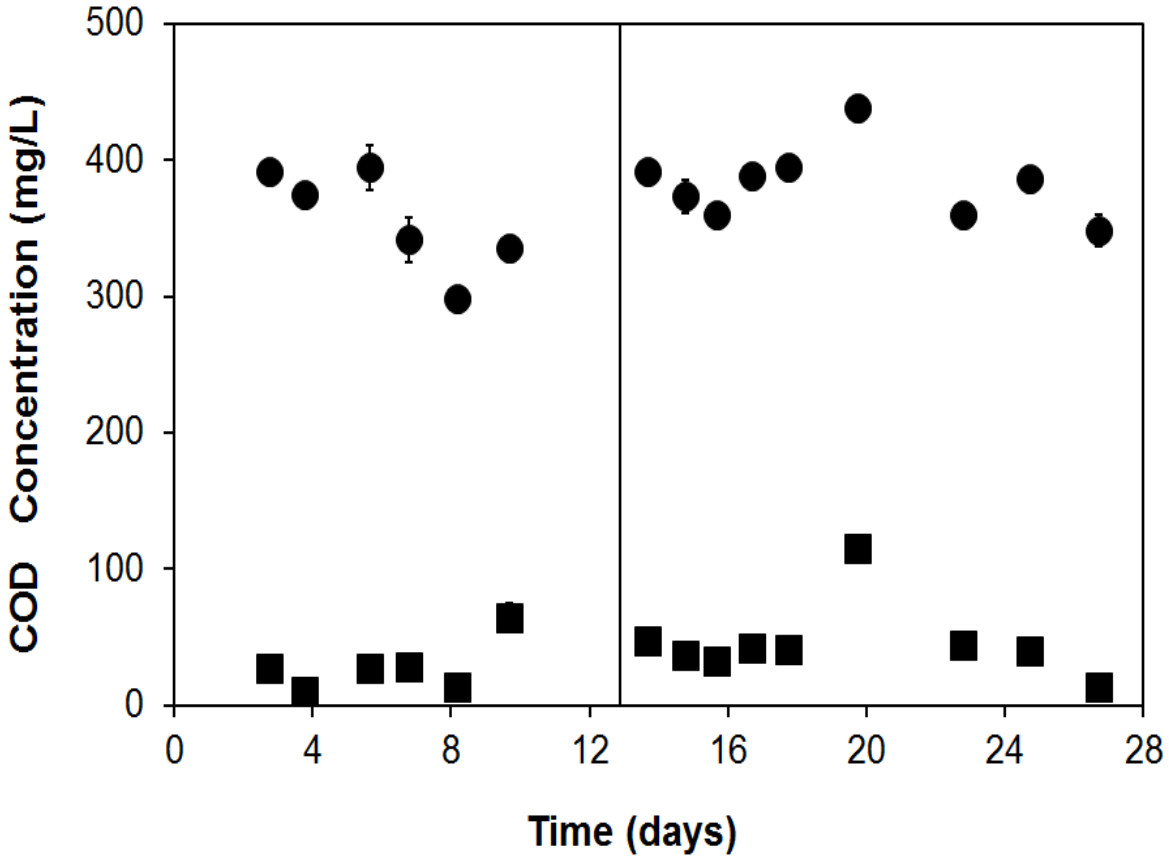

Figure 4.9. Total COD removal in the secondary treatment system when using synthetic wastewater, where (●) indicates the influent and (■) the effluent. Vertical line indicates the addition of CeO_2 to the influent wastewater.

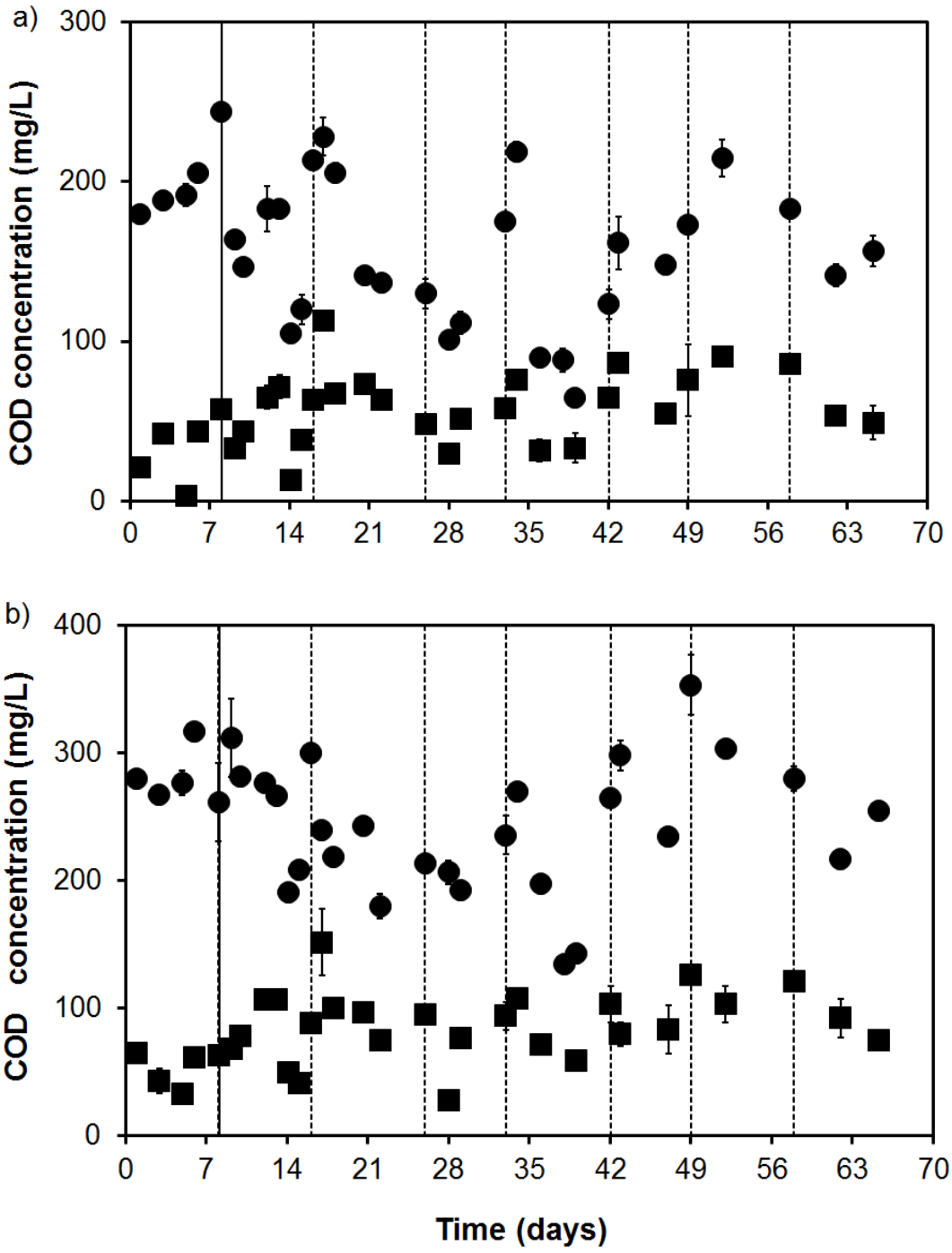

Figure 4.10. Fate of a) soluble and b) total COD in the aerobic activated sludge system when feeding real wastewater, where (●) indicates the influent and (■) the effluent. Feeding of CeO_2 to the reactor is represented by the continuous vertical line. Dashed line indicates fresh wastewater batches.

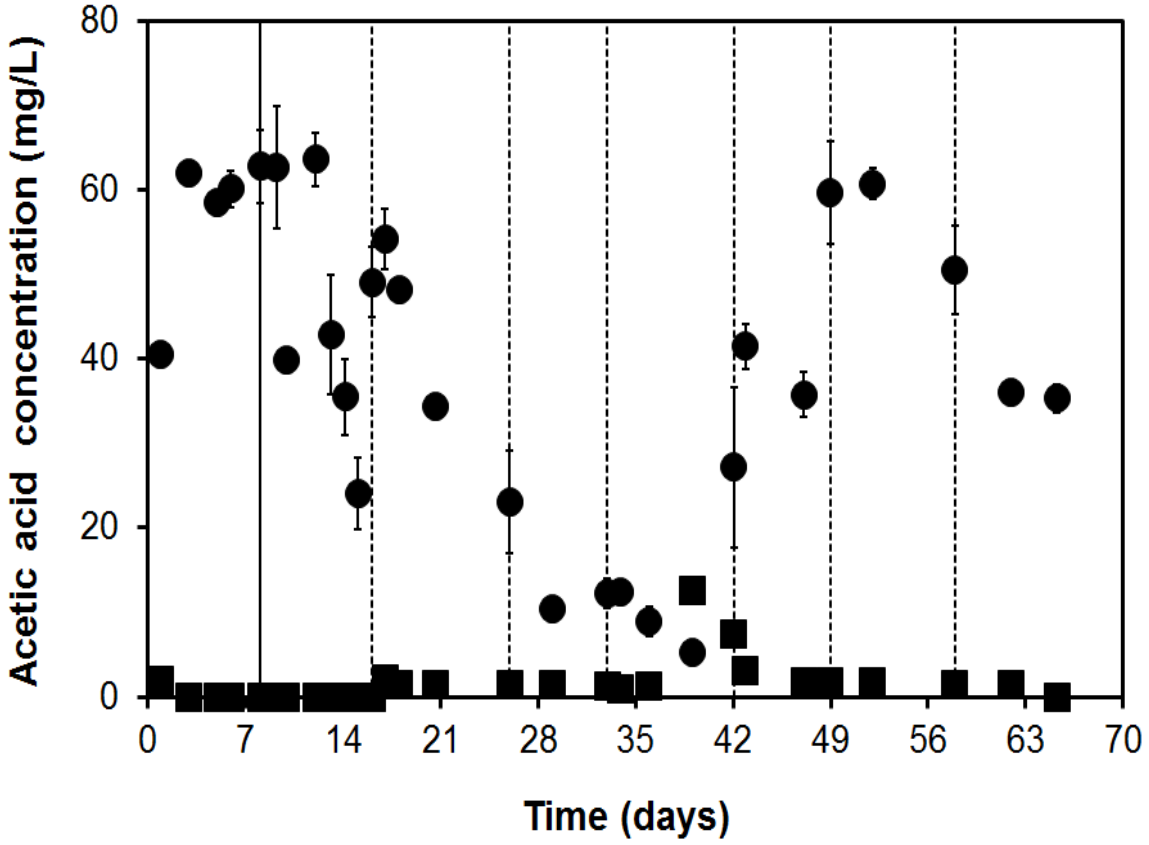

Figure 4.11. Acetate concentration in the influent, (●), and effluent, (■), of the secondary treatment when feeding real wastewater. Continuous vertical line indicates the addition of CeO_2 to wastewater. Dashed vertical lines represent fresh wastewater batches.

4.5 Discussion

4.5.1 Fate of CeO_2 during wastewater treatment

In the present study, the laboratory-scale activated sludge treatment proved to be efficient for removing CeO_2 dispersed in synthetic and real domestic wastewater as demonstrated by the high elimination achieved. When operating the secondary treatment system with real domestic wastewater only an average 3.4% of the total Ce in the influent was detected in the effluent of the clarifier. A similar behavior was observed when using synthetic wastewater, where the total Ce removal achieved was higher than 94.0%. Data obtained from Ce content in filtered samples revealed that elimination of CeO_2 < 200 nm varied significantly between experiments, achieving a higher removal in the experiment using real domestic wastewater. Approximately 98.6% of CeO_2 < 200 nm was eliminated when NPs were dispersed in real domestic wastewater. Moreover, most of these particles were < 25 nm and their elimination exceeded 92.0%. On the other hand, more than 20% of the < 200 nm particles were not removed during treatment when the reactor was fed with synthetic wastewater. The organic and/or inorganic content in the real domestic wastewater could have enhanced the further elimination of CeO_2 < 200 nm.

Previous studies exist investigating the fate of CeO_2 [26] and TiO_2 [7] NPs suspended in synthetic wastewater during simulated secondary treatment. TiO_2 NPs were more persistent than CeO_2 NPs, as the overall average removal achieved was equal to 88.0 and up to 94.0% for Ti and Ce, respectively. Moreover, Ce was highly eliminated from the influent wastewater even in the presence of biodegradable and non-biodegradable surfactants. Although these results provide an insight about the treatability of wastewater containing metal-oxide NPs, they could fail in accurately representing the behavior of the particles during treatment. Removal of Ce in the present study when using synthetic wastewater was similar to that observed by Limbach et al [26]. However, adding the NPs in real domestic wastewater resulted in higher elimination. The short duration of the previous studies did not allow the determination if the removal efficiencies were sustained with time, which was confirmed for the case of CeO_2 from the data obtained in this project.

The overall removal efficiency was greater for bulk Ce than < 200 nm or < 25 nm fraction Ce when using either synthetic or real domestic wastewater. These data suggest that elimination mainly occurred by sedimentation of agglomerated larger particles, which could have partitioned onto the sludge present in the reactor. The SEM images obtained from sludge exposed to Ce for extended time indicated mineral-like structures accumulated next to bacteria and protozoa. The occurrence of Ce in the sludge was confirmed by the EDS analysis. Evidence exists suggesting that granulated Ce could sorbed onto sludge [26]. Additionally, biosorption of ionic Ce onto aerobic granules has been demonstrated to be technically feasible [27].

4.5.2 Mechanisms for CeO_2 removal

CeO_2 was subjected to pH change as NPs were taken from the diluted stock dispersion (pH 3.10) to being mixed with wastewater (above pH 7.40). Based on colloidal chemistry, the expectation was an enhanced agglomeration of the particles as a result of the pH change, since the isoelectric point (IP) of the Ce is found between 6.75 and 7.90 [28]. Readings of the zeta-potential at the surface of the CeO_2 were higher when particles were dispersed in Milli-Q water at pH 3.11 than at pH 7.06. Particles become neutral as the pH of the dispersion approaches the IP, promoting agglomeration as the zeta-potential decreases. On the other hand, at pH values away from the IP the zeta-potential increases as result of the increased charge, causing particles to repeal each other. Data obtained from the stability test confirmed the expectations. The APS of CeO_2 agglomerates in Milli-Q water at circumneutral pH, and zeta-potential close to zero, were up to four times higher than that observed at pH 3.11. Even at acidic pH the hydrodynamic diameter of the particles was bigger than the primary particles. This observation indicates that even at low pH some aggregation is taking place. Agglomeration of commercially available NPs is common and despite the fact that aggregates can be broken by ultrasonication, it is not always possible to obtain the original small material [29]. Even though there was some agglomeration at pH 3.11, NPs largely remained in suspension over time as confirmed by the residual Ce concentration in the supernatant after incubation, which was close to that measured in the control.

The pH of the dispersion does have an important impact over aggregation of the particles, as previously discussed. However, this effect can be overcome by the presence of organic and/or inorganic compounds. Synthetic wastewater dispersed CeO_2 NPs better than Mili-Q water at pH 7.06. The presence of OM has shown to induce dispersion stability of NPs in aquatic environments as NPs are bound to acidic functional groups, providing steric and electrostatic stability [30, 31]. Evidence exists proving that NPs can bind to proteins as well. Gold NPs were stabilized in the presence of mixed peptide and polyethylene glycol monolayers; and that NPs stability increased with increasing polymer lenght [32]. CeO_2 NPs are known to bind to some proteins, even when surfaces have the same charge [33, 34]. Additionally, it has been demonstrated that certain inorganic chemicals can stabilize some NP dispersions. For example, magnetite NPs in the presence of phosphate (PO_4^{3-}) have been effectively stabilized when dispersed in different media [35, 36]. Hence, the dispersing impact of the synthetic wastewater was not surprising, since the main organic components were peptone and meat extract, and PO_4^{3-} was an important inorganic constituent of the synthetic media as well. The impact of real domestic wastewater was drastically different than that of the synthetic wastewater. Adding CeO_2 in real domestic wastewater resulted in large particle size and greater sedimentation compared to the treatment consisting of Milli-Q water at pH 7.06. Instability was promoted by the constituents of the real domestic wastewater, as its pH was equal to 7.09. It has been suggested that NPs are likely to aggregate in MWWTPs due to the organic content in the sewage [37]. Aggregation of colloidal particles in fresh

water has been shown to be enhanced by the presence of naturally occurring biopolymers, which overcome the stabilizing effect of fulvic acids [38].

During secondary treatment with synthetic wastewater NPs were highly removed. These results appear to contrast the expectations generated from the stability test, where CeO_2 was stabilized over time when dispersed in the synthetic media. However, the main difference between the activated sludge treatment and the stability test resides in that polypeptides and amino acids, from the meat extract and peptone, are degraded during the biological treatment. Hydrolysis is an important step for the biological treatment of municipal wastewater, and different enzymes have been identified serving this purpose, as bacteria can only metabolize simple molecules [39, 40]. In this study, the overall removal efficiency of CeO_2 was greater when the particles were added to real domestic wastewater compared to synthetic wastewater. This difference might be attributable to the type of organic matter and/or inorganic constituents that promoted stable dispersions in synthetic wastewater, albeit that it was subjected to degradation, and coagulation in real domestic wastewater.

The presence of biomass might enhance further removal of NPs as they become sorbed onto the sludge. Different NPs have been found to undergo biosorption onto activated sludge suggesting that this might be the major removal mechanisms of NPs from wastewater [41]. In the present study, sludge contributed significantly to the removal of CeO_2 in suspension at pH 3.00 and 6.02. However, it was observed that the soluble constituents of the sludge, dissolved during incubation, contributed by themselves to the destabilization of the nanodispersions in the absence of sludge. The extent of extra

Ce loss due to sedimentation from the supernatant of the treatment accounts in large part for the amount lost with the sludge present. Therefore, there is no strong evidence for sorption of CeO_2 onto biomass.

4.5.3 Impact of CeO_2 on the wastewater treatment process

The addition of CeO_2 into the influent wastewater did not cause disruption of the COD removal activity of the activated sludge in both experiments neither when feeding synthetic wastewater nor when using real domestic wastewater. For the latter case, small losses in COD removal were observed in the period after introducing the CeO_2 NPs compared to the values obtained before addition of the particles. This difference was mostly due to a lower COD content of the influent real domestic wastewater in the period after addition of CeO_2. Moreover, in a preliminary study performed in our research group, toxicity experiments of CeO_2 over acetate consuming activity of activated sludge have demonstrated that concentrations of up to 1000 mg CeO_2 L^{-1} had no noticeable impact on acetate consumption. Although the toxicity of many NPs to microorganisms has been explored [42, 43], information is limited assessing the impact of metal oxide NPs over biological activity in wastewater treatment processes.

4.6 Conclusions

Nanodispersions of CeO_2 are highly removed during activated sludge treatment regardless of the complexity of the organic content of the wastewater. Elimination occurred mainly by the destabilization of the particles dispersed in the wastewater, promoted by a shift in pH and, in the case of real domestic wastewater, by enhanced agglomerating effects of its constituents. The pH impacts the zeta-potential of the double layer surrounding the particles, which decreases the surface charge, resulting in agglomeration of the neutral particles. Aggregation could also occur from adhesion of NPs into proteins cause their destabilization, and hence, the formation of bigger particles.

4.7 References

1. Tiede, K., et al., *Detection and characterization of engineered nanoparticles in food and the environment.* Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 2008. 25(7): p. 795-821.
2. Rejeski, D. *State of Commercialization of Nano-enabled Consumer Products* 2009 [cited 2010 September 26th]; Available from: http://www.nanotechproject.org/inventories/consumer/.
3. Mueller, N.C. and B. Nowack, *Exposure modeling of engineered nanoparticles in the environment.* Environ Sci Technol, 2008. 42(12): p. 4447-53.

4. Kim, B., et al., *Discovery and Characterization of Silver Sulfide Nanoparticles in Final Sewage Sludge Products.* Environmental Science & Technology, 2010. 44(19): p. 7509-7514.

5. Daughton, C.G., *Non-regulated water contaminants: emerging research.* Environmental Impact Assessment Review, 2004. 24(7-8): p. 711-732.

6. Reijnders, L., *Cleaner nanotechnology and hazard reduction of manufactured nanoparticles.* Journal of Cleaner Production, 2006. 14(2): p. 124-133.

7. Kiser, M.A., et al., *Titanium nanomaterial removal and release from wastewater treatment plants.* Environ Sci Technol, 2009. 43(17): p. 6757-63.

8. Morris, J. and J. Willis, *EPA Nanotechnology White Paper* U.S.E.P. Agency, Editor. 2007: Washington p. 120.

9. Liu, X.W., G.P. Sheng, and H.Q. Yu, *Physicochemical characteristics of microbial granules.* Biotechnol Adv, 2009. 27(6): p. 1061-70.

10. Keller, A.A., et al., *Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices.* Environ Sci Technol, 2009. 44(6): p. 1962-7.

11. Keller, A.A., et al., *Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices.* Environ Sci Technol, 2010. 44(6): p. 1962-7.

12. Giasuddin, A.B., S.R. Kanel, and H. Choi, *Adsorption of humic acid onto nanoscale zerovalent iron and its effect on arsenic removal.* Environ Sci Technol, 2007. 41(6): p. 2022-7.

13. Hyung, H. and J.H. Kim, *Natural organic matter (NOM) adsorption to multi-walled carbon nanotubes: effect of NOM characteristics and water quality parameters.* Environ Sci Technol, 2008. 42(12): p. 4416-21.

14. Cordier, D.J. and J.B. Hedrick, *2008 Minerals Yearbook. Rare Earths*, U.S.G. Survey, Editor. 2010.

15. Kilbourn, B.T., *Cerium and Cerium Compounds*, in *Kirk-Othmer Encyclopedia of Chemical Technology.* . 2003, John Wiley and Sons, Inc New York.

16. Brunner, T.J., et al., *In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility.* Environ Sci Technol, 2006. 40(14): p. 4374-81.

17. Lin, W.S., et al., *Toxicity of cerium oxide nanoparticles in human lung cancer cells.* International Journal of Toxicology, 2006. 25(6): p. 451-457.

18. Schubert, D., et al., *Cerium and yttrium oxide nanoparticles are neuroprotective.* Biochem Biophys Res Commun, 2006. 342(1): p. 86-91.

19. Jung, H.J., D.B. Kittelson, and M.R. Zachariah, *The influence of a cerium additive on ultrafine diesel particle emissions and kinetics of oxidation.* Combustion and Flame, 2005. 142(3): p. 276-288.

20. Park, B., et al., *Hazard and risk assessment of a nanoparticulate cerium oxide-based diesel fuel additive - a case study.* Inhal Toxicol, 2008. 20(6): p. 547-66.

21. Golden, J., et al., *Evaluating and Treating CMP Wastewater.* Semiconductor Internationa, 2000.

22. Rittner, M., *Nanoparticles - what's now, what's next?* Chemical Engineering Progress, 2003. 99.

23. Business, C.C.B.R. *Chemical Mechanical Polishing (CMP) Equipment and Materials* 2008 [cited 2010 September 26th]; Available from: http://www.bccresearch.com/.

24. Boxall, A.B., K. Tiede, and Q. Chaudhry, *Engineered nanomaterials in soils and water: how do they behave and could they pose a risk to human health?* Nanomedicine (Lond), 2007. 2(6): p. 919-27.

25. Jarvie, H.P., et al., *Fate of silica nanoparticles in simulated primary wastewater treatment.* Environ Sci Technol, 2009. 43(22): p. 8622-8.

26. Limbach, L.K., et al., *Removal of oxide nanoparticles in a model wastewater treatment plant: influence of agglomeration and surfactants on clearing efficiency.* Environ Sci Technol, 2008. 42(15): p. 5828-33.

27. Zhang, L.L., et al., *Biosorption of rare earth metal ion on aerobic granules.* J Environ Sci Health A Tox Hazard Subst Environ Eng, 2005. 40(4): p. 857-67.

28. Hsu, J.P. and A. Nacu, *An experimental study on the rheological properties of aqueous ceria dispersions.* J Colloid Interface Sci, 2004. 274(1): p. 277-84.

29. Mandzy, N., E. Grulke, and T. Druffel, *Breakage of TiO2 agglomerates in electrostatically stabilized aqueous dispersions.* Powder Technology, 2005. 160(2): p. 121-126.

30. Illes, E. and E. Tombacz, *The effect of humic acid adsorption on pH-dependent surface charging and aggregation of magnetite nanoparticles.* Journal of Colloid and Interface Science, 2006. 295(1): p. 115-123.

31. Johnson, R.L., et al., *Natural Organic Matter Enhanced Mobility of Nano Zerovalent Iron.* Environmental Science & Technology, 2009. 43(14): p. 5455-5460.

32. Liu, Y.L., et al., *Synthesis, stability, and cellular internalization of gold nanoparticles containing mixed peptide-poly(ethylene glycol) monolayers.* Analytical Chemistry, 2007. 79(6): p. 2221-2229.

33. Horie, M., et al., *Protein Adsorption of Ultrafine Metal Oxide and Its Influence on Cytotoxicity toward Cultured Cells.* Chemical Research in Toxicology, 2009. 22(3): p. 543-553.

34. Patil, S., et al., *Protein adsorption and cellular uptake of cerium oxide nanoparticles as a function of zeta potential.* Biomaterials, 2007. 28(31): p. 4600-4607.

35. Goff, J.D., et al., *Novel Phosphonate-Functional Poly(ethylene oxide)-Magnetite Nanoparticles Form Stable Colloidal Dispersions in Phosphate-Buffered Saline.* Chemistry of Materials, 2009. 21(20): p. 4784-4795.

36. Sahoo, Y., et al., *Alkyl phosphonate/phosphate coating on magnetite nanoparticles: A comparison with fatty acids.* Langmuir, 2001. 17(25): p. 7907-7911.

37. Handy, R.D., et al., *The ecotoxicology and chemistry of manufactured nanoparticles.* Ecotoxicology, 2008. 17(4): p. 287-314.

38. Wilkinson, K.J., A. Joz-Roland, and J. Buffle, *Different Roles of Pedogenic Fulvic Acids and Aquagenic Biopolymers on Colloid Aggregation and Stability in Freshwaters.* Limnology and Oceanography, 1997. 42: p. 1714-1724

39. Lemmer, H., D. Roth, and M. Schade, *Population-density and enzyme-activities of heterotrophic bacteria in sewer biofilms and activated-sludge.* Water Research, 1994. 28(6): p. 1341-1346.

40. Frolund, B., T. Griebe, and P.H. Nielsen, *Enzymatic-activity in the activated-sludge floc matrix.* Applied Microbiology and Biotechnology, 1995. 43(4): p. 755-761.

41. Kiser, M.A., et al., *Biosorption of nanoparticles to heterotrophic wastewater biomass.* Water Research, 2010. 44(14): p. 4105-4114.

42. Hussain, S.M., et al., *Toxicity Evaluation for Safe Use of Nanomaterials: Recent Achievements and Technical Challenges.* Advanced Materials, 2009. 21(16): p. 1549-1559.

43. Brar, S.K., et al., *Engineered nanoparticles in wastewater and wastewater sludge - Evidence and impacts.* Waste Management, 2009. 30(3): p. 504-520.

CHAPTER 5

REMOVAL OF Al_2O_3 NANOPARTICLES DURING ACTIVATED SLUDGE SECONDARY TREATMENT

5.1 Abstract

Aluminum oxide (Al_2O_3) is widely used in the fabrication of ceramics, catalysts, and abrasives, among others, and it is also extensively utilized in the semiconductor industry for the chemical-mechanical polishing (CMP) of wafers. The stability of nanosized oxides in sewage is likely to be altered due to the significant changes in the water chemistry, which might result in particle agglomeration and eventually even sedimentation. In addition to particle agglomeration and gravitational settling, interactions of nanoparticles (NPs) with microorganisms involved in biological wastewater treatment might be an additional mechanism contributing to their removal. The main goal of this work was to investigate the behavior of Al_2O_3 NPs during activated sludge secondary treatment. The pH and organic content of the dispersion were investigated as possible mechanisms for the removal of the particles. Finally, the effect of Al_2O_3 on the performance of the reactor for removing the chemical oxygen demand (COD) of the wastewater was evaluated. For this purpose a laboratory-scale secondary treatment was continuously fed with real domestic wastewater containing Al_2O_3

nanoparticles. The average concentration of total Al measured in the samples was 18.10 $\pm$ 3.88 mg Al L^{-1} (34.2 $\pm$ 7.29 mg Al_2O_3 L^{-1}) in the influent and 2.10 $\pm$ 0.91 mg Al L^{-1} (3.94 $\pm$ 1.69 mg Al_2O_3 L^{-1}) in the effluent. This information suggests that the treatment system was suitable for the removal of Al_2O_3 NPs. The efficiency of the system for eliminating Al_2O_3 could be higher than that reported in this work since Al was found in the real domestic wastewater used to feed the reactor at an average concentration of 1.04 $\pm$ 0.68 mg Al L^{-1}. The elimination of particles with hydrodynamic diameter smaller than 200 nm and 25 nm was evaluated as well. However, there was no evidence of removal of these small particles since the average concentration of Al < 200 nm and < 25 nm measured in the samples was similar to that measured in the wastewater. The addition of Al_2O_3 to the wastewater greatly affected the efficiency of the treatment system for removing the total and soluble COD. The average elimination of total COD reached up to 92% before addition of the NPs and decreased down to 23% in the presence of Al_2O_3. However, COD removal efficiency increased with time to an average of 89%. Batch experiments demonstrated that pH and organic content play a major role on the aggregation and sedimentation of the particles, enhancing their removal during treatment. NPs became readily destabilized in Milli-Q water at pH 7.01 and the initial average particle size (APS) (518.0 $\pm$ 74.2 nm) measured was up to 2.4 higher than the APS of NPs dispersed in Milli-Q water at pH 3.10 (214.5 $\pm$ 8.66 nm). The initial APS of NPs dispersed in real domestic wastewater at pH 6.97 was equal to 1.03 $\pm$ 0.26 μm, suggesting that, not only the pH, but also the presence of organic matter promotes the destabilization and agglomeration of the particles.

5.2 Introduction

Engineered NPs are materials with at least one dimension of 100 nanometers (nm) or less. They are used for numerous applications in the manufacturing of electronics, cosmetics, pharmaceuticals, and biomedicine, among others [1]. Due to their small size range, the fraction of atoms exposed at the NP surfaces is significant, contributing to modifying the physical, chemical, electronic and atomic properties of the particles compared to their bulk particle counterparts[2]. Therefore, NPs can act as an effective bridge between the bulk and the molecular properties of the materials [3]. NPs provide enhanced characteristics to products, that otherwise would not be possible or cost effective, resulting in an increased utilization of these materials in the last years. There were more than a thousand nano-enable consumer products identified in 2009, produced by 50 companies distributed in 20 countries [4]. Increased concern about the presence of NPs in consumer products and the environment results from the possible toxic effects of nano-sized materials to living organisms [5-7].

Nano-metal oxides are the nanomaterials (NMs) group with most commercial applications to date [8]. Al_2O_3, also called alumina, together with other metallic NMs accounted for up to 83% of the NP market in 2005 [9]. Alumina is commonly used in the manufacturing of abrasives, catalysts, refractories and electrical insulators [10]. Moreover, Al_2O_3 is an important ceramic material that is extensively employed for electrical and biomedical applications [11]. Alumina NPs have numerous applications in the electronic, optical, and magnetic industry [12]. For microelectronic purposes, it is

used during the CMP of wafers, which is an important process to achieve even surfaces free of defects [13]. For this purpose, slurries containing metal-oxide NPs, including Al_2O_3, surfactants and additives at controlled pH, are applied to the wafers [14]. The CMP slurry market was estimated at $400 million dollars in 2003 [15]. Although Al_2O_3 NPs are of great commercial importance, the adverse effects to human health of such materials it is still not fully explored.

The very same properties that make NPs interesting for their application in products and processes raise concern about the adverse effects of such materials on biological systems [16], resulted from their small size and high surface area [17]. Toxicity of Al_2O_3 seems to increase as particle size decreases. A study comparing micro- and nano-sized Al_2O_3 showed that NPs posed higher toxicity to certain bacteria than their bulk counterparts [18]. Additionally, Al_2O_3 NPs caused considerable damage to human brain microvascular endothelial cells [19], and they might be as well responsible for the breakdown of the blood-brain barrier [20]. However, Al_2O_3 NPs toxicity seems to depend on the target cell or organisms, as relative no adverse effects were observed when HT22 cells, taken from rodent nervous system, were exposed to Al_2O_3 [21].

Although unintentional and controlled releases/discharges of engineered NPs to the environment become inevitable as their use increases in household products and industrial processes, the fate of such materials once in the environment is largely unknown. Modeling studies have suggested that a considerable fraction of these NPs can find their way to the municipal wastewater treatment plants (MWWTPs) [22, 23]. Nevertheless, there is not much information about the behavior of metal-oxide NPs

during wastewater treatment. The detection of titanium dioxide (TiO_2) NPs in the effluent of several treatment plants [24] might suggest that MWWTPs are not suitable for treating wastewater containing these emerging contaminants.

Different mechanisms have been proposed for the removal of NPs from wastewater during treatment, including sorption and entrapment onto biomass [25]. A few reports exist suggesting that the removal efficiency of the treatment for eliminating the NPs in wastewater will depend on the physicochemical characteristics of the particles and the inorganic and organic components of the wastewater. For example Limbach et al [26] observed high elimination of CeO_2 NPs dispersed in synthetic media, where the average efficiency in the simulated secondary treatment was 94%. Moreover, Jarvie et al [27] studied the sedimentation of surfactant-coated and uncoated SiO_2 NPs in a bench-scale primary treatment using screened and unscreened domestic wastewater. Only Tween-coated NPs were effectively removed during primary treatment.

In this work the fate of Al_2O_3 NPs during laboratory-scale activated sludge treatment was investigated. Results obtained in this study provide an important insight about the behavior of such NPs during wastewater treatment, as no previous reports have been published. In contrast with former studies, NPs were introduced into real domestic wastewater, and the secondary treatment was operated over extended time to simulate the operation of large scale system.

5.3 Materials and Methods

The Al_2O_3 NPs were obtained from Sigma-Aldrich (Sigma-Aldrich, St Louis, MO) as a fine powder, and according to the manufacturer the average particle size was 50 nm.

5.3.1 Stability of Al_2O_3 NPs in aqueous dispersions

The Al_2O_3 NPs were suspended in Milli-Q water and real domestic wastewater with the objective to study the impact of pH and inorganic/organic components over the stability and aggregation of the particles. For this purpose an Al_2O_3 stock dispersion containing 200.0 mg Al L^{-1} (377.9 mg Al_2O_3 L^{-1}) was prepared in 1 mM HCl by applying sonication (Daigger GEX130, 130W, Cole-Parmer Instruments, Vernon Hills, IL) for 15 min at 70% intensity. Then, 1 mL of the stock was diluted in 10 mL Milli-Q water at pH 3.10 and 7.01 as well as in 10 mL of real domestic wastewater at pH 6.97. Treatments were prepared in duplicate in falcon tubes (BD Biosciences, Bedford, MA), and in the case of wastewater, it was centrifuged for 30 min at 4000 rpm and passed through 25 nm membrane filters (Millipore, Billerica, MA) to remove any suspended solid that could interfere with the analysis. A 1 mL sample was taken after vortex mixing the treatments for 30 s, which allowed the determination of the initial zeta-potential and particle size distribution (PSD). Subsequently, treatments were incubated for 24 h without mechanical mixing, allowing the sedimentation of the aggregates. Finally, the upper 15% of the

supernatant formed in each treatment was sampled and digested to measure the remaining Al_2O_3 NPs in suspension. The treatment consisting of Milli-Q water at pH 3.10 was vigorously vortex mixed after taking the supernatant sample with the purpose of determining the total concentration of Al in the treatments.

5.3.2 Laboratory-scale secondary treatment

The fate of the Al_2O_3 NPs was studied in a laboratory-scale activated sludge treatment consisting of an 1.19 L aeration tank and a 0.66 L settler, all constructed in acrylic plastic (Figure 5.1). The real domestic wastewater was collected after primary clarification from Roger Road, a local WWTP located in Tucson, AZ, in a weekly basis and was kept under refrigeration to avoid the decomposition of the organic constituents of the water. The reactor was seeded with returned activated sludge (RAS) obtained from the same plant. In order to avoid any interference of pre-existing Al in the sludge, it was washed 7 times with Milli-Q water. The biomass concentration, measured as volatile suspended solids (VSS), in the aeration tank was set equal to 2.55 g VSS L^{-1}. The Al_2O_3 NPs were introduced into the secondary treatment system via a stock dispersion. The Al_2O_3 stock preparation was a two-step process. First a concentrated stock was prepared by dispersing the NPs in 1 mM HCl using sonication at 70% intensity for 15 min to an Al concentration of 2.00 g Al L^{-1} (3.78 g Al_2O_3 L^{-1}). Then the stock was further diluted in 1 mM HCl to 200 mg Al L^{-1} (378 mg Al_2O_3 L^{-1}). The diluted stock was kept under constant mixing to avoid aggregation of the NPs. The diluted stock was mixed with the

wastewater in the entrance of the aeration tank to achieve an Al concentration in the influent of 18.2 mg Al L^{-1} (34.2 mg Al_2O_3 L^{-1}).

The average pH and chemical oxygen demand (COD) of the influent wastewater measured after dilution with the NPs was 7.26 $\pm$ 0.22 and 313.4 $\pm$ 88.7 mg COD L^{-1}, respectively. The average flow rate was 2.99 $\pm$ 0.15 L d^{-1}, which corresponds to a hydraulic retention time (HRT) equal to 9.49 $\pm$ 0.12 h (Table 5.1). The recirculation of a fraction of the settle sludge and air and mixing was achieved by using two air pumps (Aqua, Walmart) providing a flow rate of 400 L d^{-1}.

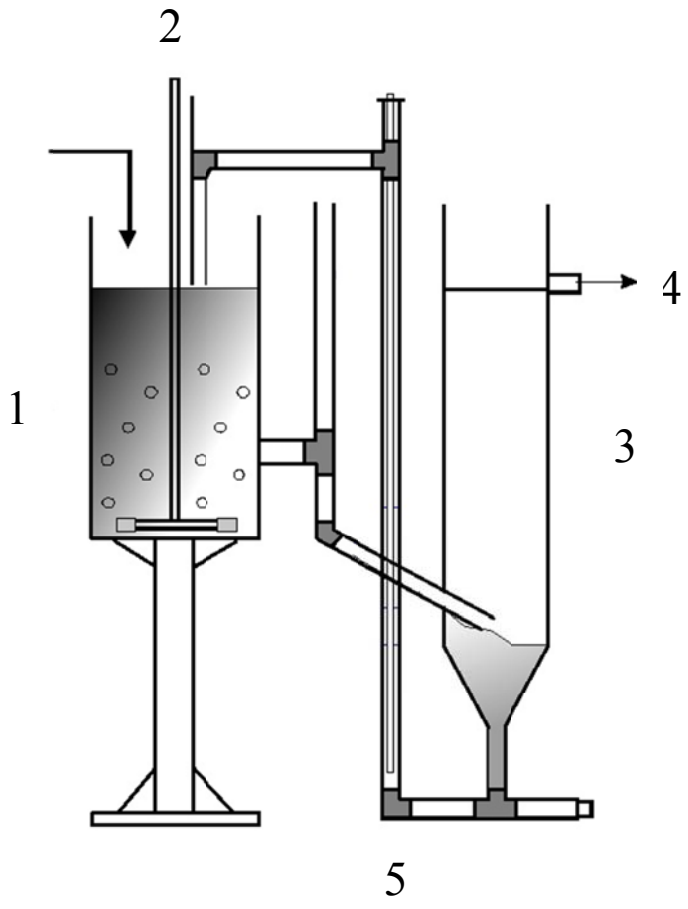

Figure 5.1. Schematic of the laboratory aerobic activated sludge treatment system. (1) Aeration tank, (2) air, (3) settler, (4) effluent, (5) recycled sludge.

The fate of the NPs and the performance of the reactor were monitored by taking samples from the diluted stock, the influent wastewater containing the NPs, and the clarified effluent. The COD and acetate concentration were measured in the influent and effluent samples. The pH was monitored in the influent and effluent samples as well. In order to maintain a relatively constant biomass concentration in the reactor, 90 mL of sludge were taken from the aeration tank and replaced with wastewater (without Al_2O_3 NPs) every other day. Since the aluminum has an inherent alkalinity, the pH of the NP stock was measured every 2 days and adjusted adding 0.03 mL of concentrated HNO_3 to a 1 L stock. Keeping the low pH values avoids agglomeration of the particles. Finally the behavior of the Al_2O_3 NPs was monitored by measuring the total concentration in the samples, as well as the concentration of Al_2O_3 that passed through a 200 nm and 25 nm membrane filters.

Images from the original sludge used to seed the reactor and samples of sludge exposed to Al_2O_3 NPs taken from the aeration tanked were obtained using scanning electron microscopy (SEM). The presence of Al was determined by energy dispersive spectroscopy (EDS).

Table 5.1. Operational parameters of the laboratory aerobic activated sludge treatment in the absence and presence of Al_2O_3 NPs.

Parameter	Real wastewater (No Al_2O_3 added)	Real wastewater (Al_2O_3 added)
Operation time (days)	24	167
Θ in the aeration tank (h)	9.41 ± 0.77	9.58 ± 0.43
Average pH In	7.37 ± 0.09	7.24 ± 0.25
Average pH Out	6.57 ± 0.27	6.76 ± 0.26
Mixed liquor volatile suspended solids concentration (g/L)	1.88 ± 0.46	2.03 ± 0.52

5.3.3 Analytical procedure

The total and soluble COD concentrations in liquid samples were measured during secondary treatment. The soluble fraction was obtained by centrifuging the samples for 10 min at 11,000 rpm. The COD was determined by mixing 2.5 mL of sample, either soluble or total, with 1.5 mL digestion solution and 3.5 mL sulfuric acid concentrated (H_2SO_4). The digestion solution consisted of (g L^{-1}): $H_2Cr_2O_7$ (10.22), Hg_2SO_4 (33.30), and H_2SO_4 (167 mL L^{-1}). The H_2SO_4 concentrated contained 10.25 g Ag_2SO_4 L^{-1}. Digestion of the resulting mixture occurred at 150ºC for 2 h. The

COD content was then obtained by measuring the absorbance of the digested samples using a spectrophotometer (Beckman-Coulter, Fullerton, CA) at a wavelength of 600 nm.

The fate of acetate, a simple organic acid, was followed in the activated sludge treatment system as well. For this purpose 1.5 mL of sample from the influent and effluent were centrifuged for 15 min at 10,000 rpm. Then, 1 mL of the formed supernatant was transferred into a 1.5 mL glass vial and amended with 0.5 μL of concentrated formic acid. Finally, acetate was measured using a gas chromatograph (GC) (7890A, Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector, and a 30 m x 0.53 mm Stabilwax-DA column (Restek, Bellefonte, PA). The carrier gas used was ultra-high purity helium at a flow rate of 16 mL min^{-1}. The liquid samples were injected into the GC by an autosampler applying a 6:1 (v/v) split ratio. The inlet and detector temperatures were equal to 250ºC and 275ºC, respectively.

As previously mentioned, the biomass content in the aeration tank was determined by measuring the VSS content. Twenty milliliters of sample were vacuumed filtered using 45 nm glass fiber filters (Whatman. Piscataway, NJ). The filters with the retentate were placed in aluminum vessels (Fisher Scientific, Pittsburgh, PA) and dried overnight at 105^{o}C. Then, the samples were ashed in a muffle furnace at 550^{o}C for 4 h. Subsequently, containers were allowed to rich ambient temperature in a dissecator and weighted.

Total Al was measured in the stability test and in the samples taken from the activated sludge treatment samples, including the diluted stock. A volume of 5 mL of sample was mixed with 5 mL of HCl 6.75 M (Fisher Scientific, Pittsburgh, PA) and digested in a Mars 5 microwave digestion oven (MDS2100, CEM Corp., Matthews, NC) at 71 PSI for 30 min. In the case of the diluted stock only, 1 mL was used for the digestion due to its high Al concentration; nevertheless it was amended with 4 mL Milli-Q water. After digestion the effluent samples were diluted with 10 mL Milli-Q water, and the diluted stock and influent digested samples were diluted with 25 mL Milli-Q water. Digested samples were placed in 15 mL falcon tubes and analyzed in an inductively coupled plasma – optical emission spectrometer (ICP-OES, Optima 2100 DV, Perkin Elmer, Waltham, MA) at a wavelength of 396 nm. The fate of Al < 200 nm and < 25 nm was monitored in the samples from the secondary treatment. For this purpose samples were passed, using a 3 mL plastic syringe, through 200 nm (Whatman, Piscataway, NJ) and 25 nm (Millipore, Billerica, MA) membrane filters. After filtration 0.015 mL HNO_3, were added to preserve the samples. Finally, 2.5 mL of the filtered sample were diluted with 2.5 mL 4% HNO_3 (v/v) and directly measured in an ICP-OES.

The APS and zeta-potential were measured in a Zeta Sizer Nano ZS (Malvern, Inc., Westborough, MA) by light scattering caused by the NPs. The equipment was set at a refractive index and absorption value equal to 1.828 and 0.01, respectively, using water as dispersant at a 25^{o}C. This operation was accomplished by filling a DTS1060C capillary vessel with 1 mL of sample, carefully to avoid air bubbles that could interfere with the analysis.

A variable that was measured in all the samples was the pH. It was determined using a probe (Orion-Thermo Scientific, Cincinnati, OH) calibrated before measurements with certified standards at pH 4.0, 7.0, and 10.0 (Orion-Thermo Scientific. Cincinnati, OH).

5.3.4 Electron microscopy

The SEM and EDS analysis of the sludge samples were executed in the University spectroscopy and imaging facilities (USIF) at the University of Arizona. The SEM the samples were fixed using formaldehyde in a 50% (v/v) ratio 4 h before the preparation of the samples. Then, samples were vacuumed dried and manually broken until forming a fine powder, which was mounted and glued into a plastic base. The specimen was finally coated with a thin layer of gold (Au) and palladium (Pd) to protect the sample from the electron beam. EDS measurements were done by directing an x-ray beam into the sample and measuring the energy reflected and measured by the detector. SEM and EDS analysis were performed in a S-4800 UHR field emission scanning microscope (Hitachi, Tokyo, Japan) at 5 kV.

5.4 Results

5.4.1 Stability of Al_3O_2 NPs

In this study, the stability of Al_2O_3 NPs dispersions in Milli-Q water, at pH 3.10 and 7.01, as well as in wastewater at its natural pH (6.97), was investigated. The study was conducted to get a better understanding about the main factors that promote the aggregation (if any) of the particles. Figure 5.2a shows the APS of the NPs at the beginning of the experiment and the supernatant after 24 h incubation without mechanical mixing. The average hydrodynamic diameter of the Al_2O_3 NPs was bigger than the 50 nm primary particle size reported by the manufacturer. At low pH, the APS was 214.5 $\pm$ 8.66 nm. The APS of the low pH dispersion remained constant over time and only changed marginally during incubation. When the NPs were suspended in Milli-Q water at neutral pH the APS right after dispersion was 518.0 $\pm$ 74.2 nm and increased to 938.6 $\pm$ 173.9 nm after 24 h of incubation. The results suggest that aggregation is considerably enhanced at circumneutral pH values.

Significant agglomeration of Al_2O_3 was observed in the presence of real domestic wastewater. At the beginning of the experiment the APS in the treatment containing wastewater was 1.03 $\pm$ 0.26 μm and the APS increased to 1.46 $\pm$ 0.09 μm over the 24 h incubation period. The data show that Al_2O_3 NPs in wastewater readily aggregate to form particles in the micron-size range. Moreover, when comparing these results to those obtained for the Milli-Q water at neutral pH treatment, it is evident that certain

constituents of the wastewater promote agglomeration of the Al_2O_3 NPs. The zeta-potential values of the samples were measured as well and these are shown in Figure 5.5b. The average zeta-potential for the treatment consisting of Milli-Q water at pH 3.10 did not vary considerably over time and it was equal to 37.8 $\pm$ 0.21 mV during the experiment. As observed with the size measurements, the zeta-potential for the Milli-Q water at pH 7.01 treatment varied with time, decreasing from an initial value of 22.4 $\pm$ 2.51 mV to only 4.73 $\pm$ 2.49 mV after 24 h. Comparing these two Milli-Q water treatments, the data suggest that low absolute values of zeta-potential promote agglomeration of the particles. An exception was observed in the treatment containing real domestic wastewater. Although the zeta-potential values at the beginning (-23.3 $\pm$ 1.61 mV) and at the end (-22.3 $\pm$ 0.82 mV) of the experiment were comparable to those measured in the Milli-Q water pH 7.01 treatment, the APS of the Al_2O_3 in wastewater was considerably higher.

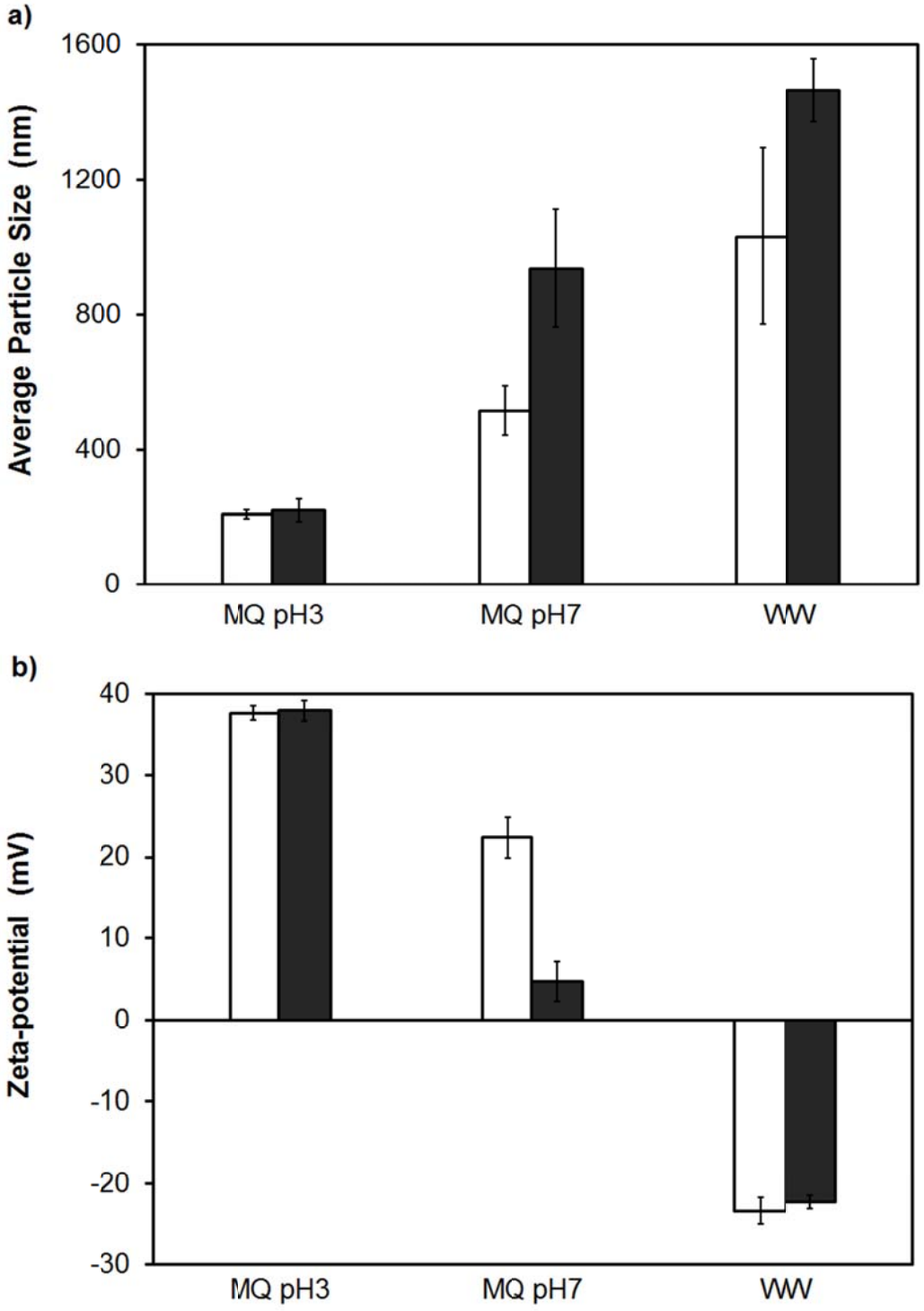

Figure 5.2. a) Average particle size and b) zeta-potential of CeO_2 in different matrices at t=0 (□) and t=24 h (■) incubation. MQ = Milli-Q water (pH 3.10 and 7.01) and WW = Real wastewater (pH 6.97).

The NP dispersion stability was also evaluated by monitoring the residual Al_2O_3 NPs remaining in suspension 24 h after incubation (Figure 5.3). To determine the maximum Al content expected in the supernatant of the treatments, the treatment at pH 3.10 was sampled after diluting the NPs and used as a control, giving a concentration equal to 17.1 $\pm$ 0.84 mg Al L^{-1} (32.3 mg Al_2O_3 L^{-1}), which was close to the designed Al concentration set at 18 mg Al L^{-1} (34 mg Al_2O_3 L^{-1}). Although the APS did not increase considerably in the treatment at pH 3.10 and remained constant over time, surprisingly around 30% of the particles sedimented during the experiment based on total Al concentration measurements. The residual Al concentrations in the treatments consisting of Millli-Q water at pH 7.01 and real wastewater were 5.71 $\pm$ 2.66 (10.8 $\pm$ 5.02) and 6.86 $\pm$ 1.39 mg Al L^{-1} (12.9 $\pm$ 2.62 mg Al_2O_3 L^{-1}), respectively, which represent an average drop off in the Al concentration of 66% for the treatment consisting of Milli-Q water at pH 7.01 and 59% for the real wastewater treatment, compared to the control (Milli-Q water at pH 3.10). Even though the final APS in the treatment containing wastewater was more than 1.5 higher, the remaining Al content in both treatments was very similar.

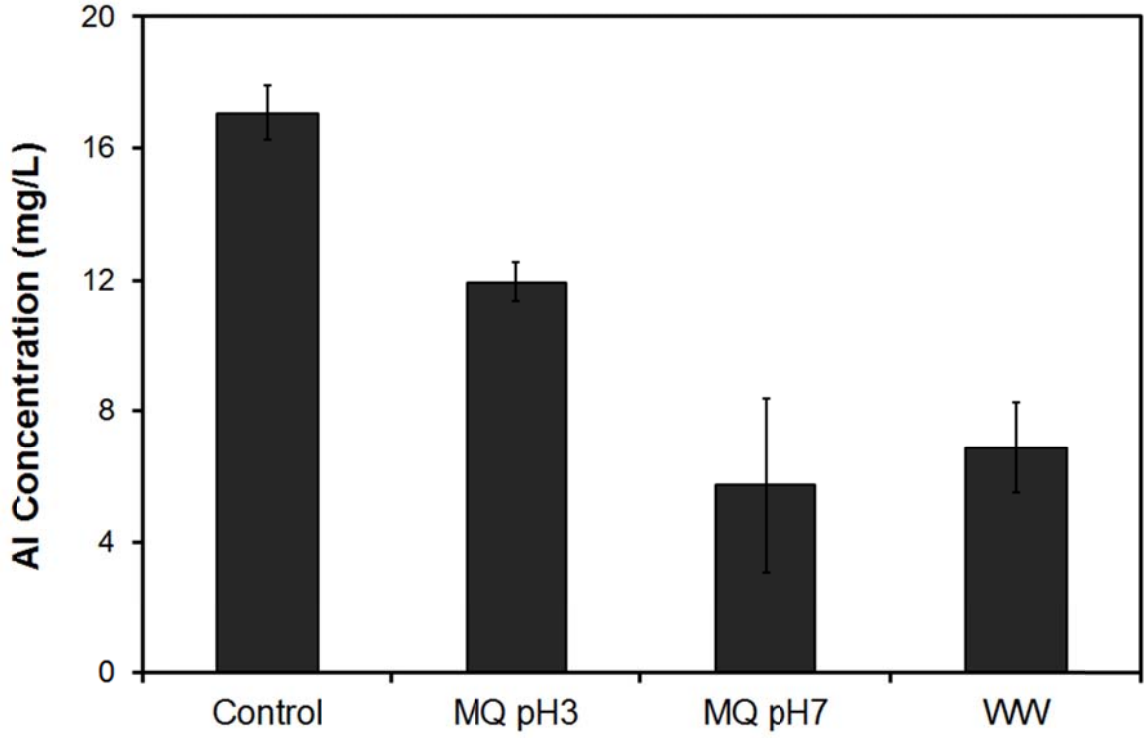

Figure 5.3. Al concentration in the supernatant from different matrices after t =24 h incubation. Control = Milli-Q water at t=0 (pH 3.10); MQ = Milli-Q water (pH 3.10 and 7.01); WW = Real wastewater (pH 6.97). The intended Al concentration at t=0 was set equal to 18 mg Al L^{-1} (34 mg Al_2O_3 L^{-1}).

5.4.2 Removal of Al_3O_2 NPs from real domestic wastewater during activated sludge treatment

The behavior of Al_2O_3 NPs suspended in real wastewater was investigated in a laboratory-scale activated sludge treatment system. Initially the secondary treatment system was fed with real domestic wastewater. After 24 days, NPs were continuously introduced into the system via a stock dispersion which was diluted with real domestic wastewater to achieve a design concentration of 18.2 mg Al L^{-1} (34.2 mg Al_2O_3 L^{-1}) in

the influent. The fate of total Al in the system is presented in Figure 5.4a. The average concentration of total Al measured in the influent was 18.10 $\pm$ 3.88 mg Al L^{-1} (34.2 $\pm$ 7.29 mg Al_2O_3 L^{-1}), whereas in the influent was found to be 2.10 $\pm$ 0.91 mg Al L^{-1} (3.94 $\pm$ 1.69 mg Al_2O_3 L^{-1}). The Al concentration in the wastewater was measured before starting the experiment to determine its natural Al background concentration. The Al content of these samples was below the detection limit of the ICP-OES (<1 part per billion). However, an average concentration of 1.04 $\pm$ 0.68 mg Al L^{-1} was found in the wastewater collected from the MWWTP during the operation of the reactor, which is considerably higher than the preliminary measurements. After stopping the secondary treatment, additional measurements revealed no detectable Al in the wastewater. This could indicate that Al was added intermittently to the wastewater during treatment in the MWWTP. The natural soluble Al content in the wastewater is indicated in Figure 5.4a by a horizontal line, and the upper and lower broken lines represent the standard deviation.

The fate of the < 200 nm and < 25 nm particles was studied by serial filtrations and is shown if Figure 5.4b. The real domestic wastewater was filtered through a 200 nm membrane filter and its Al content was measured, which is indicated by the horizontal lines. As it can be observed, the Al concentration in the influent and effluent samples taken from the secondary treatment was masked by the Al < 200 nm occurring in the real domestic wastewater. Results indicate no elimination of Al < 200 nm and Al < 25 nm during treatment since the Al content measured in the samples was similar to that observed in the real domestic wastewater.

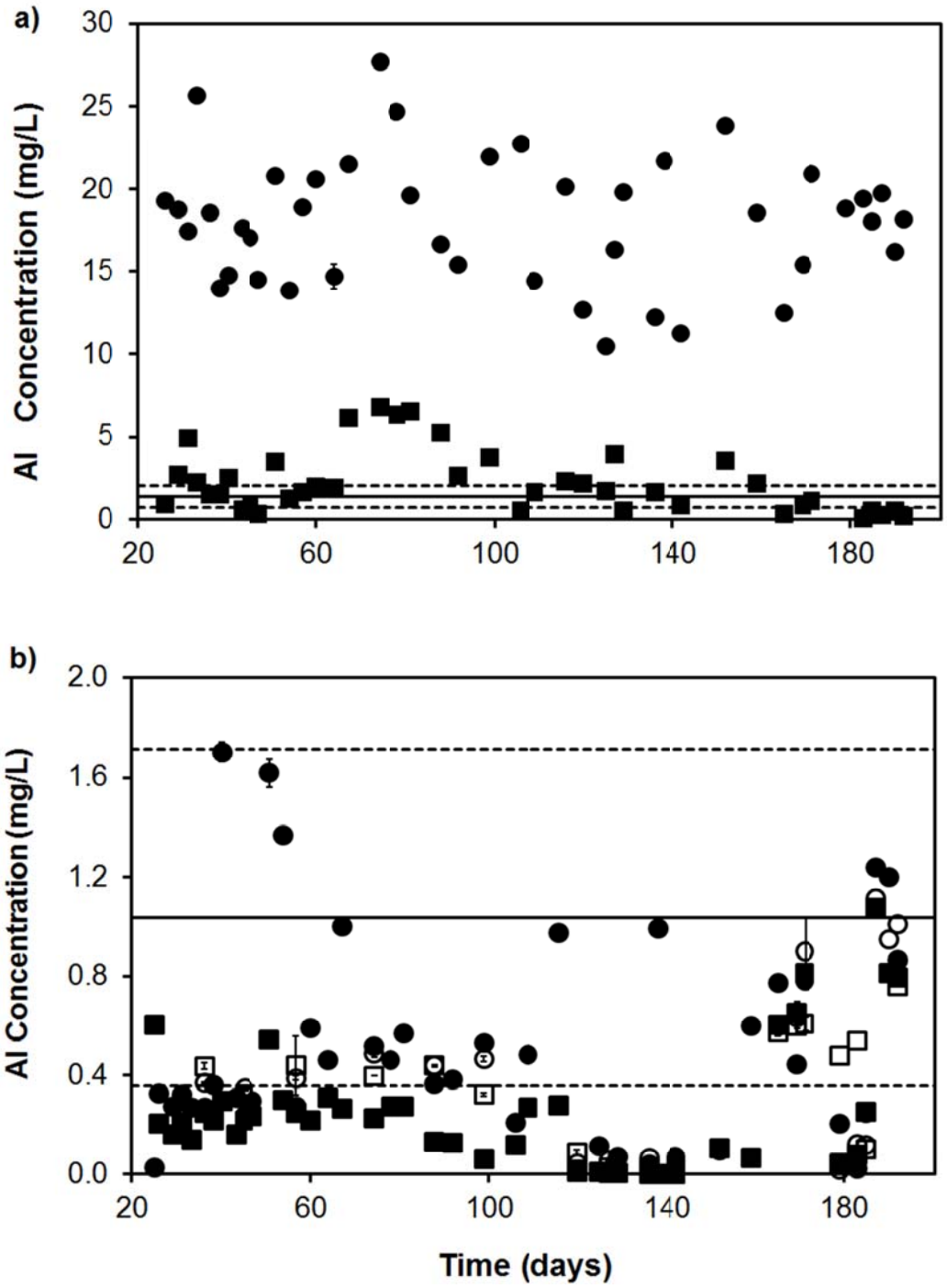

Figure 5.4. Fate of a) total unfiltered and b) filtered Al in the (●) influent and (■) effluent of activated sludge treatment. Bold markers in second panel indicate Al_2O_3 < 200 nm, while empty markers refer to Al_2O_3 < 25 nm. Horizontal line represents the average Al concentration in wastewater; broken lines indicate the standard deviation.

A sample of the original sludge used to seed the reactor was imaged and EDS analyzed together with a sample taken from aeration tank at the end of the experiment, exposed to Al_2O_3 for 167 days. Figure 5.5 shows the image of both samples and their EDS spectrum. Although the SEM shows a similar composition of the samples, consisting of bacteria, filamentous microorganisms, and extra-cellular material, the Al counts greatly varied. The presence of a small amount of Al in the original sludge could suggest the utilization of Al in the MWWTP, and might explain its presence in the wastewater used to feed the laboratory-scale secondary treatment. Even though the number of counts does not represent the concentration of an element in a sample *per se*, higher counts indicate higher amounts of the target element under study. The Al counts in the sample of sludge exposed to Al_2O_3 were up to 27 times higher than those in the original sludge, suggesting sedimentation of the particles added to the wastewater and possible entrapment or sorption onto the biomass.

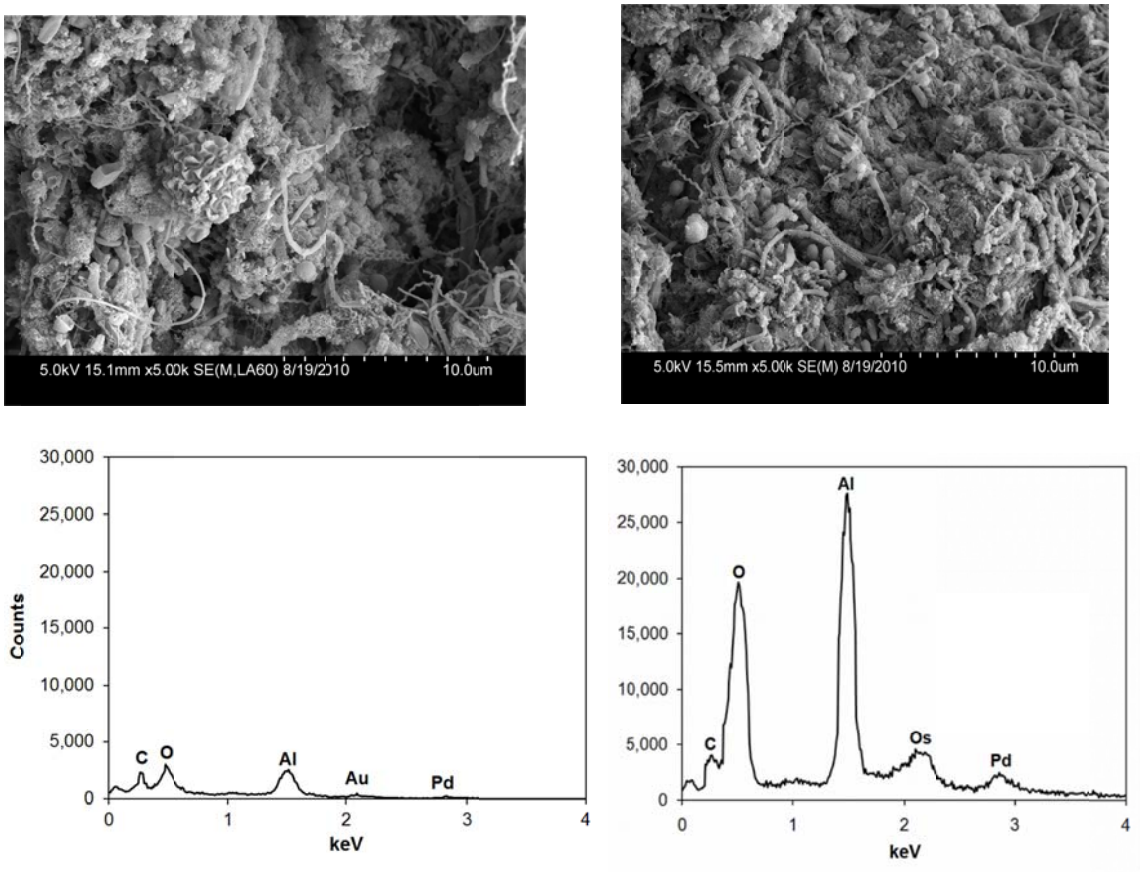

Figure 5.5. SEM image of the original sludge use to seed the reactor (left) and sludge exposed to Al_2O_3 NPs for 167 days (right). EDS spectrum of the sludge (bottom left) and the exposed sludge (bottom right).

5.4.3 Fate of the organic content in the secondary treatment

The capability of the activated sludge treatment for removing the organic content from the wastewater was evaluated by measuring the COD and acetate concentration in the influent and effluent samples. The secondary treatment system operated during 24 days without addition of the Al_2O_3 NPs in order to allow acclimatization of the biomass to the new environmental conditions. During this period the average removal of total

COD was found to be 49%, as the average influent concentration was 247.2 $\pm$ 42.35 mg COD L^{-1} and 126.3 $\pm$ 32.60 mg COD L^{-1} in the effluent (Figure 5.6a). The performance of the reactor for removing total COD gradually increased with time and from day 25 to day 38 the average total COD removal was 87%. The total COD content in the influent and effluent samples was 270.4 $\pm$ 65.54 mg COD L^{-1} and 34.29 $\pm$ 10.16 mg COD L^{-1}, respectively. However, the efficiency of the treatment system unexpectedly decreased considerably after day 40 and a significant and steady increase in the COD effluent concentration was observed until day 81. Finally, during the last 91 days of operation of the reactor, from day 98 to day 190, the COD content in the samples remained rather low and fairly constant. The average COD concentration in the influent and effluent was 331.9 $\pm$ 103.3 mg COD L^{-1} and 44.9 $\pm$ 24.7 mg COD L^{-1} respectively, resulting in an average elimination of 86%. The COD data suggest that the period of low removal efficiency could have been caused by the presence of Al_2O_3 in the reactor since the COD content in the influent wastewater showed little variation,. The elimination of the soluble COD fraction followed a similar trend as that one observed in the removal of total COD (Figure 5.6b). The performance of the reactor considerably decreased after 40 days of operation. The efficiency of the treatment system for eliminating soluble COD was more affected than for removing total COD. After 74 days, when the soluble COD content in the effluent decreased and stabilized, the average soluble COD concentration in the influent was equal to 131.4 $\pm$ 60.46 mg COD L^{-1} and that of the effluent was 30.40 $\pm$ 13.62 mg COD L^{-1}. From day 74 until the end of the experiment on day 190 the average soluble COD removal was 77%.

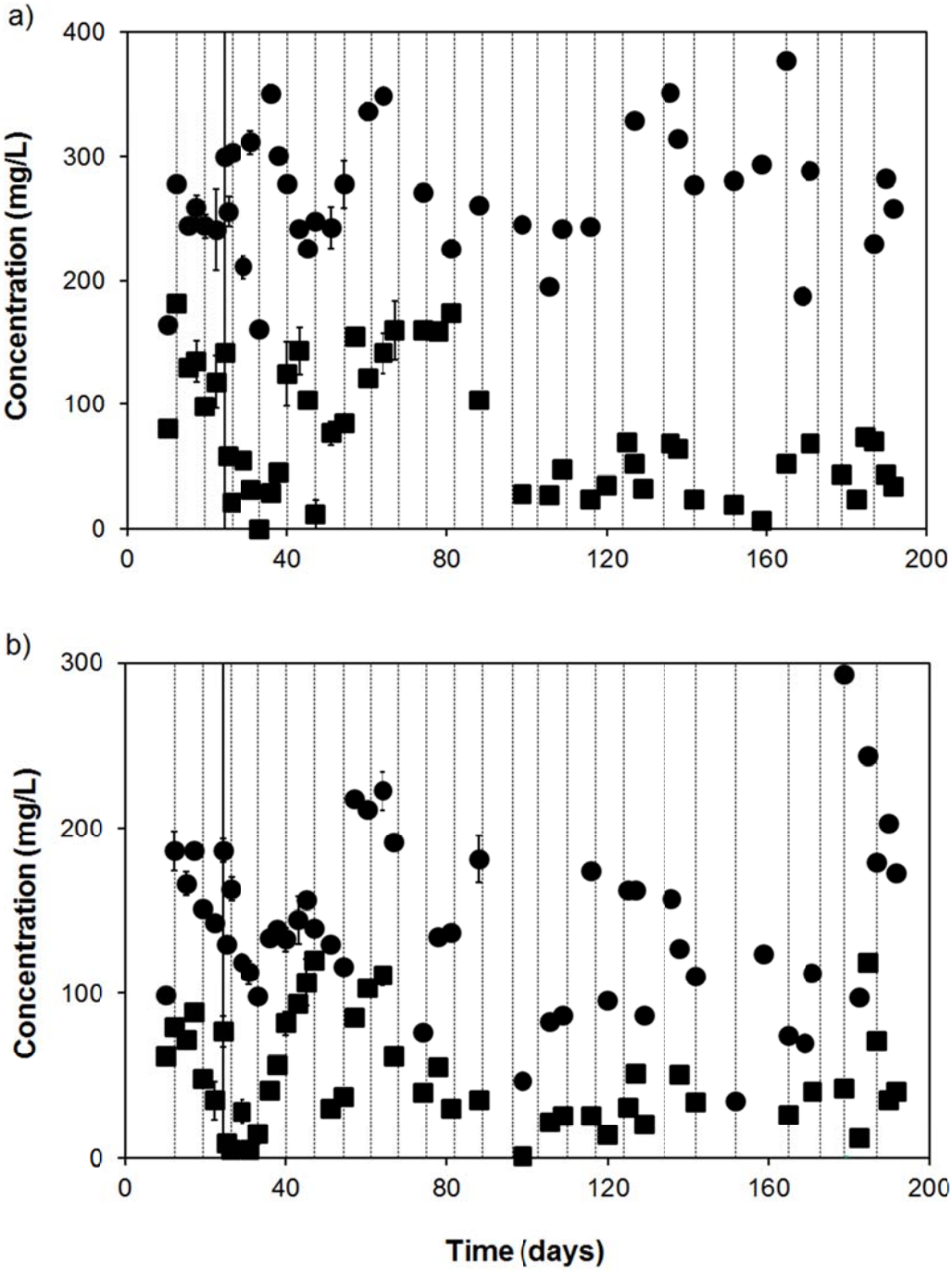

Figure 5.6. a) Total and b) soluble COD concentration in the aerobic activated sludge system, where (●) indicates the influent and (■) the effluent. Feeding of Al_2O_3 to the reactor is represented by the continuous vertical line. Doted lines indicate fresh real domestic wastewater batches.

The acetate content in the influent and effluent samples was measured and is presented in Figure 5.7. Acetate degradation is vital to maintain a circumneutral pH in the reactor, since microbial activity is decreased at low pH values. The figure shows despite the fact that acetate concentration in the influent wastewater varied significantly, it was highly removed without any lag phase and its elimination increased with time. The average acetate concentration in the influent samples was found to be 29.7 $\pm$ 17.6 mg acetate L^{-1} which reflects the great variability in the wastewater. Acetate content in the effluent was 0.93 $\pm$ 0.52 mg acetate L^{-1}, resulting in an average elimination of 96%. More importantly, the behavior observed in the removal of total and soluble COD was not detected in the case of acetate.

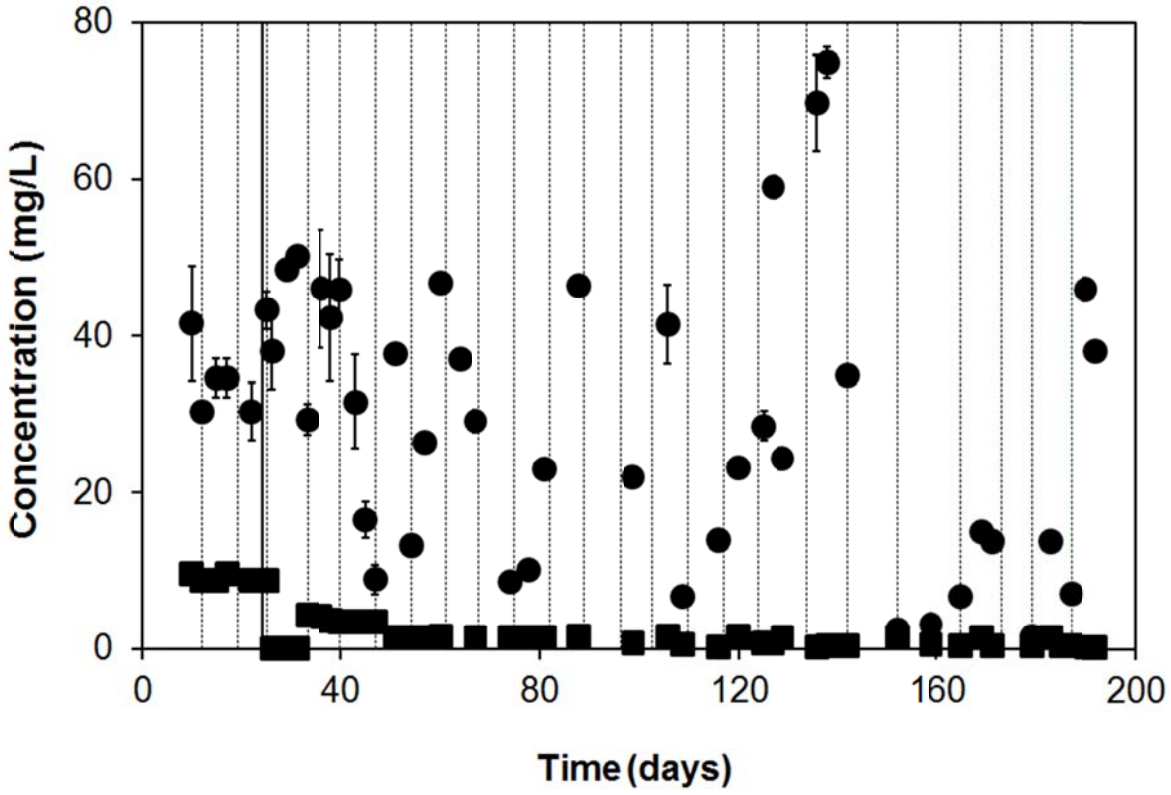

Figure 5.7. Concentration of acetate in the influent (●) and effluent (■) of the secondary activated sludge treatment. Continuous vertical lines specify the addition of Al_2O_3 to wastewater. Doted vertical lines represent fresh wastewater batches.

5.5 Discussion

5.5.1 Fate of Al_2O_3 during wastewater treatment

The data obtained in this work suggest that significant removal of Al_2O_3 can be achieved during activated sludge secondary treatment. The content of total Al was decreased from an average of 18.10 $\pm$ 3.88 mg Al L^{-1} (34.2 $\pm$ 7.29 mg Al_2O_3 L^{-1}) in the influent to only 2.10 $\pm$ 0.91 mg Al L^{-1} (3.94 $\pm$ 1.69 mg Al_2O_3 L^{-1}) in the treated effluent. However, the presence of Al in the real domestic wastewater used to feed the reactor does not allow determining the real capacity of the treatment system for removing Al_2O_3 NPs. The content of Al measured in the wastewater during the operation of the reactor was very close to that measured for Al < 200 nm and Al < 25 nm, suggesting no effective removal of the very fine particles. The presence of Al in the wastewater suggests its utilization in the local MWWTP where the wastewater batches were taken from. Alum ($Al_2(SO_4)_3$ nH_2O) is commonly used during wastewater treatment for coagulation purposes [28] and might explain the presence of Al in the wastewater. Apparent removal of soluble Al from the real domestic wastewater seemed to occurred during treatment, as the Al content for certain effluent samples was lower than that measured in the wastewater.

Different studies in laboratory-scale reactors have proved that activated sludge processes are highly efficient for the elimination of metal-oxides NPs. Limbach et al [26] observed an average removal of 94% CeO_2 NPs and Kiser et al [24] documented efficiencies of up to 88% when studying elimination of TiO_2 NPs. However, NPs were introduced into synthetic wastewater, instead of real domestic wastewater and the reactors were operated only for short periods of time, providing limited information about the behavior of the particles in a MWWTP over the long term.

The high elimination of total Al_2O_3 from the influent wastewater suggests the formation of large particles that sedimented onto the biomass in the aeration tank. The SEM image obtained from the original sludge used to seed the reactor and a sample taken from the aeration tank when the secondary treatment was stopped reveals a similar biological composition. An EDS analysis of the samples exposed the presence of Al in both cases. The presence of Al in the original sludge strengthens the possibility of its utilization at the MWWTP. Nevertheless, the number of counts was relatively small compared to the sample taken from the reactor at the end of the experiment. Additionally, these observations indicate that Al accumulated in the reactor and that biomass might play an important role in the removal of Al_2O_3 from wastewater. Evidence exists that silver sulfide NPs can bind to biological sludge during treatment in MWWTPs [29]. The sorption capacity of the biomass depends on the physical and chemical characteristics of the particles as observed by Kiser et al [30], who reported 97% removal of silver NPs but only 13% of fullerol NPs when exposed to wastewater biomass...

5.5.2 Mechanisms of Al_2O_3 removal

As Al_2O_3 is taken from the continuously stirred diluted stock dispersion to the influent real domestic wastewater, particles are subjected to a pH change from an average 3.6 up to 7.2. Such a significant change of pH was expected to cause agglomeration of the particles since the isoelectric point (IP) of the Al_2O_3 has been estimated to be at pH 7.9 [31]. At the IP, particles become neutral and the repulsion between them decreases. The zeta-potential of NPs added to Milli-Q water at pH 3.10 remained constant over a 24 h period and was equal to 37.8 ($\pm$ 0.21) mV. When NPs were suspended in Milli-Q water at pH 7.01 the zeta-potential was equal to 4.73 ($\pm$ 2.49) mV after 24 h. These results show that the absolute value of the zeta-potential is lower at high pH, and are comparable to observations by Gosh et al [31] who measured decreasing zeta-potential values on the surface of Al_2O_3 NPs with increasing pH. Moreover, data suggest that the zeta-potential can decrease with time when the pH of the dispersion is close to the IP. The zeta-potential represents the electric potential between the surface of the particle, and the bulk medium and is affected by the presence of ions in the dispersion. Charged particles repeal each other, whereas when neutralized are attracted by van der Waals forces, resulting in the formation of bigger particles. This was confirmed by the APS measured from the supernatant formed after incubation of the treatments consisting of Milli-Q water at pH 3.10 and 7.01. The average size obtained from the treatment at pH 7.01 was up to 3.4 times higher than that at pH 3.10. The data obtained indicate that the pH of the dispersion influence the zeta-potential of the particles, and hence their size

distribution. Moreover, it was observed that the absolute value of the zeta-potential decreased with time, resulting in further agglomeration. This behavior is not uncommon as increased size over time for certain NPs has been previously documented [32]. Even though particles were highly charged at pH 3.10 some degree of agglomeration was observed since the size of the Al_2O_3 NPs claimed by the manufacturer was 50 nm and the average hydrodynamic size measured after sonication was 214.5 nm. Different NPs, including TiO_2, Fe_2O_3, ZnO, and NiO, agglomerated when dispersed in water and cannot be broken down into primary particles after extended ultrasonication [33, 34]. Despite the zeta-potential and the APS remained constant during the experiment for the treatment at pH 3.10, around 30% of the particles settled over time. This observation suggests the formation of even larger agglomerates that did not remain in suspension.

The agglomeration of the Al_2O_3 NPs was influenced not only by the pH but also by certain constituents of the wastewater, since Al_2O_3 NPs diluted in real domestic wastewater at pH 6.97 showed a considerably higher APS than that at pH 7.01 in Milli-Q water. Al_2O_3 is known to bind to humic aicd and other organic compounds [35, 36], and this capacity can be increased by the presence of certain bridging compounds, including polymerin and sodium dodecylsulfate (SDS) [37, 38]. Different studies have reported the stabilization of certain NPs in the presence of organic matter and found it to be independent of the pH of the dispersion [31, 39, 40]. However evidence exist that fulvic acids and other organic polymers can destabilize colloids present in water [41, 42]. The Al content in the supernatant, after incubation, from the treatment containing wastewater was similar to that observed in Milli-Q water at pH 7.01, indicating that even larger

particles can remain in suspension by their interaction with simple organic molecules in the wastewater. The high removal efficiency achieved during secondary treatment corroborated the findings from the stability test, where the pH of the dispersion and the organic matter in the wastewater were identified as the principal mechanisms for the removal of the particles.

5.5.3 Impact of Al_2O_3 on the performance of the activated sludge treatment

The introduction of Al_2O_3 into the activated sludge secondary treatment system disrupted the overall performance of the reactor for removing the organic content from the wastewater. Microorganisms were affected by the presence of Al as demonstrated by the low total and soluble COD removal measured approximately 14 days after the feeding of Al_2O_3 NPs. The degradation of the soluble COD fraction in the reactor was more affected than the removal of total COD by the presence of Al, where at the most critical point removal decreased down to 17%. It is known that Al can be toxic to microorganisms. Toxicity can be caused by the binding of Al to cell walls, enzymes, substrates, DNA, and ATP, by the complexation of Al to nutrients, and by a decrease of the pH of the medium [43, 44] Guida et al [45] reported growth inhibition when studying the effects of aluminium nitrate to *Escherichia coli* and found it to be dependent of pH. Illmer et al [46] observed severe inhibition of nitrogen mineralization by microorganisms in soils exposed to Al The COD removal efficiency steadily increased with time and during the last 92 days of operation of the reactor remained constant at an average

elimination of 76%, a clear indication that microorganisms adapted to Al_2O_3. More importantly, accumulation of the Al particles did not cause further inhibition. Different studies have revealed that certain microorganisms can tolerate the presence of Al. *Pseudomonas fluorescens* was able to grow in in mineral media containing up to 50 mmol Al L^{-1} [47]. Fischer et al [48] observed increased resistance of a chemolithoautotrophic acidophilic bacteria, *Acidiphilium cryptum*, after precultivation in 1 mmol L^{-1} $Al_2(SO_4)_3$ and subsequent transfer to a medium containing 300 mmol L^{-1} $Al_2(SO_4)_3$. The continuous high removal of acetate from the wastewater revealed that microbial activity for acetate degradation was not affected by the presence of Al_2O_3, suggesting that the partial inhibition caused by Al affected only the degradation of higher molecular weight organics.

5.6 Conclusions

The fate of Al_2O_3 NPs was studied during activated sludge secondary treatments. The high elimination of Al was observed, suggests that Al_2O_3 in wastewater can be effectively removed in MWWTPs. The pH of the wastewater played a major role for the elimination of the Al, since it caused a decreased of the zeta-potential of the Al_2O_3 particles.. The destabilization of the nano-suspension caused by the pH of the wastewater resulted in agglomeration of the Al_2O_3, which enhanced the removal of Al. The efficiency of the treatments system for eliminating COD was affected by the presence of Al. Microorganisms responsible for the degradation of COD were partially inhibited.

However, the microbial community in the reactor adapted and recovered to the presence of Al over time.

5.7 References

1. Ju-Nam, Y. and J.R. Lead, *Manufactured nanoparticles: An overview of their chemistry, interactions and potential environmental implications.* Science of the Total Environment, 2008. 400(1-3): p. 396-414.
2. Banfield, J.F. and H. Zhang, *Nanoparticles in the Environment.* Reviews in Mineralogy and Geochemistry, 2001. 44(1): p. 1-58.
3. Henglein, A., *Physicochemical properties of small metal particles in solution - microelectrode reactions, chemisorption, composite metal particles, and the atom-to metal transition.* Journal of Physical Chemistry, 1993. 97(21): p. 5457-5471.
4. Rejeski, D. *State of Commercialization of Nano-enabled Consumer Products* 2009 [cited 2010 September 26th]; Available from: http://www.nanotechproject.org/inventories/consumer/.
5. Hussain, S.M., et al., *Toxicity Evaluation for Safe Use of Nanomaterials: Recent Achievements and Technical Challenges.* Advanced Materials, 2009. 21(16): p. 1549-1559.
6. Nel, A., et al., *Toxic potential of materials at the nanolevel.* Science, 2006. 311(5761): p. 622-7.

7. Colvin, V.L., *The potential environmental impact of engineered nanomaterials.* Nature Biotechnology, 2003. 21(10): p. 1166-1170.

8. Bell, T., *Understanding Risk Assessment of Nanotechnology*, N.N. Initiative, Editor. 2007.

9. Rittner, M.N., *Market analysis of nanostructured materials.* American Ceramic Society Bulletin, 2002. 81(3): p. 33-36.

10. Krewski, D., et al., *Human health risk assessment for aluminium, aluminium oxide, and aluminium hydroxide.* Journal of Toxicology and Environmental Health-Part B-Critical Reviews, 2007. 10: p. 1-269.

11. Pang, Y.X. and X.J. Bao, *Aluminium oxide nanoparticles prepared by water-in-oil microemulsions.* Journal of Materials Chemistry, 2002. 12(12): p. 3699-3704.

12. Holister, P. and T.E. Harper, *The nanotechnology opportunity report*. 2002, CMP Científica

13. Golden, J., et al., *Evaluating and Treating CMP Wastewater.* Semiconductor Internationa, 2000.

14. Lai, C.L. and S.H. Lin, *Electrocoagulation of chemical mechanical polishing (CMP) wastewater from semiconductor fabrication.* Chemical Engineering Journal, 2003. 95(1-3): p. 205-211.

15. Rittner, M., *Nanoparticles - what's now, what's next?* Chemical Engineering Progress, 2003. 99.

16. Karakoti, A.S., L.L. Hench, and S. Seal, *The potential toxicity of nanomaterials - The role of surfaces.* Jom, 2006. 58(7): p. 77-82.

17. Pitkethly, M.J., *Nanomaterials, the driving force.* Materials Today, 2004. 7(12): p. 20 - 29.

18. Jiang, W., H. Mashayekhi, and B.S. Xing, *Bacterial toxicity comparison between nano- and micro-scaled oxide particles.* Environmental Pollution, 2009. 157(5): p. 1619-1625.

19. Chen, L., et al., *Manufactured Aluminum Oxide Nanoparticles Decrease Expression of Tight Junction Proteins in Brain Vasculature.* Journal of Neuroimmune Pharmacology, 2008. 3(4): p. 286-295.

20. Lockman, P.R., et al., *Nanoparticle surface charges alter blood-brain barrier integrity and permeability.* Journal of Drug Targeting, 2004. 12(9-10): p. 635-641.

21. Schubert, D., et al., *Cerium and yttrium oxide nanoparticles are neuroprotective.* Biochem Biophys Res Commun, 2006. 342(1): p. 86-91.

22. Gottschalk, F., et al., *Modeled Environmental Concentrations of Engineered Nanomaterials (TiO2, ZnO, Ag, CNT, Fullerenes) for Different Regions.* Environmental Science & Technology, 2009. 43(24): p. 9216-9222.

23. Mueller, N.C. and B. Nowack, *Exposure modeling of engineered nanoparticles in the environment.* Environ Sci Technol, 2008. 42(12): p. 4447-53.

24. Kiser, M.A., et al., *Titanium nanomaterial removal and release from wastewater treatment plants.* Environ Sci Technol, 2009. 43(17): p. 6757-63.

25. Morris, J. and J. Willis, *EPA Nanotechnology White Paper* U.S.E.P. Agency, Editor. 2007: Washington p. 120.

26. Limbach, L.K., et al., *Removal of oxide nanoparticles in a model wastewater treatment plant: influence of agglomeration and surfactants on clearing efficiency.* Environ Sci Technol, 2008. 42(15): p. 5828-33.

27. Jarvie, H.P., et al., *Fate of silica nanoparticles in simulated primary wastewater treatment.* Environ Sci Technol, 2009. 43(22): p. 8622-8.

28. Vanbenschoten, J.E. and J.K. Edzwald, *Chemical aspects of coagulation using aluminum salts .1. Hydrolytic reactions of alum and polyaluminum chloride.* Water Research, 1990. 24(12): p. 1519-1526.

29. Kim, B., et al., *Discovery and Characterization of Silver Sulfide Nanoparticles in Final Sewage Sludge Products.* Environmental Science & Technology, 2010. 44(19): p. 7509-7514.

30. Kiser, M.A., et al., *Biosorption of nanoparticles to heterotrophic wastewater biomass.* Water Research, 2010. 44(14): p. 4105-4114.

31. Ghosh, S., et al., *Colloidal Behavior of Aluminum Oxide Nanoparticles As Affected by pH and Natural Organic Matter.* Langmuir, 2008. 24(21): p. 12385-12391.

32. Tso, C.P., et al., *Stability of metal oxide nanoparticles in aqueous solutions.* Water Science and Technology, 2010. 61(1): p. 127-133.

33. Mandzy, N., E. Grulke, and T. Druffel, *Breakage of TiO2 agglomerates in electrostatically stabilized aqueous dispersions.* Powder Technology, 2005. 160(2): p. 121-126.

34. Zhang, Y., et al., *Stability of commercial metal oxide nanoparticles in water.* Water Res, 2008. 42(8-9): p. 2204-12.

35. Tombacz, E., et al., *Effect of pH and ionic strength on the interaction of humic acid with aluminium oxide.* Colloid and Polymer Science, 2000. 278(4): p. 337-345.

36. Pommerenk, P. and G.C. Schafran, *Adsorption of inorganic and organic ligands onto hydrous aluminum oxide: Evaluation of surface charge and the impacts on particle and NOM removal during water treatment.* Environmental Science & Technology, 2005. 39(17): p. 6429-6434.

37. Iorio, M., et al., *Sorption of phenanthrene by dissolved organic matter and its complex with aluminum oxide nanoparticles.* Environmental Pollution, 2008. 156(3): p. 1021-1029.

38. Valsaraj, K.T., et al., *Reusable adsorbents for dilute solution separation. 1. Adsorption of phenanthrene on surfactant-modified alumina.* Separation and Purification Technology, 1998. 13(2): p. 137-145.

39. Keller, A.A., et al., *Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices.* Environ Sci Technol, 2010. 44(6): p. 1962-7.

40. Illes, E. and E. Tombacz, *The effect of humic acid adsorption on pH-dependent surface charging and aggregation of magnetite nanoparticles.* Journal of Colloid and Interface Science, 2006. 295(1): p. 115-123.

41. Wilkinson, K.J., A. Joz-Roland, and J. Buffle, *Different Roles of Pedogenic Fulvic Acids and Aquagenic Biopolymers on Colloid Aggregation and Stability in Freshwaters.* Limnology and Oceanography, 1997. 42: p. 1714-1724

42. Lafuma, F., K. Wong, and B. Cabane, *Bridging of colloidal particles through adsorbed polymers.* Journal of Colloid and Interface Science, 1991. 143(1): p. 9-21.

43. Illmer, P. and C. Erlebach, *Influence of Al on growth, cell size and content of intracellular water of Arthrobacter sp PI/1-95.* Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology, 2003. 84(3): p. 239-246.

44. Pina, R.G. and C. Cervantes, *Microbial interactions with aluminium.* Biometals, 1996. 9(3): p. 311-316.

45. Guida, L., et al., *Aluminum toxicty and binding to Escherichia-coli.* Archives of Microbiology, 1991. 156(6): p. 507-512.

46. Illmer, P., K. Marschall, and F. Schinner, *Influence of available aluminum on soil-microorganisms.* Letters in Applied Microbiology, 1995. 21(6): p. 393-397.

47. Appanna, V.D., M. Kepes, and P. Rochon, *Aluminum tolerance in Pseudomonas-fluorescens ATCC-13525 - Involvement of a gelatinous pilip-rich residue.* Fems Microbiology Letters, 1994. 119(3): p. 295-301.

48. Fischer, J., et al., *Inducible aluminum resistance of Acidiphilium cryptum and aluminum tolerance of other acidophilic bacteria.* Archives of Microbiology, 2002. 178(6): p. 554-558.

CONCLUSIONS

Organic and inorganic contaminants present in gasoline plumes and landfill leachates are known to be persistent in nature and toxic to living organisms. In this dissertation different biological techniques were explored for their potential use for the treatment of landfill leachates and contaminated groundwater.

Different inocula were used to study the anaerobic degradation of toluene, benzene, m-xylene, and *cis*-DCE using nitrate and chlorate as alternative electron acceptors. Toluene degradation occurred under nitrate and methanogenic reducing conditions; however higher energy yield was obtained from the reduction of nitrate, since toluene removal occurred at higher rate in the presence of nitrate. In this case, toluene degradation was linked to denitrification as nitrite was formed in significant amounts. Although various respikes and transfers were performed in treatments that exhibited toluene removal, enrichment cultures were not obtained. The fact that toluene removal stopped after just a few transfers suggests that degradation occurred via cometabolism. Microorganisms capable of toluene removal using chlorate as electron acceptor were not observed.

m-Xylene can be degraded under methanogenic conditions after extended incubation. Moreover, microorganisms can adapt to the presence of m-xylene over time, as demonstrated by the fast degradation of the fresh m-xylene respiked to treatments that showed m-xylene removal. Pure cultures could be obtained after serial transfers.

Surprisingly microorganisms responsible for the degradation of m-xylene using nitrate or chlorate as electron acceptors were not found in the inocula tested. Benzene can be degraded in the absence of oxygen when nitrate and chlorate are used as electron acceptors. Nevertheless, this is not a common process since removal was observed after extended incubation only in a few experiments. Microorganisms responsible for benzene degradation under methanogenic conditions were not found in the inocula used in the experiments. *cis*-DCE proved to be recalcitrant in anaerobic environments. Microorganisms capable of using *cis*-DCE as carbon source were not present in the inocula tested.

The treatability of a synthetic landfill leachate containing volatile fatty acids, phenol, p-cresol and ammonia was investigated in an anaerobic – aerobic system configuration. The high performance of the reactors demonstrates that the system can be used for the treatment of landfill leachates. The upflow anaerobic sludge blanket (UASB) is suitable for removing the organic content of the leachate, as indicated by the high elimination of the chemical oxygen demand. During anaerobic treatment in the UASB, organics were converted to methane gas. Microorganisms in the reactor can rapidly adapt to phenol and p-cresol since these contaminants were removed with no lag phase. Volatile fatty acids are highly degraded in the UASB and their removal increases with time. The persistence of ammonia in the leachate after treatment indicates that anaerobic processes are not suitable for its removal.

The aerobic downflow hanging sponge (DHS) column proved to be suitable for the nitrification of ammonia, as demonstrated by the high removal achieved during treatment. The extremely low organic content of the influent suggests that the consortia of microorganisms responsible for nitrification are autotrophs. Oxidation of ammonia in the DHS is divided in two major processes: 1) oxidation of ammonia to nitrite and 2) oxidation of nitrite to nitrate. Ammonia oxidizing bacteria, responsible for partial nitrification to nitrite, dominate during the first days of operation; however ammonia oxidizers and nitrite oxidizers dominate over time, demonstrated by the complete nitrification of ammonia achieved during extended operation of the reactor.

The fate of different oxide nanoparticles during domestic wastewater was studied in this dissertation. The adverse effects of oxide nanoparticles are not well understood; however evidence suggests that these materials pose a risk to human health. To date, it is still unknown if the current wastewater treatment processes are suitable for the removal of nanoparticles, or if these materials escape in the treated effluents.

The behavior of cerium oxide nanoparticles was investigated during activated sludge secondary treatment of synthetic and real domestic wastewater. Data shows that the secondary treatment is adequate for the elimination of the particles in the influent wastewater. Efficiency was higher when using real wastewater, suggesting that inorganic/organic components lacking in the synthetic wastewater enhance further removal of particles. Particles agglomerate when diluted in synthetic and real wastewater during treatment since less than 15%, at best, of the total ceria showed to be smaller than

200 nm. Performance of the secondary treatment system for removing the small particles (<200 and <25 nm) is not as high as that observed for total ceria.

Removal of ceria is dependent of the pH and organic content of the wastewater. Circumneutral pH values enhance agglomeration of the particles, resulting in the formation of unstable aggregates. Depending on the kind of organic content that is in the wastewater, particles can be stabilized or destabilize. In the synthetic wastewater, peptone seems to stabilize the particles, whereas the complex organic matter found in real wastewater causes the aggregation of the particles. When particles agglomerate tend to sediment. The detection of ceria in the sample of sludge taken from the aeration tank at the end of the experiment suggests accumulation of the material over time. The presence of ceria in the reactor did not cause microbial inhibition. The chemical oxygen demand and acetate were continuously removed during treatment.

In a similar experiment aluminum oxide was added to real domestic wastewater and its removal investigated during secondary treatment. Aluminum oxide is highly removed during treatment. The pH and organic matter enhance the agglomeration and sedimentation of the particles, as observed for ceria. Aluminum oxide causes partial inhibition to microorganisms responsible for the removal of the organic content in the wastewater. Adaptation occurs over time since efficiency gradually increased, despite accumulation of the material in the reactor. Acetate degradation was not inhibited by the presence of aluminum oxide.

REFERENCES

Adamczyk, Z., et al., *Structure and ordering in localized adsorption of particles.* Journal of Colloid and Interface Science, 1990. 140(1): p. 123-137.

Agrawal, L.K., et al., *Treatment of raw sewage in a temperate climate using a UASB reactor and the hanging sponge cubes process.* Water Science and Technology, 1997. 36(6-7): p. 433-440.

Aiyuk, S., et al., *Anaerobic and complementary treatment of domestic sewage in regions with hot climates - A review.* Bioresource Technology, 2006. 97(17): p. 2225-2241.

Akunna, J.C., C. Bizeau, and R. Moletta, *Nitrate reduction by anaerobic sludge using glucose at various nitrate concentrations – ammonification, denitrification and methanogenic activities.* Environmental Technology, 1994. 15(1): p. 41-49.

Alagappan, G. and R. Cowan, *Substrate inhibition kinetics for toluene and benzene degrading pure cultures and a method for collection and analysis of respirometric data for strongly inhibited cultures.* Biotechnology and Bioengineering, 2003. 83(7): p. 798-809.

Alargova, R.G., S. Deguchi, and K. Tsujii, *Stable colloidal dispersions of fullerenes in polar organic solvents.* Journal of the American Chemical Society, 2001. 123(43): p. 10460-10467.

Albaiges, J., F. Casado, and F. Ventura, *Organic indicators of groundwater pollution by a sanitary-landfill.* Water Research, 1986. 20(9): p. 1153-1159.

Alkalay, D., et al., *Anaerobic treatment of municipal sanitary landfill leachates: the problem of refractory and toxic components.* World Journal of Microbiology & Biotechnology, 1998. 14(3): p. 309-320.

Amal, R., et al., *Structure and kinetics of aggregating colloidal haematite.* Colloids and Surfaces, 1990. 46(1): p. 1-19.

Andreozzi, R., et al., *Advanced oxidation processes (AOP) for water purification and recovery.* Catalysis Today, 1999. 53(1): p. 51-59.

Appanna, V.D., M. Kepes, and P. Rochon, *Aluminum tolerance in Pseudomonas-fluorescens ATCC-13525 – Involvement of a gelatinous lipid-rich residue.* Fems Microbiology Letters, 1994. 119(3): p. 295-301.

Araki, N., et al., *Behaviors of nitrifiers in a novel biofilm reactor employing hanging sponge-cubes as attachment site.* Water Science and Technology, 1999. 39(7): p. 23-31.

ATSDR, *Toxicological profile for 1,2-dichloroethene*, U.S.D.O.H.A.H. SERVICES, Editor. 1996: Atlanta.

ATSDR, *Toxicological profile for toluene*, U.S.D.O.H.A.H. SERVICES, Editor. 2000: Atlanta.

ATSDR, *Toxicological profile for xylene*, U.S.D.O.H.A.H. SERVICES, Editor. 2007: Atlanta.

ATSDR, *Toxicological profile for benzene*, U.S.D.O.H.A.H. SERVICES, Editor. 2007: Atlanta.

ATSDR, *Toxicological profile for cresols*, U.S.D.O.H.A.H. SERVICES, Editor. 2008: Atlanta.

ATSDR, *Toxicological profile for phenol*, U.S.D.O.H.A.H. SERVICES, Editor. 2010: Atlanta.

Atwater, J.W., et al., *Experiments using Daphnia to measure landfill leachate toxicity.* Water Research, 1983. 17(12): p. 1855-1861.

Banfield, J.F. and H. Zhang, *Nanoparticles in the Environment.* Reviews in Mineralogy and Geochemistry, 2001. 44(1): p. 1-58.

Battersby, N.S. and V. Wilson, *Survey of the anaerobic biodegradation potential of organic chemicals in digesting sludge.* Appl Environ Microbiol, 1989. 55(2): p. 433-9.

Baun, A., et al., *Toxicity testing of organic chemicals in groundwater polluted with landfill leachate.* Environmental Toxicology and Chemistry, 1999. 18(9): p. 2046-2053.

Baun, A., et al., *Xenobiotic organic compounds in leachates from ten Danish MSW landfills - chemical analysis and toxicity tests.* Water Research, 2004. 38(18): p. 3845-3858.

Beek, B. and W. Reineke, *Aerobic and Anaerobic Biodegradation Potentials of Microorganisms*, in *Biodegradation and Persistance*. 2001, Springer Berlin / Heidelberg. p. 1-161.

Bell, T., *Understanding Risk Assessment of Nanotechnology*, N.N. Initiative, Editor. 2007.

Berrueta, J. and L. Castrillon, *Anaerobic treatment of leachates in UASB reactors.* Journal of Chemical Technology and Biotechnology, 1992. 54(1): p. 33-37.

Bicego, M.C., et al., *Assessment of contamination by polychlorinated biphenyls and aliphatic and aromatic hydrocarbons in sediments of the Santos and Sao Vicente Estuary System, Sao Paulo, Brazil.* Marine Pollution Bulletin, 2006. 52(12): p. 1804-1816.

Bicego, M.C., et al., *Results from a 15-year study on hydrocarbon concentrations in water and sediment from Admiralty Bay, King George Island, Antarctica.* Antarctic Science, 2009. 21(3): p. 209-220.

Boll, M., G. Fuchs, and J. Heider, *Anaerobic oxidation of aromatic compounds and hydrocarbons.* Curr Opin Chem Biol, 2002. 6(5): p. 604-11.

Boopathy, R., *Factors limiting bioremediation technologies.* Bioresource Technology, 2000. 74(1): p. 63-67.

Botton, S. and J.R. Parsons, *Degradation of BTX by dissimilatory iron-reducing cultures.* Biodegradation, 2007. 18(3): p. 371-81.

Boxall, A.B., K. Tiede, and Q. Chaudhry, *Engineered nanomaterials in soils and water: how do they behave and could they pose a risk to human health?* Nanomedicine (Lond), 2007. 2(6): p. 919-27.

Boyd, S.A., et al., *Anaerobic biodegradation of phenolic-compounds in digested sludge.* Applied and Environmental Microbiology, 1983. 46(1): p. 50-54.

Brackmann, R. and G. Fuchs, *Enzymes of anaerobic metabolism of phenolic-compounds – 4-hydorxybenzoyl-CoA reductase (dehydroxylating) from a denitrifying Pseudomonas species.* European Journal of Biochemistry, 1993. 213(1): p. 563-571.

Bradley, P.M., *Microbial degradation of chloroethenes in groundwater systems.* Hydrogeology Journal, 2000: p. 104-111.

Brar, S.K., et al., *Engineered nanoparticles in wastewater and wastewater sludge - Evidence and impacts.* Waste Management, 2009. 30(3): p. 504-520.

Brunner, T.J., et al., *In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility.* Environ Sci Technol, 2006. 40(14): p. 4374-81.

Bryant, M.P., *Microbial methane production – Theoretical aspects.* Journal of Animal Science, 1979. 48(1): p. 193-201.

Business, C.C.B.R. *Chemical Mechanical Polishing (CMP) Equipment and Materials* 2008 [cited 2010 September 26th]; Available from: http://www.bccresearch.com/.

Chakraborty, R., *Anaerobic degradation of monoaromatic hydrocarbons.* Appl Microbial Biotechnol, 2004. 64: p. 437-446.

Chang, Y.J., N. Nishio, and S. Nagai, *Characteristics of granular methanogenic sludge grown on phenol synthetic medium and methanogenic fermentation of phenolic waste-water in a UASB reactor.* Journal of Fermentation and Bioengineering, 1995. 79(4): p. 348-353.

Chapell, F.H.B., P. M., *Selecting Remediation Goals by Assessing the Natural Attenuation Capacity of Groundwater Systems.* Bioremediation Journal, 1998: p. 227-238.

Chen, L., et al., *Manufactured Aluminum Oxide Nanoparticles Decrease Expression of Tight Junction Proteins in Brain Vasculature.* Journal of Neuroimmune Pharmacology, 2008. 3(4): p. 286-295.

Chiang, C.Y., et al., *Aerobic degradation of benzene, toluene, and xylene in a sandy aquifer - data-analysis and computer modeling.* Ground Water, 1989. 27(6): p. 823-834.

Christensen, T.H., et al., *Attenuation of landfill leachate pollutants in aquifers.* Critical Reviews in Environmental Science and Technology, 1994. 24(2): p. 119-202.

Christensen, T.H., et al., *Biogeochemistry of landfill leachate plumes.* Applied Geochemistry, 2001. 16(7-8): p. 659-718.

Chuang, H.P., et al., *Effective partial nitrification to nitrite by down-flow hanging sponge reactor under limited oxygen condition.* Water Research, 2007. 41(2): p. 295-302.

Coates, J.D., et al., *Anaerobic benzene oxidation coupled to nitrate reduction in pure culture by two strains of Dechloromonas.* Nature, 2001. 411(6841): p. 1039-43.

Colvin, V.L., *The potential environmental impact of engineered nanomaterials.* Nature Biotechnology, 2003. 21(10): p. 1166-1170.

Cordier, D.J. and J.B. Hedrick, *2008 Minerals Yearbook. Rare Earths*, U.S.G. Survey, Editor. 2010.

Cozzarelli, I.M. and A.L. Baehr, *Volatile fuel hydrocarbons and MTBE in the environment*, in *Environmental geochemistry*, B.S. Lollar, Editor. 2005, Elsevier. p. 433 - 468.

Danovaro, R., et al., *Sunscreens cause coral bleaching by promoting viral infections.* Environmental Health Perspectives, 2008. 116(4): p. 441-447.

Daughton, C.G., *Non-regulated water contaminants: emerging research.* Environmental Impact Assessment Review, 2004. 24(7-8): p. 711-732.

Dean, B.J., *Recent findings on the genetic toxicology of benzene, toluene, xylenes and phenols.* Mutation Research, 1985. 154(3): p. 153-181.

Dias, J.M., et al., *Waste materials for activated carbon preparation and its use in aqueous-phase treatment: A review.* Journal of Environmental Management, 2007. 85(4): p. 833-846.

Diaz, E.E., et al., *Phenotypic properties and microbial diversity of methanogenic granules from a full-scale upflow anaerobic sludge bed reactor treating brewery wastewater.* Applied and Environmental Microbiology, 2006. 72(7): p. 4942-4949.

Distefano, T.D., J.M. Gossett, and S.H. Zinder, *Hydrogen as an electron-donor for dechlorination of tetrachloroethene by an anaerobic mixed culture.* Applied and Environmental Microbiology, 1992. 58(11): p. 3622-3629.

El Deeb, K.Z., et al., *Distribution and sources of aliphatic and polycyclic aromatic hydrocarbons in surface sediments, fish and bivalves of Abu Qir Bay (Egyptian Mediterranean Sea).* Bulletin of Environmental Contamination and Toxicology, 2007. 78(5): p. 373-379.

Elmen, J., et al., *Kinetics of toluene degradation by a nitrate-reducing bacterium isolated from a groundwater aquifer.* Biotechnol Bioeng, 1997. 55(1): p. 82-90.

EPA. *Drinking Water Contaminants*. 2009 [cited 2011; Available from: http://water.epa.gov/drink/contaminants/upload/mcl-2.pdf.

EPA. *National Recommended Water Quality Criteria* 2009; Available from: http://water.epa.gov/scitech/swguidance/waterquality/standards/current/upload/nrwqc-2009.pdf.

EPA. *Municipal Solid Waste (MSW) in the United States*. 2009 [cited 2010; Available from: http://www.epa.gov/epawaste/nonhaz/municipal/msw99.htm.

EPA, U. *UST/LUST Program Status In Arizona*. 2008 [cited 2009 August 4, 2009].

Evans, P.J., et al., *Metabolites formed during anaerobic transformation of toluene and o-xylene and their proposed relationship to the initial steps of toluene mineralization.* Appl Environ Microbiol, 1992. 58(2): p. 496-501.

Evans, P.J., et al., *Quantification of denitrification by strain T1 during anaerobic degradation of toluene.* Appl Microbiol Biotechnol, 1992. 37(1): p. 136-40.

Evans, P.J., D.T. Mang, and L.Y. Young, *Degradation of toluene and m-xylene and transformation of o-xylene by denitrifying enrichment cultures.* Appl Environ Microbiol, 1991. 57(2): p. 450-4.

Evans, W.C. and G. Fuchs, *Anaerobic degradation of aromatic compounds.* Annu Rev Microbiol, 1988. 42: p. 289-317.

Fang, H.H.P. and G.M. Zhou, *Degradation of phenol and p-cresol in reactors.* Water Science and Technology, 2000. 42(5-6): p. 237-244.

Farhadian, M., et al., *In situ bioremediation of monoaromatic pollutants in groundwater: a review.* Bioresour Technol, 2008. 99(13): p. 5296-308.

Fatta, D., A. Papadopoulos, and M. Loizidou, *A study on the landfill leachate and its impact on the groundwater quality of the greater area.* Environmental Geochemistry and Health, 1999. 21(2): p. 175-190.

Fernandez, N., et al., *Analysis of microbial community during biofilm development in an anaerobic wastewater treatment reactor.* Microbial Ecology, 2008. 56(1): p. 121-132.

Finnegan, M.P., H.Z. Zhang, and J.F. Banfield, *Phase stability and transformation in titania nanoparticles in aqueous solutions dominated by surface energy.* Journal of Physical Chemistry C, 2007. 111(5): p. 1962-1968.

Fischer, J., et al., *Inducible aluminum resistance of Acidiphilium cryptum and aluminum tolerance of other acidophilic bacteria.* Archives of Microbiology, 2002. 178(6): p. 554-558.

Foresti, E., *Anaerobic treatment of domestic sewage: established technologies and perspectives.* Water Science and Technology, 2002. 45(10): p. 181-186.

Freese, S.D., et al., *Comparison of ozone and hydrogen peroxide/ozone for the treatment of eutrophic water.* Water Science and Technology, 1999. 39(10-11): p. 325-328.

Fries, M.R., et al., *Isolation, characterization, and distribution of denitrifying toluene degraders from a variety of habitats.* Appl Environ Microbiol, 1994. 60(8): p. 2802-10.

Fritsche, W. and M. Hofrichter, *Aerobic Degradation by Microorganisms*. Biotechnology. 2008: Wiley-VCH Verlag GmbH. 144-167.

Frolund, B., T. Griebe, and P.H. Nielsen, *Enzymatic-activity in the activated-sludge floc matrix.* Applied Microbiology and Biotechnology, 1995. 43(4): p. 755-761.

Fukushi, K. and T. Sato, *Using a Surface Complexation Model To Predict the Nature and Stability of Nanoparticles.* Environmental Science & Technology, 2005. 39(5): p. 1250-1256.

Garcia, H., J.L. Rico, and P.A. Garcia, *Comparison of anaerobic treatment of leachates from an urban-solid-waste landfill at ambient temperature and at 35 degrees C.* Bioresource Technology, 1996. 58(3): p. 273-277.

Gasparro, F.P., M. Mitchnick, and J.F. Nash, *A review of sunscreen safety and efficacy.* Photochemistry and Photobiology, 1998. 68(3): p. 243-256.

Ghosh, S., et al., *Colloidal Behavior of Aluminum Oxide Nanoparticles As Affected by pH and Natural Organic Matter.* Langmuir, 2008. 24(21): p. 12385-12391.

Giasuddin, A.B., S.R. Kanel, and H. Choi, *Adsorption of humic acid onto nanoscale zerovalent iron and its effect on arsenic removal.* Environ Sci Technol, 2007. 41(6): p. 2022-7.

Goff, J.D., et al., *Novel Phosphonate-Functional Poly(ethylene oxide)-Magnetite Nanoparticles Form Stable Colloidal Dispersions in Phosphate-Buffered Saline.* Chemistry of Materials, 2009. 21(20): p. 4784-4795.

Gogate, P.R. and A.B. Pandit, *A review of imperative technologies for wastewater treatment I: oxidation technologies at ambient conditions.* Advances in Environmental Research, 2004. 8(3-4): p. 501-551.

Golden, J., et al., *Evaluating and Treating CMP Wastewater.* Semiconductor Internationa, 2000.

Gottschalk, F., et al., *Modeled Environmental Concentrations of Engineered Nanomaterials (TiO2, ZnO, Ag, CNT, Fullerenes) for Different Regions.* Environmental Science & Technology, 2009. 43(24): p. 9216-9222.

Gray, D., et al., *Spray irrigation of landfill leachate: estimating potential exposures to workers and bystanders using a modified air box model and generalised source term.* Environ Pollut, 2005. 133(3): p. 587-99.

Guerin, T.F., et al., *An application of permeable reactive barrier technology to petroleum hydrocarbon contaminated groundwater.* Water Res, 2002. 36(1): p. 15-24.

Guida, L., et al., *Aluminum toxicity and binding to Escherichia-coli.* Archives of Microbiology, 1991. 156(6): p. 507-512.

Haarstad, K. and T. Maehlum, *Important aspects of long-term production and treatment of municipal solid waste leachate.* Waste Management & Research, 1999. 17(6): p. 470-477.

Hall, C., et al., *Hydrocarbons and the evolution of human culture.* Nature, 2003. 426(6964): p. 318-322.

Hanarp, P., et al., *Control of nanoparticle film structure for colloidal lithography.* Colloids and Surfaces a-Physicochemical and Engineering Aspects, 2003. 214(1-3): p. 23-36.

Handy, R.D., R. Owen, and E. Valsami-Jones, *The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs.* Ecotoxicology, 2008. 17(5): p. 315-325.

Handy, R.D., et al., *The ecotoxicology and chemistry of manufactured nanoparticles.* Ecotoxicology, 2008. 17(4): p. 287-314.

Hart, S.L. and M.B. Milstein, *Creating sustainable value.* Academy of Management Executive, 2003. 17(2): p. 56-67.

He, F. and D.Y. Zhao, *Preparation and characterization of a new class of starch-stabilized bimetallic nanoparticles for degradation of chlorinated hydrocarbons in water.* Environmental Science & Technology, 2005. 39(9): p. 3314-3320.

He, J., et al., *Isolation and characterization of Dehalococcoides sp. strain FL2, a trichloroethene (TCE)- and 1,2-dichloroethene-respiring anaerobe.* Environ Microbiol, 2005. 7(9): p. 1442-50.

Healy, J.B. and L.Y. Young, *Catechol and phenol degradation by a methanogenic population of bacteria.* Applied and Environmental Microbiology, 1978. 35(1): p. 216-218.

Healy, J.B. and L.Y. Young, *Anaerobic biodegradation of eleven aromatic compounds to methane.* Appl Environ Microbiol, 1979. 38(1): p. 84-9.

Heider, J., et al., *Anaerobic bacterial metabolism of hydrocarbons.* Fems Microbiology Reviews, 1998. 22(5): p. 459-473.

Henglein, A., *Physicochemical properties of small metal particles in solution – Microelectrode reactions, chemisorption, composite metal particles, and the atom-to-metal transition.* Journal of Physical Chemistry, 1993. 97(21): p. 5457-5471.

Holister, P. and T.E. Harper, *The nanotechnology opportunity report*. 2002, CMP Científica

Holliger, C. and A.J.B. Zehnder, *Anaerobic biodegradation of hydrocarbons.* Current Opinion in Biotechnology, 1996. 7(3): p. 326-330.

Horie, M., et al., *Protein Adsorption of Ultrafine Metal Oxide and Its Influence on Cytotoxicity toward Cultured Cells.* Chemical Research in Toxicology, 2009. 22(3): p. 543-553.

Hsu, J.P. and A. Nacu, *An experimental study on the rheological properties of aqueous ceria dispersions.* J Colloid Interface Sci, 2004. 274(1): p. 277-84.

Huheey, J.E., E.A. Keiter, and R.L. Keiter, *Inorganic Chemistry:Principles of Structure and Reactivity*. 4th ed. 1993, New York: Prentice Hall. 964.

Hussain, S.M., et al., *Toxicity Evaluation for Safe Use of Nanomaterials: Recent Achievements and Technical Challenges.* Advanced Materials, 2009. 21(16): p. 1549-1559.

Hutchins, S.R.S., G. W.; Kovacst, D. A.; and Smith, G. A., *Biodegradation of Aromatic Hydrocarbons by Aquifer Microorganisms under Denitrifying Conditions.* Environ. Sci. Technol., 1991: p. 68-76.

Hyung, H. and J.H. Kim, *Natural organic matter (NOM) adsorption to multi-walled carbon nanotubes: effect of NOM characteristics and water quality parameters.* Environ Sci Technol, 2008. 42(12): p. 4416-21.

Illes, E. and E. Tombacz, *The effect of humic acid adsorption on pH-dependent surface charging and aggregation of magnetite nanoparticles.* Journal of Colloid and Interface Science, 2006. 295(1): p. 115-123.

Illmer, P. and C. Erlebach, *Influence of Al on growth, cell size and content of intracellular water of Arthrobacter sp PI/1-95.* Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology, 2003. 84(3): p. 239-246.

Illmer, P., K. Marschall, and F. Schinner, *Influence of available aluminum on soil-microorganisms.* Letters in Applied Microbiology, 1995. 21(6): p. 393-397.

Iorio, M., et al., *Sorption of phenanthrene by dissolved organic matter and its complex with aluminum oxide nanoparticles.* Environmental Pollution, 2008. 156(3): p. 1021-1029.

Ishikawa, Y., Y. Katoh, and H. Ohshima, *Colloidal stability of aqueous polymeric dispersions: Effect of pH and salt concentration.* Colloids and Surfaces B-Biointerfaces, 2005. 42(1): p. 53-58.

James, K.J. and M.A. Stack, *The impact of leachate collection on air quality in landfills.* Chemosphere, 1997. 34(8): p. 1713-1721.

Jarvie, H.P., et al., *Fate of silica nanoparticles in simulated primary wastewater treatment.* Environ Sci Technol, 2009. 43(22): p. 8622-8.

Jiang, W., H. Mashayekhi, and B.S. Xing, *Bacterial toxicity comparison between nano- and micro-scaled oxide particles.* Environmental Pollution, 2009. 157(5): p. 1619-1625.

Jimenez, L., R. Alzaga, and J.M. Bayona, *Determination of organic contaminants in landfill leachates: A review.* International Journal of Environmental Analytical Chemistry, 2002. 82(7): p. 415-430.

Jindrova, E., et al., *Bacterial aerobic degradation of benzene, toluene, ethylbenzene and xylene.* Folia Microbiologica, 2002. 47(2): p. 83-93.

Johnson, R.L., et al., *Natural Organic Matter Enhanced Mobility of Nano Zerovalent Iron.* Environmental Science & Technology, 2009. 43(14): p. 5455-5460.

Ju-Nam, Y. and J.R. Lead, *Manufactured nanoparticles: An overview of their chemistry, interactions and potential environmental implications.* Science of the Total Environment, 2008. 400(1-3): p. 396-414.

Jung, H.J., D.B. Kittelson, and M.R. Zachariah, *The influence of a cerium additive on ultrafine diesel particle emissions and kinetics of oxidation.* Combustion and Flame, 2005. 142(3): p. 276-288.

Kamat, P.V. and D. Meisel, *Nanoscience opportunities in environmental remediation.* Comptes Rendus Chimie, 2003. 6(8-10): p. 999-1007.

Karakoti, A.S., L.L. Hench, and S. Seal, *The potential toxicity of nanomaterials - The role of surfaces.* Jom, 2006. 58(7): p. 77-82.

Karn, B., T. Kuiken, and M. Otto, *Nanotechnology and in situ remediation: a review of the benefits and potential risks.* Environ Health Perspect, 2009. 117(12): p. 1813-31.

Kazumi, J.C., M. E.; Suflita, J. M.; Lovley, D. R.; and Young, L. Y., *Anaerobic Degradation of Benzene in Diverse Anoxic Environments.* Environ. Sci. Technol., 1997: p. 813-818.

Keller, A.A., et al., *Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices.* Environ Sci Technol, 2009. 44(6): p. 1962-7.

Keller, A.A., et al., *Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices.* Environ Sci Technol, 2010. 44(6): p. 1962-7.

Kennedy, E.J. and E.M. Lentz, *Treatment of landfill leachate using sequencing batch and continuous flow upflow anaerobic sludge blanket (UASB) reactors.* Water Research, 2000. 34(14): p. 3640-3656.

Kennes, C., R. Mendez, and J.M. Lema, *Methanogenic degradation of p-cresol in batch and in continuous UASB reactors.* Water Research, 1997. 31(7): p. 1549-1554.

Kettunen, R.H. and J.A. Rintala, *Performance of an on-site UASB-reactor treating leachate at low temperature.* Water Research, 1998. 32(3): p. 537-546.

Kilbourn, B.T., *Cerium and Cerium Compounds*, in *Kirk-Othmer Encyclopedia of Chemical Technology*. . 2003, John Wiley and Sons, Inc New York.

Kim, B., et al., *Discovery and Characterization of Silver Sulfide Nanoparticles in Final Sewage Sludge Products.* Environmental Science & Technology, 2010. 44(19): p. 7509-7514.

Kiser, M.A., et al., *Biosorption of nanoparticles to heterotrophic wastewater biomass.* Water Research, 2010. 44(14): p. 4105-4114.

Kiser, M.A., et al., *Titanium nanomaterial removal and release from wastewater treatment plants.* Environ Sci Technol, 2009. 43(17): p. 6757-63.

Kjeldsen, P., et al., *Present and long-term composition of MSW landfill leachate: A review.* Critical Reviews in Environmental Science and Technology, 2002. 32(4): p. 297-336.

Kjeldsen, P., et al., *Characterization of an old municipal landfill (Grindsted, Denmark) as a groundwater pollution source: landfill history and leachate composition.* Waste Management & Research, 1998. 16(1): p. 3-13.

Kjeldsen, P.B., M. A.; Rooker, A. P.; Baun, A.; Ledin, A.; and Christensen, T. H., *Present and Long-Term Composition of MSW Landfill Leachate: A Review.* Critical Reviews in Environmental Science and Technology, 2002: p. 297–336.

Knoll, G. and J. Winter, *ANAEROBIC DEGRADATION OF PHENOL IN SEWAGE-SLUDGE - BENZOATE FORMATION FROM PHENOL AND CO2 IN THE PRESENCE OF HYDROGEN.* Applied Microbiology and Biotechnology, 1987. 25(4): p. 384-391.

Krewski, D., et al., *Human health risk assessment for aluminium, aluminium oxide, and aluminium hydroxide.* Journal of Toxicology and Environmental Health-Part B-Critical Reviews, 2007. 10: p. 1-269.

Krieger, C.J., et al., *A stable organic free radical in anaerobic benzylsuccinate synthase of Azoarcus sp strain T.* Journal of Biological Chemistry, 2001. 276(16): p. 12924-12927.

Kuchovsky, T. and O. Sracek, *Natural attenuation of chlorinated solvents: a comparative study.* Environmental Geology, 2007. 53(1): p. 147-157.

Kung, H.H., *Transition metal oxides: surface chemistry and catalysis*. 1991, Amsterdam: Elsevier.

Kunukcu, Y.K., *In situ bioremediation of groundwater contaminated with petroleum constituents using oxygen release compounds (ORCs).* Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering, 2007. 42(7): p. 839-845.

Lafuma, F., K. Wong, and B. Cabane, *Bridging of colloidal particles through adsorbed polymers.* Journal of Colloid and Interface Science, 1991. 143(1): p. 9-21.

Lai, C.L. and S.H. Lin, *Electrocoagulation of chemical mechanical polishing (CMP) wastewater from semiconductor fabrication.* Chemical Engineering Journal, 2003. 95(1-3): p. 205-211.

Langenhoff, A.A.M., et al., *Microbial reduction of manganese coupled to toluene oxidation.* Fems Microbiology Ecology, 1997. 22(2): p. 119-127.

Leahy, J.G. and R.R. Colwell, *Microbial-degradation of hydrocarbons in the environment.* Microbiological Reviews, 1990. 54(3): p. 305-315.

Lema, J.M., R. Mendez, and R. Blazquez, *Characteristics of landfill leachates and alternatives for their treatment – A review.* Water Air and Soil Pollution, 1988. 40(3-4): p. 223-250.

Lemmer, H., D. Roth, and M. Schade, *Population-density and enzyme-activities of heterotrophic bacteria in sewer biofilms and activated-sludge.* Water Research, 1994. 28(6): p. 1341-1346.

Lendvay, J.M.L., et al., *Bioreactive Barriers: A Comparison of Bioaugmentation and Biostimulation for Chlorinated Solvent Remediation.* Environ. Sci. Technol, 2003: p. 1422-1431.

Lettinga, G. and L.W.H. Pol, *UASB-process desing for various types of wastewaters.* Water Science and Technology, 1991. 24(8): p. 87-107.

Lettinga, G., et al., *Use of the upflow sludge blanket (USB) reactor for biological wastewater-treatment, especially for anaerobic treatment.* Biotechnology and Bioengineering, 1980. 22(4): p. 699-734.

Leuthner, B., et al., *Biochemical and genetic characterization of benzylsuccinate synthase from Thauera aromatica: a new glycyl radical enzyme catalysing the first step in anaerobic toluene metabolism.* Mol Microbiol, 1998. 28(3): p. 615-28.

Li, X.L., et al., *Metal nanoparticles stabilized by cubic silsesquioxanes for catalytic hydrogenations.* Catalysis Letters, 2007. 118(1-2): p. 151-158.

Limbach, L.K., et al., *Removal of oxide nanoparticles in a model wastewater treatment plant: influence of agglomeration and surfactants on clearing efficiency.* Environ Sci Technol, 2008. 42(15): p. 5828-33.

Lin, W.S., et al., *Toxicity of cerium oxide nanoparticles in human lung cancer cells.* International Journal of Toxicology, 2006. 25(6): p. 451-457.

Liu, W.-T., *Nanoparticles and Their Biological and Environmental Applications.* Journal of Bioscience and Bioengineering, 2006. 102.

Liu, X.W., G.P. Sheng, and H.Q. Yu, *Physicochemical characteristics of microbial granules.* Biotechnol Adv, 2009. 27(6): p. 1061-70.

Liu, Y.L., et al., *Synthesis, stability, and cellular internalization of gold nanoparticles containing mixed peptide-poly(ethylene glycol) monolayers.* Analytical Chemistry, 2007. 79(6): p. 2221-2229.

Lo, I.M.C., *Characteristics and treatment of leachates from domestic landfills.* Environment International, 1996. 22(4): p. 433-442.

Lockman, P.R., et al., *Nanoparticle surface charges alter blood-brain barrier integrity and permeability.* Journal of Drug Targeting, 2004. 12(9-10): p. 635-641.

Logan, B.E. and J. Wu, *Enhanced Toluene Degradation Under Chlorate-Reducing Conditions by Bioaugmentation of Sand Columns with Chlorate- and Toluene-Degrading Enrichments* Bioremediation Journal, 2002. 6(2): p. 87-95.

Long, T.C., et al., *Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): Implications for nanoparticle neurotoxicity.* Environmental Science & Technology, 2006. 40(14): p. 4346-4352.

Machdar, I., et al., *Combination of a UASB reactor and a curtain type DHS (downflow hanging sponge) reactor as a cost-effective sewage treatment system for developing countries.* Water Science and Technology, 2000. 42(3-4): p. 83-88.

Macleod, F.A., S.R. Guiot, and J.W. Costerton, *Layered structure of bacterial aggregates produced in an upflow anaerobic sludge bed and filter reactor.* Applied and Environmental Microbiology, 1990. 56(6): p. 1598-1607.

Mandzy, N., E. Grulke, and T. Druffel, *Breakage of TiO2 agglomerates in electrostatically stabilized aqueous dispersions.* Powder Technology, 2005. 160(2): p. 121-126.

Mangimbulude, J.C., et al., *Seasonal dynamics in leachate hydrochemistry and natural attenuation in surface run-off water from a tropical landfill.* Waste Manag, 2009. 29(2): p. 829-38.

McConnell, W.P., et al., *Electronic and optical properties of chemically modified metal nanoparticles and molecularly bridged nanoparticle arrays.* Journal of Physical Chemistry B, 2000. 104(38): p. 8925-8930.

Mor, S., et al., *Leachate characterization and assessment of groundwater pollution near municipal solid waste landfill site.* Environ Monit Assess, 2006. 118(1-3): p. 435-56.

Morgan, P., S.T. Lewis, and R.J. Watkinson, *Biodegradation of benzene, toluene, ethylbenzene and xylenes in gas-condensate-contaminated ground-water.* Environ Pollut, 1993. 82(2): p. 181-90.

Morris, J. and J. Willis, *EPA Nanotechnology White Paper* U.S.E.P. Agency, Editor. 2007: Washington p. 120.

Mueller, N.C. and B. Nowack, *Exposure modeling of engineered nanoparticles in the environment.* Environ Sci Technol, 2008. 42(12): p. 4447-53.

Murray, H.E. and J.N. Beck, *Concentrations of Synthetic organic chemicals in leachate from a municipal landfill.* Environ Pollut, 1990. 67(3): p. 195-203.

National Nanotechnology Initiative. [cited 2010 February 2010]; Available from: http://www.nano.gov.

Nel, A., et al., *Toxic potential of materials at the nanolevel.* Science, 2006. 311(5761): p. 622-7.

Nutt, M.O., J.B. Hughes, and S.W. Michael, *Designing Pd-on-Au bimetallic nanoparticle catalysts for trichloroethene hydrodechlorination.* Environ Sci Technol, 2005. 39(5): p. 1346-53.

Nyer, E.K., *Groundwater Treatment Technology*. 2nd Edition ed. 1992, New York: Van Nostrand Reinhold. 320.

Obare, S.O. and G.J. Meyer, *Nanostructured materials for environmental remediation of organic contaminants in water.* J Environ Sci Health A Tox Hazard Subst Environ Eng, 2004. 39(10): p. 2549-82.

Okabe, S., H. Satoh, and Y. Watanabe, *In situ analysis of nitrifying biofilms as determined by in situ hybridization and the use of microelectrodes.* Applied and Environmental Microbiology, 1999. 65(7): p. 3182-3191.

Pang, Y.X. and X.J. Bao, *Aluminium oxide nanoparticles prepared by water-in-oil microemulsions.* Journal of Materials Chemistry, 2002. 12(12): p. 3699-3704.

Pankow, J.F., R.L. Johnson, and J.A. Cherry, *Air sparging in gate wells in cutoff walls and trenches for control of plumes of volatile organic-compounds (VOCS).* Ground Water, 1993. 31(4): p. 654-663.

Pant, P. and S. Pant, *A review: advances in microbial remediation of trichloroethylene (TCE).* J Environ Sci (China), 2009. 22(1): p. 116-26.

Park, B., et al., *Hazard and risk assessment of a nanoparticulate cerium oxide-based diesel fuel additive - a case study.* Inhal Toxicol, 2008. 20(6): p. 547-66.

Patil, S., et al., *Protein adsorption and cellular uptake of cerium oxide nanoparticles as a function of zeta potential.* Biomaterials, 2007. 28(31): p. 4600-4607.

Pavlostathis, S.G. and E. Giraldogomez, *Kinetics of anaerobic treatment – A critical-review.* Critical Reviews in Environmental Control, 1991. 21(5-6): p. 411-490.

Paxeus, N., *Organic compounds in municipal landfill leachates.* Water Science and Technology, 2000. 42(7-8): p. 323-333.

Phelps, C.D. and L.Y. Young, *Anaerobic biodegradation of BTEX and gasoline in various aquatic sediments.* Biodegradation, 1999. 10(1): p. 15-25.

Pina, R.G. and C. Cervantes, *Microbial interactions with aluminium.* Biometals, 1996. 9(3): p. 311-316.

Pis, J.J., et al., *Preparation of active carbons from coal .1. Oxidation of coal.* Fuel Processing Technology, 1996. 47(2): p. 119-138.

Pitkethly, M.J., *Nanomaterials, the driving force.* Materials Today, 2004. 7(12): p. 20 - 29.

Playne, M.J. and B.R. Smith, *Toxicity of organic reagents to anaerobic-bacteria.* Biotechnology and Bioengineering, 1983. 25(5): p. 1251-1265.

Plotkin, S. and N.M. Ram, *Multiple bioassays to assess the toxicity of a sanitary landfill leachate.* Arch Environ Contam Toxicol, 1984. 13(2): p. 197-206.

Pommerenk, P. and G.C. Schafran, *Adsorption of inorganic and organic ligands onto hydrous aluminum oxide: Evaluation of surface charge and the impacts on particle and NOM removal during water treatment.* Environmental Science & Technology, 2005. 39(17): p. 6429-6434.

Rabus, J.H.a.R., *Initial reactions of anaerobic metabolism of alkylbenzenes in denitrifying and sulfate-reducing bacteria.* Arch Microbiol, 1998: p. 377–384.

Rabus, R. and F. Widdel, *Anaerobic degradation of ethylbenzene and other aromatic hydrocarbons by new denitrifying bacteria.* Arch Microbiol, 1995. 163(2): p. 96-103.

Reijnders, L., *Cleaner nanotechnology and hazard reduction of manufactured nanoparticles.* Journal of Cleaner Production, 2006. 14(2): p. 124-133.

Reinhard, M.H., G. D.; Steinle-Darling, E.; and LeBron, C. A., *In Situ Biotransformation of BTEX Compounds Under Methanogenic Conditions.* Groud Water Monitoring and Remediation, 2005: p. 50-59.

Reinhart, D.R., *A review of recent studies on the source of hazardous compounds emitted from solid-waste landfills – A United-States experience.* Waste Management & Research, 1993. 11(3): p. 257-268.

Reinhart, D.R. and F.G. Pohland, *The assimilation of organic hazardous wastes by municipal solid-waste landfills.* Journal of Industrial Microbiology, 1991. 8(3): p. 193-200.

Rejeski, D. *State of Commercialization of Nano-enabled Consumer Products* 2009 [cited 2010 September 26th]; Available from: http://www.nanotechproject.org/inventories/consumer/.

Renou, S., et al., *Landfill leachate treatment: Review and opportunity.* Journal of Hazardous Materials, 2008. 150(3): p. 468-493.

Revuz, D. and M. Yor, *Continuous martingales and Brownian motion*. 1991: Springer-Verlag.

Ridgway, H.F., et al., *Identification and catabolic activity of well-derived gasoline-degrading bacteria from a contaminated aquifer.* Applied and Environmental Microbiology, 1990. 56(11): p. 3565-3575.

Rittmann, B.E. and P.L. McCarty, *Environmental biotechnology : principles and applications*. 2001, Boston: McGraw-Hill.

Rittner, M., *Nanoparticles - what's now, what's next?* Chemical Engineering Progress, 2003. 99.

Rittner, M.N., *Market analysis of nanostructured materials.* American Ceramic Society Bulletin, 2002. 81(3): p. 33-36.

Roychoudhury, A.N. and G.L. Merrett, *Redox pathways in a petroleum contaminated shallow sandy aquifer: Iron and sulfate reductions.* Sci Total Environ, 2006. 366(1): p. 262-74.

Sahoo, Y., et al., *Alkyl phosphonate/phosphate coating on magnetite nanoparticles: A comparison with fatty acids.* Langmuir, 2001. 17(25): p. 7907-7911.

Salem, Z., et al., *Evaluation of landfill leachate pollution and treatment.* Desalination, 2008. 220(1-3): p. 108-114.

Scherer, M.M., et al., *Chemistry and microbiology of permeable reactive barriers for in situ groundwater clean up.* Crit Rev Microbiol, 2000. 26(4): p. 221-64.

Schink, B., *Energetics of syntrophic cooperation in methanogenic degradation.* Microbiology and Molecular Biology Reviews, 1997. 61(2): p. 262-&.

Schubert, D., et al., *Cerium and yttrium oxide nanoparticles are neuroprotective.* Biochem Biophys Res Commun, 2006. 342(1): p. 86-91.

Schwarzbauer, J., et al., *Occurrence and alteration of organic contaminants in seepage and leakage water from a waste deposit landfill.* Water Res, 2002. 36(9): p. 2275-87.

Seaton, A. and K. Donaldson, *Nanoscience, nanotoxicology, and the need to think small.* Lancet, 2005. 365(9463): p. 923-4.

Semmler, M., et al., *Diffusional deposition of charged latex particles on water-solid interfaces at low ionic strength.* Langmuir, 1998. 14(18): p. 5127-5132.

Seyfried, B., et al., *Initial reactions in the anaerobic oxidation of toluene and m-xylene by denitrifying bacteria.* Appl Environ Microbiol, 1994. 60(11): p. 4047-52.

Shim, H. and T.K. Wood, *Aerobic degradation of mixtures of chlorinated aliphatics by cloned toluene-o-xylene monooxygenase and toluene o-monooxygenase in resting cells.* Biotechnol Bioeng, 2000. 70(6): p. 693-8.

Shim, H. and S.T. Yang, *Biodegradation of benzene, toluene, ethylbenzene, and o-xylene by a coculture of Pseudomonas putida and Pseudomonas fluorescens immobilized in a fibrous-bed bioreactor.* J Biotechnol, 1999. 67(2-3): p. 99-112.

Sikkema, S.D., E.; Ahlert, R. and Kosson, D., *Identification and Quantification of Volatile Organic Species During Microbial Treatment of Leachate.* Environmental Progress, 1988. 7: p. 77-83.

Slack, R.J., J.R. Gronow, and N. Voulvoulis, *Household hazardous waste in municipal landfills: contaminants in leachate.* Sci Total Environ, 2005. 337(1-3): p. 119-37.

Smith, P.H. and R.A. Mah, *Kinetics of acetate metabolism during sludge digestion.* Applied Microbiology, 1966. 14(3): p. 368-&.

Stams, A.J.M., *Metabolic interactions between anaerobic-bacteria in methanogenic environments.* Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology, 1994. 66(1-3): p. 271-294.

Steinhauer, M.S. and P.D. Boehm, *The composition and distribution of saturated and aromatic-hydrocarbons in nearshore sediments, river sediments, and coastal peat of the Alaskan Beaufort sea – Implications for detecting anthropogenic hydrocarbon inputs.* Marine Environmental Research, 1992. 33(4): p. 223-253.

Strous, M., et al., *Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (Anammox) process in different reactor configurations.* Water Research, 1997. 31(8): p. 1955-1962.

Szewzyk, U., R. Szewzyk, and B. Schink, *Methanogenic degradation of hydroquinone and catechol via reductive dehydroxylation to phenol.* Fems Microbiology Ecology, 1985. 31(2): p. 79-87.

Tagawa, M., et al., *Influence of surface-properties of particles n their adhesion and removal.* Colloid and Polymer Science, 1989. 267(5): p. 434-439.

Takahata, Y., et al., *Rapid intrinsic biodegradation of benzene, toluene, and xylenes at the boundary of a gasoline-contaminated plume under natural attenuation.* Appl Microbiol Biotechnol, 2006. 73(3): p. 713-22.

Tandukar, M., et al., *A low-cost municipal sewage treatment system with a combination of UASB and the "fourth-generation" downflow hanging sponge reactors.* Water Sci Technol, 2005. 52(1-2): p. 323-9.

Tandukara, M., O. Akiyoshib, and H. Hidekia, *Nitrogen removal by down-flow hanging-sponge reactor operated as a post treatment unit for UASB treating domestic sewage.* Proceedings of the Water Environment Federation, 2007: p. 5296-5303.

Tawfik, A., A. Ohashi, and H. Harada, *Sewage treatment in a combined up-flow anaerobic sludge blanket (UASB)-down-flow hanging sponge (DHS) system.* Biochemical Engineering Journal, 2006. 29(3): p. 210-219.

Tchobanoglous, G., F.L. Burton, and H.D. Stensel, *Wastewater Engineering: Treatment and Reuse*. 4th ed. 2002: McGraw-Hill 1848.

Thauer, R.K., K. Jungermann, and K. Decker, *Energy-conservation in chemotropic anaerobic bacteria.* Bacteriological Reviews, 1977. 41(1): p. 100-180.

Thomas, J.M., et al., *Biodegradation of BTEX in subsurface minerals contaminated with gasoline – Granger, Indiana.* Water Science and Technology, 1990. 22(6): p. 53-62.

Tiede, K., et al., *Detection and characterization of engineered nanoparticles in food and the environment.* Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 2008. 25(7): p. 795-821.

Tombacz, E., et al., *Effect of pH and ionic strength on the interaction of humic acid with aluminium oxide.* Colloid and Polymer Science, 2000. 278(4): p. 337-345.

Tso, C.P., et al., *Stability of metal oxide nanoparticles in aqueous solutions.* Water Science and Technology, 2010. 61(1): p. 127-133.

Uemura, S., et al., *Removal of organic substances and oxidation of ammonium nitrogen by a down-flow hanging sponge (DHS) reactor under high salinity conditions.* Bioresource Technology, 2010. 101(14): p. 5180-5185.

Underwood, A.J.V., *Industrial synthesis of hydrocarbons from hydrogen and carbon monoxide.* Industrial and Engineering Chemistry, 1940. 32: p. 449-454.

USGS. *Chlorinated Solvents Contamination*. 2008 [cited 2011; Available from: http://toxics.usgs.gov/investigations/chlorinated_solvents.html.

Valsaraj, K.T., et al., *Reusable adsorbents for dilute solution separation. 1. Adsorption of phenanthrene on surfactant-modified alumina.* Separation and Purification Technology, 1998. 13(2): p. 137-145.

Van Stempvoort, D. and K. Biggar, *Potential for bioremediation of petroleum hydrocarbons in groundwater under cold climate conditions: A review.* Cold Regions Science and Technology, 2008. 53(1): p. 16-41.

Vanbenschoten, J.E. and J.K. Edzwald, *Chemical aspects of coagulation using aluminum salts. 1. Hydrolytic reactions of alum and polyaluminum chloride.* Water Research, 1990. 24(12): p. 1519-1526.

Vermerris, W. and R. Nicholson, *Phenolic Compound Biochemistry* 2006: Springer. 276.

Verstraete, W. and P. Vandevivere, *New and broader applications of anaerobic digestion.* Critical Reviews in Environmental Science and Technology, 1999. 29(2): p. 151-173.

Wang, P., et al., *Landfill leachate treatment with combined UASB and Fenton coagulation.* Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering, 2000. 35(10): p. 1981-1988.

Wardak, A., et al., *Environmental regulation of nanotechnology and the TSCA.* Ieee Technology and Society Magazine, 2007. 26(2): p. 48-56.

Widdel, F. and R. Rabus, *Anaerobic biodegradation of saturated and aromatic hydrocarbons.* Current Opinion in Biotechnology, 2001. 12(3): p. 259-276.

Wigginton, N.S., K.L. Haus, and M.F. Hochella, Jr., *Aquatic environmental nanoparticles.* J Environ Monit, 2007. 9(12): p. 1306-16.

Wilkins, K., *Volatile organic compounds from household waste.* Chemosphere, 1994. 29(1): p. 47-53.

Wilkinson, K.J., A. Joz-Roland, and J. Buffle, *Different Roles of Pedogenic Fulvic Acids and Aquagenic Biopolymers on Colloid Aggregation and Stability in Freshwaters.* Limnology and Oceanography, 1997. 42: p. 1714-1724

Wilson, L.P. and E.J. Bouwer, *Biodegradation of aromatic compounds under mixed oxygen/denitrifying conditions: a review*. J Ind Microbiol Biotechnol, 1997. 18(2-3): p. 116-30.

Wiszniowski, J., et al., *Landfill leachate treatment methods: A review*. Environmental Chemistry Letters, 2006. 4(1): p. 51-61.

Yasuhara, A., et al., *Organic components in leachates from hazardous waste disposal sites*. Waste Management & Research, 1999. 17(3): p. 186-197.

Young, L.Y. and M.D. Rivera, *Methanogenic degradation of 4 phenolic-compounds.* Water Research, 1987. 21(7): p. 859-861.

Zhang, L.L., et al., *Biosorption of rare earth metal ion on aerobic granules.* J Environ Sci Health A Tox Hazard Subst Environ Eng, 2005. 40(4): p. 857-67.

Zhang, Y., et al., *Stability of commercial metal oxide nanoparticles in water*. Water Res, 2008. 42(8-9): p. 2204-12.

Zinder, S.H., *Physiological Ecology of Methanogens*, in *Methanogensis: Ecology, Physiology, Biochemistry & Genetics* J.G. Ferry, Editor. 1993, Chapman and Hall, Inc.: New York. p. 128 - 205.